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CHEMICAL MANIPULATION OF WHITE CLOVER (*Trifolium repens* L.) GROWN FOR SEED PRODUCTION

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ABSTRACT

The effects of chemical manipulation through the use of plant growth regulators on white clover (*Trifolium repens* L.) cv. Grasslands Pitau grown for seed were investigated in this study, using both sward and individual plant trials.

A white clover seed crop was established in autumn 1988, certified breeders seed of cv. Grasslands Pitau being sown at 3 kg/ha in 45 cm rows. Three plant growth regulators, chlormequat chloride (1.5 and 3.0 kg a.i./ha), paclobutrazol (0.5 and 1.0 kg a.i./ha) and triapenthenol (0.5 and 1.0 kg a.i./ha) were applied at two growth stages; during reproductive initiation (11 October) or at the appearance of the first visible bud (8 November). A further plant growth regulator, daminozide (2.0 and 4.0 kg a.i./ha) was applied only in November. Chlormequat chloride, daminozide and triapenthenol did not significantly affect node production, inflorescence production or seed yield, although thousand seed weight (TSW) was reduced. Paclobutrazol significantly reduced petiole length and increased the number of nodes/m^2, but did not affect dry matter production. The October application of paclobutrazol at 1.0 kg a.i./ha significantly increased potential harvestable seed yield by 71 % through increasing the number of inflorescences produced, but the 57 % increase following the November application at the same rate did not differ significantly from the control. Actual seed yield differences (+25 and 26 %) were also not significant.

In the following season (1989/1990), three of the plant growth regulators (chlormequat chloride at 3.0 kg a.i./ha, paclobutrazol at 1.0 kg a.i./ha, triapenthenol at 1.0 kg a.i./ha) were applied using the same site as for the 1988/1989 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 (September), during peak reproductive initiation (October) or when reproductive buds/early flowers were first visible (November). Chlormequat chloride did not affect either vegetative or reproductive growth and development. Triapenthenol initially retarded growth (e.g. by reducing petiole length), but this effect was only transiatory, and was no longer evident 3 weeks after application. Although triapenthenol applied in November increased inflorescence number at peak flowering, seed yield was not increased. Triapenthenol applied in October did not affect inflorescence number at peak flowering, but reduced TSW. Paclobutrazol applied in September, October and November reduced petiole length and leaf size,

but only application in November increased both node and stolon production. Application in October and November increased inflorescence numbers at peak flowering and harvest respectively, but seed yield was not increased. Data recorded from plots sprayed with paclobutrazol the previous season (1988/1989) provided no evidence of growth retardation through soil residual activity.

In an attempt to clarify the effects of paclobutrazol on white clover growth and development, individual plants grown from seeds selected at random from a lot of certified breeders seed were established as spaced plants (80 x 80 cm) in the field in spring of 1990. Paclobutrazol was applied at 1.0 kg a.i./ha on 6 November 1990 (when more than 75 % of the plants were initiating reproductive buds at their terminal buds) or 23 November 1990 (when more than 50 % of the plant population had reproductive buds visible on their stolons). Petiole length and leaf size were initially reduced, but beginning two months after application, vigorous regrowth occurred, to the extent that paclobutrazol treated plants became as tall as the control plants. However, retardation effects occurred again at harvest. Total plant dry matter and root:shoot ratios were not affected by paclobutrazol. Chlorophyll content/unit leaf area and leaf thickness increased following paclobutrazol application, but increases were not correlated. Seed yield and yield components did not differ from that of the control plants, mainly because plant to plant variation was very large, irrespective of treatment.

To attempt to reduce this source of variation, a further spaced plant trial was established in 1991/1992 using plants produced by clonal propagation from three distinct genotypes from within cv. Grasslands Pitau. Paclobutrazol was applied at the same rate and time as in the previous season, and while not affecting the number of nodes developed along stolons or inflorescence initiation at the stolon apices, it did significantly increase stolon production in all three genotypes through increasing secondary, tertiary and to a lesser extent quaternary branch numbers. However, not all these extra stolons were able to produce inflorescences, and this ability varied significantly with genotype. As a consequence, inflorescence number and potential harvestable seed yield were significantly increased only in one genotype following paclobutrazol application. However, paclobutrazol reduced seed abortion and increased seed weight in all three genotypes.

In individual plants, inflorescence growth and development from emergence to the seed ripening stage occurred more quickly in paclobutrazol treated plants than untreated plants. A simulated sward trial was used in 1990/1991 to determine whether the previous failures to significantly increase actual seed yield were because paclobutrazol treated plots had ripened earlier than control plots, and as a consequence more seed had been shed by the time of harvest. However, no significant paclobutrazolXharvest time interactions for seed yield or seed yield components were recorded. These results suggest that paclobutrazol did not affect seed maturity in a sward situation. Irrespective of treatment, greatest seed yield came from harvesting 25 days after peak flowering, but this did not differ significantly from harvesting 35 days after peak flowering. Delaying harvest to 40 and 45 days after peak flowering significantly reduced seed yield. As in previous sward trials, paclobutrazol application significantly increased inflorescence numbers, but large (+56 %) differences in potential harvestable and actual seed yield were statistically not significant. In each case, high data variation (CV > 30 %) was recorded. Factors responsible for the failure of apparent biological increases to be statistically real are briefly discussed.

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CHAPTER 1

GENERAL INTRODUCTION

White clover (*Trifolium repens* L.) is one of the world's most important temperate pasture plants. It has a distribution ranging from the Arctic to the subtropics and has a wide altitudinal range, being reported from sea level to up to 6000 m in the Himalaya Range of India (Williams, 1987a). The likely centre of origin of white clover was in the Mediterranean region (Vavilov, 1951), and it spread naturally throughout Europe, Western Asia and Northern Africa. It has been introduced to other temperate regions including China, Mongolia, Korea, Japan, the Americas, Southern Africa and Australasia (Williams, 1987a). The species also grows wild in high altitude areas near the equator such as Guatemala, Costa Rica, Venezuela, Colombia, Peru, Bolivia, Brazil and Hawaii (Williams, 1987a).

White clover is important in farming systems for at least two reasons. The first reason is its ability to fix atmospheric nitrogen through symbiosis with root nodule *Rhizobium* bacteria (Crush, 1987). This nitrogen becomes immediately available for white clover growth, and in a mixed sward, ultimately available for associated grass growth through the excretion of nitrogenous compounds from nodules of white clover, the decay of white clover root nodules and aerial plant parts, or through fertility recycling in the excreta (dung and urine) of grazing animals which consume the herbage (Butler and Bathurst, 1956). Secondly, it has long been established that white clover has advantages over grass in terms of quality for animal feeding. In general, it has less fibre than grasses, a higher ratio of soluble and insoluble carbohydrate, higher protein content and a higher level of digestibility (Minson *et al.*, 1964; Rattray and Joyce,1969; Ulyatt, 1971; Ulyatt *et al.*, 1977). These factors result in more milk production and increased animal growth rates (Johns, 1966; Brown, 1990), and white clover dominant pastures are also superior to grass dominant ones for flushing breeding ewes (Leonard *et al.*, 1985). Even when a

comparison was made between herbage legume species, sheep fed with white clover produced higher liveweight gains than those fed with other species such as red clover (*Trifolium pratense* L.), lucerne (*Medicago sativa* L.), sainfoin (*Onobrychis viciifolia* Scop.) and Maku lotus (*Lotus uliginosus* Schkuhr.) (John and Lancashire, 1981).

The worldwide importance of white clover is also shown in the annual world production of some 9000 tonnes of seed, sufficient to sow up to 3 million hectares (Clifford and Rolston, 1990). Around 60 % of this seed is produced in the Canterbury region (43°-45°S) of New Zealand (Clifford and Rolston, 1990). The New Zealand average seed yield is only 230 kg/ha (Rolston and Clifford, 1989). Similarly in the United Kingdom and Denmark, the national average seed yield is low, i.e. 136 kg/ha (Evans *et al.*, 1986) and 348 kg/ha (Nordestgaard and Andersen, 1991) respectively. However, some good specialist seed growers are producing between 500-900 kg/ha (Rolston and Clifford, 1989), while in research trials, Clifford (1985a) reported a yield of 1330 kg/ha. This low average seed yield of white clover is because seed yield can fluctuate widely within and between seasons as commonly occurs in many herbage legume species (Hampton, 1990). Poor crop management is also a major factor which contributes to low average seed yields (Davies, 1981; Hampton, 1990). Some growers are still practicing the catch-cropping system of growing white clover and ryegrass pasture mixtures for seed of either species between periods when the sward is used for grazing (Hebblethwaite, 1980; Wilman *et al.*, 1991). Seed yields obtained from this system have usually been lower than those from specialist seed crops (Zaleski, 1970). Clifford (1987) has demonstrated that there are distinctly different growth requirements needed to produce 'seed' rather than 'feed'. The potential seed yield of white clover represents the cumulative expression of four principal components of yield: inflorescence numbers per unit area, floret numbers per inflorescence, seed numbers per floret and seed weight (Zaleski, 1970; Van Bogaert, 1977; Huxley *et al.*, 1979; Maldonado, 1985; Evans *et al.*, 1986; Clifford, 1987; Thomas, 1987c). The growth and development of these components are strongly affected by environmental factors as

well as genetic variability within the species (Thomas, 1987c; Hampton, 1990), and this may result in fluctuating seed yields. In addition, white clover's indeterminate growth and responses to the environment result in plants flowering over an extended period, during which inflorescences with different ripeness categories can be present simultaneously on an individual plant (Norris, 1984; Marshall and Hides, 1986, 1989, 1991b; Thomas, 1987c; Hollington *et al.*, 1989; Marshall *et al.*, 1989). This condition makes it difficult to optimize harvest date. However, the potential for high yield is already there, i.e. 1500 kg (conservatively estimated by Clifford, 1987), and with the correct crop management practices, Clifford (1987) has demonstrated that consistently high seed yields can be produced from high herbage producing white clover cultivars.

With the increasingly wide use of chemicals in agricultural practice, there are also several options for using plant growth regulators to manipulate plant growth and development for successfully improved seed production, as has been demonstrated in grasses (Hampton, 1988a). The present study was aimed at investigating the use of plant growth regulators in white clover grown for seed. The study consisted of several experiments. The effect of selected plant growth regulators on growth, development and seed yield of a first and second year crop is reported in Chapters 3 and 4 respectively. Based on the results reported in these two chapters, the effect of paclobutrazol on growth, development and seed yield was then closely examined using individual plants propagated from both seeds and clones of three different genotypes. This work is reported in Chapter 5. The effect of harvest time in relation to the use of paclobutrazol in white clover seed production is reported in Chapter 6. Finally, Chapter 7 reports an investigation which was carried out to examine the effect of residual paclobutrazol on the growth, development and seed yield of white clover. In addition, a review of the species and factors affecting white clover reproductive growth and development, management for seed production and plant growth regulators used in this study, is presented in Chapter 2. The last chapter in this study (Chapter 8) comprises a general discussion and conclusions and recommendations for future research.

CHAPTER 2

LITERATURE REVIEW

This review will concentrate on aspects considered to be relevant to the present study on white clover seed production. Reproductive growth and development in white clover, and factors that affect this are discussed. In addition, particular emphasis is given to seed crop management practices and the possibility of chemical manipulation of reproductive growth and development using plant growth regulators.

2.1. THE WHITE CLOVER PLANT

White clover (*Trifolium repens* L.) is a perennial herbage legume which is classified taxonomically in tribe Trifolieae of the sub-family Papilionoideae of the family Leguminosae. Information about the origin, breeding, characteristics and agronomic potential of 232 of the many white clover cultivars worldwide has been summarized by Caradus (1986).

Complete descriptions of structure and vegetative growth and development of white clover have been presented by Thomas (1987a,b). White clover has a procumbent solid stolon which is the basic structure of the plant. Its final form is the result of the initiation, growth and development of leaf, axillary bud and internode primordia in the apical bud. The procumbent stolon consists of a series of internodes separated by nodes. Each node bears a trifoliate leaf with an erect petiole, two root primordia and an axillary bud (during purely vegetative growth) which is capable of growing into a lateral stolon.

Stolons can grow indefinitely at their tips, rooting at their nodes, dying off at their bases gradually and developing lateral branches from their axillary buds. In this way a mature white clover plant consists of several stolons of various origins and of

different chronological and developmental ages. This can create a problem when attempting to describe the morphology of the plant, in particular, under sward conditions. To overcome this problem, Thomas (1987a) suggested that each plant should be described using four categories (see also Figure 2.1):

- Main stolon : A stolon which bears (or has produced) at least eight leaves with unfolded leaflets along its main axis *and* possesses adventitious roots at one or more nodes or at its base.
- Axillary bud : A vegetative shoot in a leaf axil which has produced fewer than two leaves with unfolded leaflets.
- Lateral branch : Any vegetative axillary outgrowth which has produced two or more leaves with unfolded leaflets. The apical bud of such a branch is often referred to by agronomists as containing an 'active meristem' (even when it is not growing).
- Lateral stolon : A lateral branch growing from a main stolon, or from another lateral stolon, which has produced three or four leaves with unfolded leaflets *and* has a stem which, from its base to the node of attachment of its youngest leaf with unfolded leaflets, is at least twice as long as the stipules of its subtending leaf, *or* has produced five to seven leaves with unfolded leaflets and developed its own adventitious root system *or* has produced more than seven leaves with unfolded leaflets but possesses no adventitious root system.

2.2. REPRODUCTIVE GROWTH AND DEVELOPMENT, AND FACTORS THAT AFFECT GROWTH AND DEVELOPMENT

Reproductive growth and development in white clover proceeds in four stages:

- inflorescence initiation
- inflorescence growth and development
- anthesis, pollination and fertilization, and
- seed development

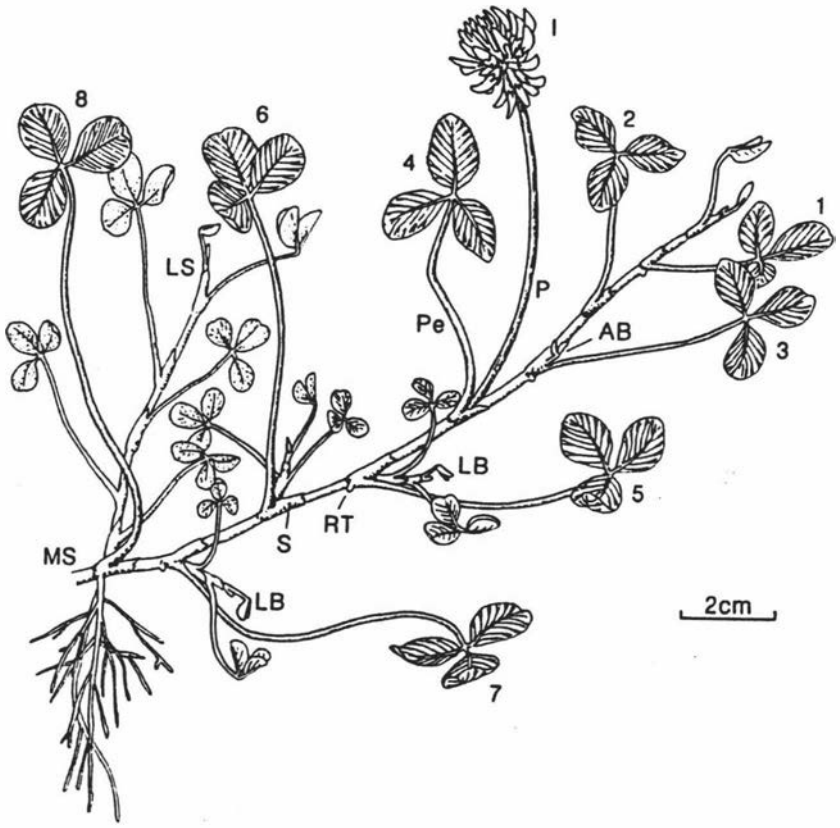


Figure 2.1. Drawing of a main stolon (MS) of white clover showing axillary buds (AB), lateral branches (LB) and a lateral stolon (LS). S= stipule, Pe = petiole, RT = nodal root primordium, I = inflorescence, P = peduncle. Emerged leaves on the main stolon and the nodes bearing them, are numbered 1 to 8 (Thomas, 1987a).

2.2.1. Inflorescence initiation

Inflorescences in white clover form at the stolon apex. Axillary bud primordia may develop into either an inflorescence or a secondary stolon, but never both at the same site (Thomas, 1962, 1980a, 1987c). During vegetative growth the youngest axillary bud primordium appears in the axil of the third youngest leaf primordium.

The transition from the vegetative to the reproductive phase results in bud formation in the axil of the youngest leaf primordium, as opposed to the third youngest axil in the vegetative state. The youngest leaf primordium and its subtended bud together form a so-called 'double ridge structure'. This precocious axillary bud then grows into an inflorescence primordium. Differences between stolon tips during vegetative and reproductive stages are presented in Plates 2.1 and 2.2.

The change in axillary bud initiation to either an inflorescence or a secondary stolon is under the control of the environment, with the two most important controlling factors being temperature and daylength. Another factor, available mineral nutrients, affects the degree to which plants are able to respond to temperature and daylength (Thomas, 1987c).

White clover is generally regarded as a long-day plant (LDP) because many cultivars have been reported to be LDPs (see Thomas, 1987c). However, this gives a rather misleading conception of its physiological response to the environment, firstly because inflorescence initiation is strongly influenced by low temperatures as well as by long days, and secondly because many cultivars behave more as short-long-day plants (SLDPs) than as long-day plants (Thomas, 1987c).

Regardless of daylength, white clover initiates inflorescences in response directly or indirectly to low temperatures (Thomas, 1987c). In plants showing a direct response, initiation occurs during naturally cool conditions in autumn, winter and early spring. Indirect responses are classically referred to as vernalization. In these cases low temperature pre treatment enhances inflorescence initiation in subsequent warm conditions. Many white clover cultivars show a direct response to low temperatures (Laude *et al.*, 1958; Britten, 1960, 1961; Zaleski, 1964; Thomas, 1979, 1980a, 1982, 1987c), but few cultivars show an indirect response to low temperatures, e.g. Kent Wild White (Haggar, 1961) and some lesser indications in Spanish C1067, Louisiana S1 and Tamar (Thomas, 1982).

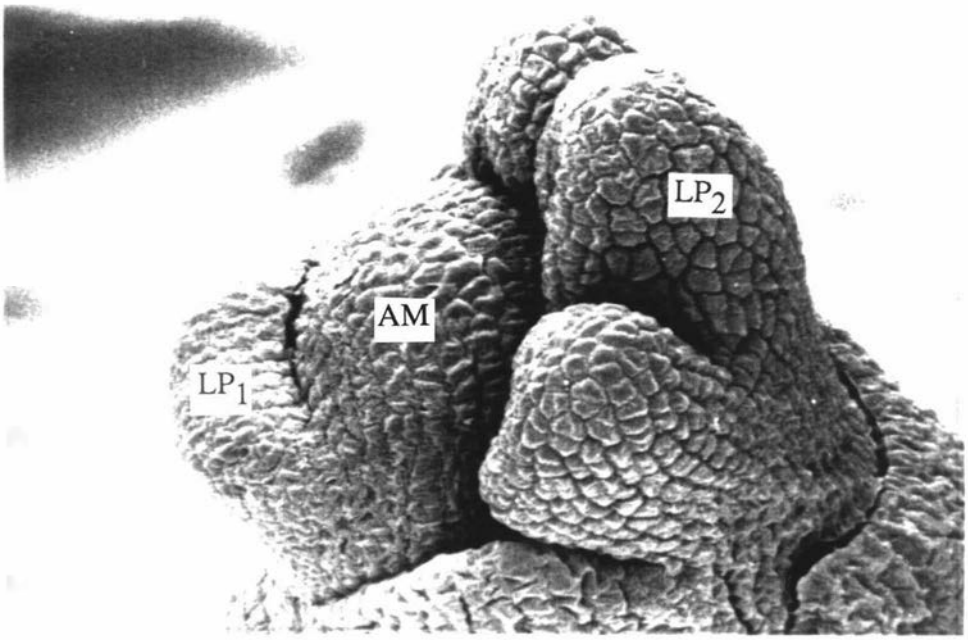


Plate 2.1. Scanning electron micrograph of the apical meristem and adjacent tissues at a vegetative stolon tip (Seed Technology Centre, 1992). LP_1 and LP_2 = the youngest leaf primordia AM = the apical meristem

Plate 2.2. Scanning electron micrograph of the apical meristem and adjacent tissues at a reproductive stolon tip (reproduced from Maldonado, 1985). IP = the inflorescence primordium, AM = the apical meristem

The mechanism of action of low temperatures is still being investigated. Thomas (1981a, 1987c) hypothesized that inflorescence initiation in white clover is influenced by inhibitors as well as promoters, and it seems likely that low temperatures probably act either by increasing the level of promoters or by decreasing the level of initiation antagonists (inhibitors). These promoters and inhibitors may be associated with growth regulating compounds excluding inorganic and organic nutrients, also known as plant growth hormones. However, which hormones act as promoters or inhibitors for inflorescence initiation in white clover are not fully known. No published reports are available of the influence of auxins and cytokinins on inflorescence initiation in white clover. On the other hand, attempts to induce inflorescence initiation in white clover by gibberellins have resulted in contradictary findings. Cohen and Dovrat (1976) reported that application of GA_3 under short days caused Tamar (a low-latitude white clover cultivar originating from Israel) to flower more intensively as the dose of GA_3 was increased, but it did not affect the flowering of Tammisto (a high-latitude white clover cultivar originating from Finland). However, flowering intensity of GA_3-treated Tamar stolons was always lower than that of Tamar stolons growing in long days without GA_3 application. Neither cultivar responded to GA_{4+7}. Thomas (1987c) also reported that GA_3 application in warm short days had proved unsuccessful for inducing inflorescence initiation in long-day clones of Grasslands Huia, but GA_3 application hastened the start of inflorescence initiation by two to three weeks and doubled inflorescence numbers per stolon in clones of several high-latitude cultivars growing in the open during winter in Palmerston North. Thus, GA_3 effects only occurred in clones in which the critical daylength was lowered by exposure to low temperature, but not in those in which it was unaffected. Cohen and Dovrat (1976) suggested that the level of inhibitory compounds also played a role as a regulatory factor in inflorescence initiation. They found that the level of inhibitors in Tammisto was almost twice as high under short days than under long days, but was not affected by daylength in Tamar. Therefore, they hypothesized that the presence of a relatively high level of inhibitors in Tammisto when grown under short days interfered with the activity of gibberellins in their effect on flowering.

However, application of abscisic acid (which was detected amongst the inhibitors) and chlormequat chloride (which interfered with gibberellin biosynthesis) under long days did not affect flowering. Thus, possibly inhibitors other than abscisic acid are involved in the prevention of flowering.

White clover cv. Grasslands Huia initiates inflorescences when transferred from short days to long days at 21°C (Thomas, 1961a, 1979, 1987c). These plants do so for approximately three weeks and then lose their competence to initiate inflorescences if maintained in long days. Exposure of these non-initiated plants to a period of short days enables them to initiate inflorescences again when transferred back into long days. Therefore, white clover is regarded as a short-long-day plant. A similar cessation of inflorescence initiation in the natural environment in summer, at a time when photoperiods exceed the critical duration for the start of initiation, has been reported for several lines growing in USA, England and New Zealand (Gibson, 1957; Thomas, 1981b, 1982) and a wild population growing in arctic Norway (Thomas, 1980b). However, a similar loss of competence to initiate inflorescences does not seem to occur in all lines of white clover. The Ladino white clover cv. Pilgrim, for example, initiates inflorescences as long as the photoperiod exceeds the critical daylength (Laude *et al.*, 1958).

Response to long days is also influenced by temperature. However, the data available seem conflicting. Roberts and Struckmeyer (1938, 1939) reported that the intensity of flowering of three unspecified cultivars of white clover in glasshouses in long days increased as minimum dark temperatures decreased, while Ridley and Laude (1968) found that inflorescence initiation in Ladino white clover was stronger at higher than lower dark temperatures. On the other hand, Thomas (1987c) reported that results obtained from plants of cv. Grasslands Huia surprisingly agreed with both the finding of Roberts and Struckmeyer and that of Ridley and Laude. He suggested that the apparent discrepancy might have been caused by the use of an inadequate number and range of temperature treatments in the two previous experiments, although he stated that the results could not be strictly compared as his

experiments used a 12 h photoperiod instead of a 16 h photoperiod. The different results might also have arisen from their use of different lines of white clover. When plants were grown in 16 h days, higher temperatures were found to favour inflorescence initiation in lines showing a strong long day response (such as Ladino, the Kalinin line and cv. Grasslands Huia), while having the opposite effect on others that have a weaker long day response, e.g. Tamar and Spanish C1067 (Thomas, 1980a, 1982).

In spite of some uncertainties regarding interpretation of responses to temperature, Thomas (1987c) suggested an explanation of the mechanism of photoperiodic control using the following hypothesis: during the light period a promoter (P) forms in the leaves and this is later translocated to the stolon apices. Reactions stimulated by P at the stem apex are enhanced by high temperatures in the dark (Ridley and Laude 1968). Long light periods also act via the leaves to exert an inhibitory influence (I) which is greater at higher light temperatures. The strength of inflorescence initiation at the stem apex depends on the ratio of P to I (Thomas, 1981a). The action of P at the stem apex is additionally antagonized by long dark periods, the effect of which is decreased by low dark temperatures (Thomas, 1987c).

The relative importance of long days and low temperatures seems to vary with the geographical origin of cultivars, plants of higher latitude origin tending to be more sensitive to long days and those from lower latitudes tending to be more responsive to low temperatures (Thomas, 1980a, 1982, 1987c). In response to low temperatures, however, some New Zealand cultivars, i.e. cv. Grasslands Huia and cv. Grasslands Pitau, have been regarded as an intermediate type between the two groups because the time of low temperature inducing initiation in these cultivars is intermediate between that in low latitude plants and high latitude plants. Nevertheless, they do initiate in response to natural long days in November (typical of the high latitude group).

There is very little information on the effect of mineral nutrition on inflorescence initiation in response to daylength or temperature. One study by Thomas (1980a) using three different cultivars, i.e. Grasslands Huia, Grasslands Pitau and the Spanish C 1067, showed that nitrogen supported inflorescence initiation in white clover grown in 16 h photoperiods, particularly under cooler conditions, but there was little effect in 12 h photoperiods at any temperature. These results point strongly to a difference in nitrogen requirements between initiation in short days as a direct response to low temperatures, and initiation in response to long days. In another investigation, Thomas (1987c) found that when plants of cv. Grasslands Huia clone C were fed different daily quantities of a complete mineral nutrient solution, there was a clear positive correlation between the amount of nutrient given and the number of flower heads initiated per stolon, when plants were transferred from short days to long days. However, it is not known in this case whether nutrient level affected the short day pretreatment or the long day initiation process or both.

Axillary buds within the apical bud of a stolon grow in an uninhibited fashion, initiating leaf primordia at approximately the same rate as the apical meristem itself (Thomas, 1962, 1987b). Thus, at the time of transition to the reproductive phase 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. However, this does not happen. Initiation of inflorescence primordia occurs only at the terminal apical meristem on stolons and on elongating lateral branches (Thomas, 1980a). There seems to be a correlative inhibition within the apical buds which prevents inflorescence formation on axillary buds, and this inhibition is associated with the presence of the organs (leaf primordium and the tissues distal to it) at and immediately behind the apical meristem (Thomas, 1987c). Removal of these organs allows the initiation of an inflorescence primordium in the axil of the next formed leaf primordium on the youngest remaining axillary bud when the stolon is placed in long days.

2.2.2. Inflorescence growth and development

An inflorescence starts to grow and develop when an inflorescence primordium emerges from its stipular sheath a few days after the emergence of its subtending leaf. The subsequent behaviour of the stolon tip after this emergence varies with the genotype of the individual plant and with the environmental conditions. Most commonly however, the formation of precocious axillary buds at one or two nodes is followed by the formation of two or three leaf primordia without precocious buds, and this sequence is repeated for three or more cycles (Thomas, 1980a, 1987c). In field stands, development is greatest for the inflorescence closest to the stolon base, commonly with no inflorescence at the next youngest node, a much delayed inflorescence development at the next successive node, and usually a maximum of two to three inflorescences per stolon (Clifford, 1985b). In all cases, however, inflorescences form only in a lateral, axillary, position; the terminal stem apical meristem on a stolon always remaining vegetative (Thomas, 1980a).

Inflorescence emergence and growth, inflorescence and floret size, and the abortion of developing inflorescences and florets are strongly affected by environmental factors. Both temperature and daylength strongly affect the rate of inflorescence emergence and growth. The rate of inflorescence emergence is completely dependent on the rate of leaf emergence (Thomas, 1987c). When the rate of leaf emergence is as slow as one per fortnight, as it is in mid-winter in New Zealand (Thomas, 1981c), and petal emergence does not occur until the 9th node after initiation (Thomas, 1987c), it will take 18 weeks from initiation to 'first petal colour'. On the other hand, when the rate of leaf emergence is as high as two per week, as it is in summer (Thomas, 1979), the period from inflorescence initiation to first petal emergence can be as short as four weeks. The rate of inflorescence growth is decreased by low temperatures and to a lesser extent by short days (Thomas, 1961b). On the other hand, inflorescence size (indicated by the number of florets per inflorescence) and floret size (indicated by corolla length and ovule numbers per carpel) are decreased by high temperatures (Thomas, 1961b). Under natural

conditions, floret numbers per inflorescence are decreased from spring to summer (Clifford, 1979; Thomas, 1981d). In addition, low light intensities and warm short photoperiods seem to favour the abortion of developing inflorescences and florets (Thomas, 1961b, 1987c; Dunn *et al.*, 1962). This was partly explained by Pasumarty *et al.*, (1991), who reported that shading the inflorescence resulted in an increase in partitioning of photoassimilates to the peduncle at the expense of the developing flower head, which might explain why flower heads were aborted while peduncles often continued to grow.

Calcium (Ca), phosphorus (P), nitrogen (N), potassium (K), sulphur (S), boron (B) and molybdenum (Mo) are five major and two minor elements that tend to dominate the nutritional limitations to white clover growth and development (Clifford and Rolston, 1990). Marshall *et al.*, (1991) reported that reproductive growth and development in white clover was directly influenced by boron (B), boron deficiency resulting in fewer reproductive stolons and inflorescences produced, smaller inflorescences developed, fewer seed numbers per floret set, less nectar produced and reduced peduncle length. However, not many publications reported direct effects of the deficiencies of other mineral nutrients on reproductive growth. In general, deficiencies in these mineral nutrients have detrimental effects on vegetative growth and development which subsequently presumably will affect reproductive growth and development. For example, phosphorus deficiency will result in dwarfed prostrate growth, nitrogen deficiency in general yellowing, leaf necrosis and death, calcium in collapse of the petiole, with the leaf initially remaining green and turgid, potassium in leaf necrosis and death, and sulphur in leaf necrosis and death (Dunlop and Hart, 1987). However, high levels of N, P and K also result in excessive vegetative growth and reduced inflorescence numbers (Clifford and Rolston, 1990). Regardless of the amount, however, a function of mineral nutrients in the soil is modified by moisture availability for plant uptake. Clifford (1986a) found that moisture stress did not affect inflorescence emergence and growth but reduced seed yield by decreasing seed numbers per floret and thousand seed weight. On the contrary, high soil moisture increased vegetative

growth at the expense of inflorescence production. Thus, soil of medium fertility which does not lose moisture readily is most suitable for the growth of white clover seed crops (Zaleski, 1970). Clifford (1985a) suggested that crops should be sown where fertility is low to medium (Olsen P 6-15) and watered on a basis of no more than 50 % plant available moisture where possible.

2.2.3. Anthesis, pollination and fertilization

Once a fertile inflorescence has reached maturity, the formation of ripe seeds is dependent on the successful completion of the steps from pollination through to fertilization, endosperm formation and embryogenesis (Thomas, 1987c). When anthesis, or opening of florets occurs, this is a sign that pollination is about to begin. In warm summer conditions, a whole inflorescence requires 7 or 8 days for completion of anthesis (Erith, 1924; Clifford 1986a). Unfortunately, each floret may not have a self pollination capability. White clover has a well developed genetic gametophytic self incompatibility mechanism (Williams, 1931, Atwood, 1940, 1941; Williams, 1987b) with only a small proportion of plants in a population being quite strongly self compatible. Thus cross pollination is essential for significant seed set within a population. Bees, particularly honey bees (*Apis mellifera* L.), are the most important natural pollinating agents responsible for this (Erith, 1924).

The foraging behaviour of bees is influenced by weather conditions and the attraction of nectar secretion (which is also influenced by environment). During unfavourable weather (wet, cool and windy) bees tend to forage nearby, or remain in the hive, while warm and dry weather favours bee foraging (Free, 1970). Both honey bees and bumble bees (*Bombus hortorum* L., *Bombus ruderatus* L. and *Bombus terrestris* L.) prefer to visit inflorescences that contain nectar (Oertel, 1961; Heinrich, 1979; Morse, 1980). White clover produces only rather small quantities of nectar compared with many other forage legumes (Weaver, 1965). Nectar is reported to be absent at the time of floret opening, to accumulate slowly over the next 24 hours, and then to continue to build up over the next few days (Vansell,

1951). Nectar secretion is greater on a sunny than a dull day, reflecting the fact that the nectar sugars are products of photosynthesis, which in turn is influenced by sunlight (Shuel, 1952).

Once a floret has been pollinated, compatible pollen grains germinate quickly on the stigma. The tube nucleus and generative cell then pass into the growing pollen tube where the latter divides to give rise to two male gametes (Chubirko, 1965). Temperature has a strong influence on pollen germination and pollen tube growth, which are faster at high temperatures than low temperatures (Chen and Gibson, 1973). Fertilization takes place 15-20 hours after pollination under summer conditions (Erith, 1924). Cross pollination usually results in fertilization. However, the effectiveness of fertilization is also influenced by the fertility of pollen and ovules. Cool conditions increase pollen sterility (Thomas, 1961b, 1981d), while low light intensities increase ovule sterility (Pasumarty and Thomas, 1990).

2.2.4. Seed development

Seed development commences soon after fertilization. The development involves a highly coordinated sequence of events (Thomas, 1987c). A series of cell divisions within the fertilized ovum cell form the complete structure of the embryo. At the same time a series of cell divisions within the fertilized two polar nuclei cells form the endosperm. Extensive changes also occur in the integuments of the ovule to give rise to the testa of the seed.

Under natural conditions, the development of an ovule into a mature seed following pollination and fertilization comprises three stages (Hyde, 1950):

a. The growth stage:

   This stage occupies a period of 10 days after pollination during which there is a rapid increase in seed weight. The growth rate of the ovule is logarithmic and is presumably determined by the rate of cell division in the embryo and seed coat.

The seed moisture content is high and constant at approximately 79 % of the fresh weight. The seed during this first period is non viable.

b. The stage of food reserve accumulation:

This second stage takes up a period of 10-14 days following the first stage, or 20-24 days after pollination. It is characterized by a constant rate of growth and is presumably determined by the rate at which food reserves can be transferred from the parent plant to the seed. The dry weight of the seed during this stage increases about three times, reaching a maximum at the end of the period. The actual amount of water in the seed decreases slightly, but seed moisture content changes from 79 % to 63 %. At the end of this stage the seed is structurally complete and attains total viability and vigour, being physiologically mature.

c. The ripening stage:

This stage lasts from three to seven days from the completion of the second stage. During this period the seed dries out rapidly and shrinks in size. The dry weight changes very little, but the fresh weight decreases by over a half as the moisture content decreases from 63 % to 10 %. The seed finally reaches equilibrium with the relative humidity of the surrounding environment.

Failure of fertilized ovules to develop into seeds could result from failure of endosperm to grow and/or arrested development of embryos (Thomas, 1987c). The abortion of some developing seeds might arise as a result of competition for nutrients within the inflorescence (Atwood, 1940). Fertilized ovule or immature seed abortion mainly occurs during the first week after pollination, and beyond this period, only a very small proportion of the total seeds abort (Pasumarty *et al.*, 1992a,b). Ovule sterility is the major cause for the high percentage of abortion in the early stage of seed development (Pasumarty *et al.*, 1992b). Shading which occurs before fertilization increases ovule sterility (Pasumarty and Thomas, 1990), and shading which occurs after fertilization causes the abortion of developing seeds due to nutrient shortage (Pasumarty *et al.*, 1992b). This nutrient shortage occurs because shading changes the assimilate partitioning in favour of the peduncle at the expense of the inflorescence head (Pasumarty *et al.*, 1991), or directly reduces ***in situ***

photosynthesis (Pasumarty, 1987). Clifford (1986a, 1987) suggested that when moisture stress, which reduced the plant uptake of mineral nutrients, occurred at the time of active flowering, floral expression would be maintained at the expense of fertilized ovule numbers and their subsequent provisioning. However, white clover seed production is sensitive to excessive irrigation. An excess of 25 mm in water supplied was responsible for a 30 % decrease in seed yield, mainly due to a lower number of ripe inflorescences (Deschamps and Wery, 1988). Nevertheless, Clifford (1986b) found that an irrigation system that maintained mean plant available soil moisture at about 25 % could increase seed yield of white clover cv. Grasslands Kopu by 53 % compared with unirrigated treatments. This effect occurred as a result of a 22 % increase in inflorescence density, a 27 % reduction in ovule abortion and a 4 % increase in thousand seed weight.

2.3. SEED PRODUCTION

2.3.1. Seed yield and seed yield components

The potential seed yield of white clover represents the cumulative expression of four principal components of yield: inflorescence numbers per unit area, floret numbers per inflorescence, seed numbers per floret and seed weight (Zaleski, 1970; Van Bogaert, 1977; Huxley *et al.*, 1979; Maldonado, 1985; Evans *et al.*, 1986; Clifford, 1987; Thomas, 1987c). In addition, the proportion of ripe inflorescences harvested is another important factor that contributes to total seed yield in a protracted flowering plant such as white clover (Van Bockstaele and Rijckaert, 1988; Hollington *et al.*, 1989; Marshall *et al.*, 1989; Nordestgaard, 1989). High seed yields are only achieved if the crop is harvested when the number of ripe inflorescences is at a maximum, as floret numbers per inflorescence, seed numbers per floret and seed weight remain constant over the harvest period and are not influenced by harvest date (Hollington *et al.*, 1989).

Seed yield of white clover varies from year to year. This is because the growth and development of seed yield components are strongly affected by environmental factors (see section 2.2) as well as genotype (Thomas, 1987c). Regardless of the genetic variation however, seed yield might be considerably increased if the optimum conditions required for each component of seed yield were more fully understood. In optimum conditions suitable for initiation, growth and development, a large number of inflorescences each bearing a large number of florets which contain a high number of fertile ovules as well as fertile pollen would be formed. Subsequently a high percentage of these florets would undergo anthesis, be efficiently pollinated and fertilized and ultimately produce seed of maximum weight, vigour and germination capacity.

2.3.2. Main obstacles for seed production

The first obstacle that limits white clover seed production is that the optimum conditions for each component of seed yield differ, so that the overall optimum for seed production must be a compromise between them (Thomas, 1987c). The most favourable conditions for almost all seed yield components only occur during late spring and early summer (see Table 2.1).

Secondly, indeterminate growth and responses to the environment result in plants flowering over an extended period during which inflorescence buds, blooming inflorescences, young pods and mature pods ready to dehisce can be present simultaneously on an individual plant (Norris, 1984; Marshall and Hides, 1986, 1989, 1991b; Thomas, 1987c; Hollington *et al.*, 1989; Marshall *et al.*, 1989). This extended flowering period and the resultant range of flower ripeness categories in the crop makes it difficult to optimize harvest date. Early harvesting can result in unripe inflorescences being gathered but a late harvest can miss the older, earlier produced inflorescences which may have fallen below cutting height, or from which seed may have sprouted.

Table 2.1. Optimal conditions for components of seed yield (Thomas, 1987c; *Pasumarty and Thomas, 1990).

Seed yield components	Contributing factors	Environmental conditions favouring yield components	Most favourable time of year				
			early spring	mid spring	late spring	early summer	mid summer
Inflorescence no./m²	No. of active stem apices	High light & nutrient					
	Strength of flowering stimulus	High light, warm (≥ 25°C)					
		long days, *or* cool (≤ 10°C)					
	Plant vigour	High nutrient					
Rate of inflorescence emergence	Rate of leaf emergence	Warm, moist, long days					
Floret no./inflorescence	(?) Inflorescence primordium size	Cool, moist, long days					
Ovule no./floret	(?) Inflorescence primordium size	Cool-mild, moist					
Pollen fertility		Warm (≥ 20°C)					
Ovule fertility		High light*					
Ovule fertilization	Bee (pollinator) activity	Warm					
Seed weight	Seed size	High nutrient & light					
Seed ripening	Inflorescence exposure	High light, warm, dry					
	Lack of peduncle lodging	Unknown					

Thirdly, white clover is an apically dominant plant. Therefore, inflorescences are determined by the number of potential sites, i.e. actively growing stolon tips and the ability of these to respond to the floral stimulus (Thomas, 1987c). However, efforts to increase stolon tip production, such as the provision of additional mineral nutrients (Haggar *et al.*, 1963; Clifford, 1987; Clifford and Rolston, 1990) and irrigation (Adachi and Suzuki, 1968; Deschamps and Wery, 1988), can result in excessive foliage growth and subsequently reduced inflorescence density because of the shading effect of competitive leaves.

2.3.3. Management for high seed yield

The problem related to seed crop management practices is the dual purpose use of the white clover plant, involving the production of herbage for animal feeding and the production of seed in the same farming year. White clover can be grown for seed either in monoculture or with a companion grass, where the seed is taken as a catch crop between periods when the sward is utilized for grazing. Generally, the seed yields obtained by this method have been lower than when white clover has been grown in monoculture (Zaleski, 1970), although Wilman *et al.*, (1991) recently reported that sowing a companion grass (*Lolium perenne* L.) did not affect white clover seed yield. There are distinctly different growth requirements needed to produce 'seed' rather than 'feed' (Clifford, 1987). Therefore, for seed production, white clover should be grown alone as a specialist crop.

Thomas (1987c) stated that there was no single most important component of seed yield. However, in practice, the most significant component is commonly considered to be the number of inflorescences produced per unit area (Zaleski, 1970; Huxley *et al.*, 1979; Clifford, 1987; Van Bockstaele and Rijckaert, 1988; Hollington *et al.*, 1989; Marshall *et al.*, 1989; Yakuts and Kurchak, 1991). Thus, management practices are directed to result in the high, uniformly distributed stolon tip density needed by the time of closing the crop so that there is a short but prolific flowering span.

Due to its slow establishment and non aggressive growth, establishment of a satisfactory white clover plant population is of prime importance in order to achieve high seed yields. Direct drilling into cereal stubble after a burn-off in autumn has proved satisfactory for establishment of white clover seed crops (Montgomery, 1983; McCartin, 1985). Direct drilling minimizes soil disturbance and hence does not bring dormant weed seed to the surface.

Because of white clover's poor ability to support upward growth expression, the surface area available for stolon expression is an over-riding factor in maintaining uninterrupted vegetative growth (Clifford, 1987). Thus, wider row spacings encourage a high number of vigorous primary stolons and consequently a high number of inflorescences. Clifford (1980, 1985b) reported that a row spacing of 30 and 45 cm increased seed yield of cv. Grasslands Huia and cv. Grasslands Pitau when compared with the commonly used 15 cm row spacing.

Increasing the density of planting can lead to reduced inflorescence numbers per unit area as a result of overcrowding (Zaleski, 1961; Marshall and James, 1988). However, excessively low seeding rates also lead to fewer initiation sites per unit area and thereby reduce inflorescence density (Clifford, 1977). A seeding rate of 3 kg/ha at 30 cm row spacing can give a high seed yield as it promotes competition between primary stolons at the expense of secondary stolon development (Clifford, 1985b, 1987). In Russia, Perepravo and Zolotarev (1988) reported that in a trial where white clover was sown at different seeding rates from 1 to 10 kg/ha at 15, 30 or 60 cm row spacing, crops sown at a seeding rate of 2 kg/ha at 30 cm row spacing were superior to those sown in other treatments in terms of both yield components and seed yield.

White clover should be sown at sites with a moderate soil fertility for high seed yield (Clifford, 1985a, 1987). Calcium, phosphorus, nitrogen, potassium, sulphur, molybdenum and boron are five major and two minor elements that tend to dominate the nutritional limitations to white clover seed yield (Clifford and Rolston,

1990; Marshall *et al.*, 1991). Severe deficiencies in these elements result in poor vegetative and reproductive growth and development, but high levels of N, P and K cause excessive vegetative growth which tends to diminish seed yield potential. Therefore, the decision on whether to apply fertilizer or not should be based on the following considerations:

- Calcium : The recommended soil acidity level range for white clover seed production sites is pH 5.5-6.0 (Clifford and Rolston, 1990).
- Phosphorus : Sites with Olsen P levels of 6-15 have been found to give the highest seed yield (Clifford, 1985b; Clifford and Rolston, 1990). Harvestable seed yield potential declines as available soil P levels rise.
- Nitrogen : In most cases, plant requirements for nitrogen are more than satisfied by the clover roots' symbiotic relationship with nitrogen fixing rhizobia. However, in sites with low soil N, deficiencies can readily be amended by application of a nitrogenous fertilizer (Clifford and Rolston, 1990). In addition, to sustain controlled growth for a desired period, the use of fertilizer nitrogen is preferred rather than risking over-application of P.
- Potassium : Sites with exchangeable K of 20 ppm are adequate for producing high seed yields (Clifford and Rolston, 1990). High K levels can limit plant nectar secretion potential.
- Sulphur : Sites with exchangeable S of 6 ppm give high seed yield and no advantages to added S above this level have been demonstrated (Clifford and Rolston, 1990). It is of note that correction for S deficiency will depress Mo levels (Clifford and White, 1986). Thus, a knowledge of Mo levels is desired where correction for S deficiency is contemplated.

- Molybdenum : The major role of Mo is to ensure efficient nitrogen fixation by the root nodule rhizobia. Its main function in this symbiotic relationship is in the union with the root to ensure adequate plant carbohydrate transfer for bacterial use. 0.5 to 1.0 ppm Mo in herbage is considered adequate for this requirement (Clifford and Rolston, 1990).
- Boron : Boron is important for reproductive growth and development. In pot trials, an available boron concentration of 1 mg/l is necessary to optimize reproductive growth and development (Marshall *et al.*, 1991). In the field, the requirements may be higher than this level as rainfall can cause boron leaching and the increase in soil acidity followed by lime application to correct such acidity is likely to reduce boron availability (Gupta, 1979).

Plant nutrient uptake from the soil is influenced by soil moisture level. The easiest way to control seed yield problems in high fertility soils is by not applying irrigation to limit plant nutrient uptake (Clifford, 1987). On the other hand, when moisture stress and the first visual symptoms of 'wilting', which is a sign of a declined photosynthesis efficiency (Upchurch *et al.*, 1955), occur, irrigation water can be applied on a basis of no more than 50 % plant available moisture where possible (Clifford, 1985a). It is preferable to maintain mean plant available soil moisture at about 25 % for high yield (Clifford, 1986a).

It is also preferable not to graze the crop at all. Grazing reduces inflorecence numbers per unit area at harvest due to main stolon removal, particularly in large-leaved white clover cultivars (Marshall and Hides, 1990). In the absence of grazing, any excess growth can be controlled by topping, silage or haying techniques. These growth control systems further limit secondary stolon development while protecting primary stolons from undue loss (Montgomery, 1983; Clifford, 1987). However, defoliation prior to flowering has been reported to have beneficial effects on

inflorescence production and other components of seed yield of white clover (Zaleski, 1970; Thomas, 1981b; Hides *et al.*, 1984; Marshall *et al.*, 1987; Hollington *et al.*, 1989; Marshall *et al.*, 1989), although defoliation can also severely reduce seed yield (Maldonado, 1985; Van Bockstaele and Rijckaert, 1988; Nordestgaard, 1989). The difference in these results might have arisen from different timing and techniques for the defoliation used.

Closing date is taken to be the final time when the vegetative growth is controlled either by cutting, grazing or by the use of chemicals. According to Thomas (1987c), the aim in deciding closing date is to ensure that as many inflorescences as possible are already initiated but not yet emerged at the stolon tips at the time of closing. These inflorescences then emerge over a short period of time in the immediately following weeks to give the maximum inflorescence density and the minimum range of inflorescence ages. In the New Zealand environment, mid November closing gives the best seed yield (Clifford, 1979, 1980; Maldonado, 1985). Closing too early results in longer flowering spans, and hence fewer inflorescences mature at any one time. On the other hand, if closing is too late, inflorescences are smaller and the density of inflorescences is reduced. However, on lighter soils or dryland situations, where shortage of moisture can limit plant growth, a mid October closing is recommended (Montgomery, 1983).

The effects of diseases caused by fungi, bacteria, viruses and mycoplasma-, spiroplasma- and rickettsia-like organisms on white clover grown for pastures, and their control have been well documented by Latch and Skipp (1987), but not many reports of the effects of specific white clover diseases on white clover seed production have been published. Nevertheless, the impact of white clover diseases in grazed pastures is seldom dramatic enough to alarm farmers and agronomists. Among those diseases, those caused by fungal leaf pathogens, i.e. *Pseudopeziza* leaf spot (caused by *Pseudopeziza trifolii* (Biv.) Fckl.) and pepper spot (caused by *Leptosphaerulina trifolii* (Rost.) Petrak), may reduce white clover seed yield

(Foulkes and Clifford, 1989). However, these diseases can be effectively controlled by fungicide application.

The pests of white clover and their control have been recently reviewed by Gaynor and Skipp (1987). Among those pests, there are several important ones which can directly cause detrimental effects on white clover seed production because they are seed and flower feeding insects. These pests are the clover seed weevil (*Apion dichroum* Bedel), the clover head weevil (*Hypera meles* F.), the clover case-bearer moth (*Coleophora spp*) and the Ladino clover seed midge (*Dasineura gentneri* Pritchard). Other pests cause leaf damage, e.g. porina (*Wiseana spp*), blue green lucerne aphid (*Acyrthosiphon kondoi* Shinji), pea aphid (*Acyrthosiphon pisum* Harris), two-spotted mite (*Tetranychus urticae* Koch) and spider mite (*Tetranychus turkestani* Ugarov and Nikolski), and root damage, e.g. grass grub (*Costelytra zealandica* White), which in turn will affect reproductive growth and development. These pests can be controlled with one of four main methods: chemical, cultural, biological and varietal. However, the most important recent development has been the attempt to integrate a number of different control measures into an integrated pest management system (Kain *et al.*, 1982).

The assessment of optimum harvest date is difficult (see section 2.3.2). As the time for full seed development in individual florets from pollination to mature seed is 26 ± 5 days depending on weather conditions (Hyde, 1950; Hyde *et al.*, 1959), the most consistent method of determining an optimum harvest date may be associated with the time after peak flowering. Clifford (1985b) recommended harvesting six weeks after peak flowering to maximize seed yields with low seed losses. However, Holloway (1987) found that the best seed yield was obtained from harvesting 28 days after peak flowering. Harvest delay resulted in a linear decline in seed yields.

Seed yields in white clover are reduced significantly by both climatic losses and harvesting losses (Holloway, 1987). A number of climatic factors such as strong winds, heavy rain with large raindrops or hot, dry conditions can result in climatic

losses. Inflorescences can fall below harvesting height in wet weather (Mohamed, 1981; Evans *et al.*, 1986). Inflorescence shedding due to hot, dry conditions results in the loss of seed pods and/or individual seeds (Holloway, 1987). Seed loss increases with time of exposure to unfavourable climatic conditions and plant breakdown. Therefore, to minimize seed loss, seed must be harvested as soon as it is ripe. Harvesting losses are mainly due to machinery inefficiencies and design (Clifford and McCartin, 1985). These can be as high as 40 %. Crop condition at harvesting (green or desiccated) and time of harvesting (morning or afternoon) are also important factors that affect harvesting losses. In Canterbury, lowest losses (5 %) were recorded from a desiccated crop cut in the morning with a double reciprocating knife mower (Clifford and McCartin, 1985).

With the increasingly wide use of chemicals in agricultural practice, there are also several options for using plant growth regulators for improved seed production. This is discussed in the following section.

2.4. CHEMICAL MANIPULATION OF REPRODUCTIVE GROWTH AND DEVELOPMENT WITH THE USE OF PLANT GROWTH REGULATORS

2.4.1. Plant growth regulators

Chemicals that are used to alter physiological or morphological characteristics of plants are called plant growth regulators (PGRs). They include hormones (growth regulating compounds produced naturally by the plant, excluding inorganic and organic nutrients) and synthetic chemicals. Both are effective at very low concentrations. For practical purposes, plant growth regulators can be defined as "either natural or synthetic compounds that are applied directly to a target plant to alter its life processes or its structure to improve quality, increase yields or facilitate harvesting" (Nickell, 1982).

In the future, only synthetic plant growth regulators will play a major role in agriculture. This is because by taking greater advantage of the enormous capacity of industry, new synthetic plant growth regulators which are more efficient than those available today will be discovered. Also, unlike the natural hormones which are present and function in virtually all plants, the synthetic plant growth regulators may be specific for certain crops and used to alter specific physiology functions (Ory and Rittig, 1984).

A classification of plant growth regulators into groups can be carried out according to their principle of action. In most cases the principle of action is the intended influencing of the plant's hormone status according to the following possibilities (Jung, 1984):

- supply of a phytohormone participating in the existing equilibrium or of an analogous compound;
- enhancing or inhibiting hormone biosynthesis with exogenous compounds (precursors or synergists, and antagonists or inhibitors);
- varying availability of a phytohormone at the site of action by influencing its transport and catabolism.

At the present time, the compounds interfering with gibberellin biosynthesis (GA antagonists), also known as plant growth retardants, are of special interest because they have already found extensive application particularly in some areas of world cereal cultivation and for use in orchard management to improve efficiency of fruit production (Nickell, 1982; Hoad, 1982; Thomas, 1982; Jung, 1984; Quinlan, 1987; Rademacher, 1990). There are two main groups of plant growth retardants (Sauter, 1984): the onium compound group, e.g. chlormequat chloride (CCC), mepiquat chloride (DPC) and daminozide (Alar-85), and the nitrogen heterocyclic group, e.g. ancymidol, tetcyclacis and 'triazoles' which include paclobutrazol (PP_{333}) and triapenthenol (RSW-0411).

The mechanisms and spectra of action of those two groups are different (Dicks, 1980; Schott *et al.*, 1984; Hedden, 1990). For GA antagonists of the onium type, it is assumed that they inhibit the cyclization of geranylgeranyl pyrophosphate to copalyl pyrophosphate in the course of GA biosynthesis, while the nitrogen heterocyclic group compounds inhibit the sequential oxidation of *ent*-kaurene to *ent*-kaurenoic acid (Figure 2.2).

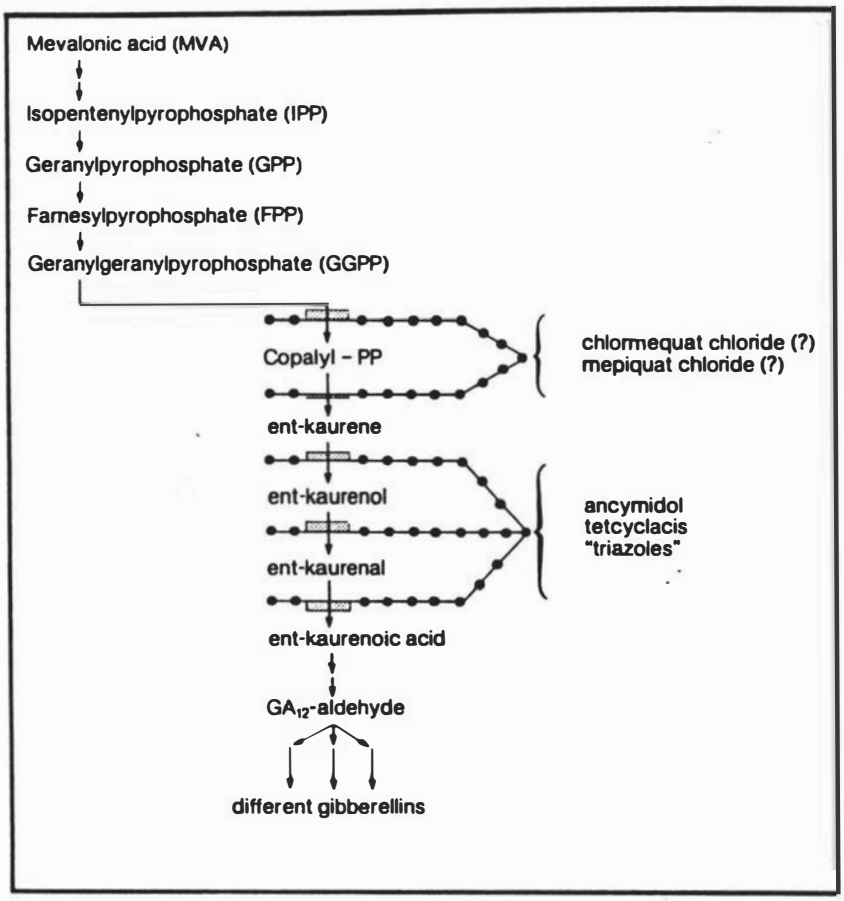


Figure 2.2. Pattern of gibberellin biosynthesis and points of inhibition by plant growth retardants (Schott *et al.*, 1984).

2.4.1.1. Paclobutrazol

Paclobutrazol [(2RS,3RS)-1-(-4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)-pentan-3-ol] or PP_{333}, also commercially known as Cultar, Parlay, Bonzy or Clipper, is a member of the triazole group. Paclobutrazol has two asymmetric carbon atoms and therefore can exist as four isomers, i.e. two diastereoisomeric pairs of enantiomers. The commercial material contains about 98% of the 2RS,3RS diastereoisomer; and of this the 2S,3S enantiomer is the most potent growth retardant, while the 2R,3R compound has considerable fungicidal activity (Sugavanam,1984; Hedden, 1990).

The mode of action of paclobutrazol involves inhibition of the endogenous formation of GAs by blocking cytochrome P-450 type oxygenases that catalyze the reactions between *ent*-kaurene and *ent*-kaurenoic acid (Schott *et al.*, 1984; Burden *et al.*, 1987; Hedden, 1990). The applied paclobutrazol reaches the target site, the active sub-apical meristem, by transport in the xylem from the roots following soil application or directly to the young sub-apical shoot tissue following foliage spraying (Lever, 1986). Paclobutrazol is not transported in the phloem (Quinlan and Richardson, 1986; Wang *et al.*, 1986). Therefore, activation is influenced by soil moisture. Paclobutrazol is more active when rainfall is high (Shearing and Batch, 1982; Hampton and Hebblethwaite, 1984b). The activity of reduced growth by paclobutrazol lasts between 27-82 days after application in apple trees (Irving and Pallesen, 1989) and several weeks in amenity grasses (Shearing and Batch, 1982). However, paclobutrazol has soil residual properties and therefore carry over effects in the following year have been reported in some species (Froggatt *et al.*, 1982; Hampton, 1988b; Tabora, 1991).

There are a wide range of uses for paclobutrazol (Davis *et al.*, 1988; Rademacher, 1990) including control of lodging in small grains and rice (*Oryza sativa* L.), control of excessive vegetative shoot growth in orchard trees, growth reduction in other trees, growth regulation of grasses, improvement of quality in

ornamental plants, improved resistance to drought and low temperatures in cucumber (*Cucumis sativus* L.) and zucchini squash (*Cucurbita pepo* L.), and improved resistance to fungal infections in melon (*Cucumis melo* L.) seedlings. The use of paclobutrazol in herbage legumes is discussed separately in section 2.4.2.

2.4.1.2. Triapenthenol

Triapenthenol [(E)-(RS)-1-cyclohexyl-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl)-1-penten-3-ol], also known as RSW-0411, is a recently discovered triazole plant growth regulator (Lürssen and Reiser, 1985). As a member of the triazole group, the mode and site of action of triapenthenol is similar to paclobutrazol (Lürssen and Reiser, 1985; Hedden, 1990). Triapenthenol when applied as a foliar spray to apple trees is as effective as paclobutrazol, but its growth control capability is short lived, i.e. between 27 - 40 days following application (Irving and Pallesen, 1989). When applied as a root drench, Curry and Reed (1989) also found that the effects of triapenthenol on the growth of 4-week-old apple seedlings were short-lived compared to paclobutrazol. These results suggest that the environmental half-life of triapenthenol is less than that of paclobutrazol. Triapenthenol is metabolized more quickly in plant tissues and is taken up or transported to the meristematic tissue more slowly by either the root or xylar tissues (Curry and Reed, 1989). There has been no reported soil residual activity of triapenthenol.

Triapenthenol has potential for controlling excessive shoot growth in orchard trees (Irving and Pallesen, 1989). Compared to paclobutrazol, the short-term effectiveness of triapenthenol could be of advantage where soil residue from paclobutrazol might undesirably reduce growth in subsequent seasons. The effects of triapenthenol in reducing plant growth and increasing seed yield through decreasing apical dominance, promoting branching or tillering, increasing flowers, preventing lodging, improving winter hardiness or by reducing pod abortion or pod shatter, have been reported to be consistent in field beans (*Vicia faba* L.) (Hack *et al.*, 1985), oil seed rape (*Brassica napus* L.) (Hack *et al.*, 1985; Child *et al.*, 1987),

rice (*Oryza sativa* L.) (Hack *et al.*, 1985; Lürssen and Reiser, 1987) and perennial ryegrass (*Lolium perenne* L) (Wiltshire *et al.*, 1989). The use of triapenthenol in herbage legumes is discussed in section 2.4.2.

2.4.1.3. Chlormequat chloride

Chlormequat chloride (2-chloroethyl-trimethylammonium chloride) or CCC is a member of the onium compound group. Chlormequat chloride retards plant growth by inhibiting the endogenous formation of gibberellins. However, its site of action differs from the triazole group. It inhibits the cyclization of geranylgeranyl pyrophosphate to copalyl pyrophosphate in the course of GA biosynthesis (Dicks, 1980; Schott *et al.*, 1984; Hedden, 1990). Chlormequat chloride is rapidly degraded in the soil by enzyme activity (Anon., 1982), and thus does not have soil residual activities.

Chlormequat chloride is primarily used in cereal crops such as wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and oats (*Avena sativa* L.). Its effect on plants is to shorten plant stems by about 20%, prevent stem break and lodging, and increase grain yields by both increasing the number of fertile tillers and improving the synchrony of tiller growth and development within plants (Kust, 1986). Chlormequat chloride has also been tried for increasing herbage legume seed yield. This is discussed in section 2.4.2.

2.4.1.4. Daminozide

Daminozide [butanedioic acid mono(2,2-dimethylhydrazide)], also known as Alar-85, or B-Nine, was introduced in 1962. Daminozide is a hydrazine derivative which is an effective plant growth retardant. It inhibits the endogenous formation of GAs (Riddell *et al.*, 1962). Daminozide is usually administered as a foliar spray. However, its persistence is not great and re-application may sometimes be necessary

(Davis and Andersen, 1989). Daminozide has extremely short persistence in the soil since it is rapidly consumed by microorganisms and leaves no noxious residues (Sullivan, 1977).

The major use of daminozide in agriculture is to regulate fruiting and post-harvest quality of fruit crops, and it is also used to modify the stem length and shape of ornamental plants (Andersen, 1979; Davis and Andersen, 1989). Daminozide is effective in controlling shoot elongation, inducing flower bud initiation, increasing fruit set and preventing pre-harvest drop. Daminozide has also been used to try and increase seed yield of herbage legumes, and this is discussed in section 2.4.2. Daminozide is no longer registered for use on crops in New Zealand due to concern about the possible carcinogenic effects of daminozide (Davis and Andersen, 1989)

2.4.2. The use of plant growth regulators in herbage legume seed production

Herbage legume seed yields may be increased by using plant growth regulators to manipulate growth, development and flowering pattern, either directly by increasing inflorescence numbers and potential seed yield, or indirectly by encouraging greater stolon or shoot production and increasing the potential number of sites for inflorescence production. Plant growth regulators have been used successfully to manipulate growth and increase yield in a number of grass species (Hampton, 1988a) through decreasing apical dominance, promoting tillering, preventing lodging, synchronizing flowering and delaying leaf senescence. On the other hand, based on the preliminary results of several workers, the application of plant growth regulators in herbage legume species can give different and inconsistent results.

Seed yields of red clover (*Trifolium pratense* L.) were increased by applying daminozide at the beginning of the bud formation stage (Puri and Laidlaw, 1983; Trofimova *et al.*, 1987) and at the start of inflorescence emergence (Rybak and Walczak, 1988; Christie and Choo, 1990) through increasing inflorescence numbers.

On the other hand, Niemeläinen (1987) reported that the application of daminozide, triapenthenol, chlormequat chloride, ethephon, ethephon+chlormequat chloride or ethephon+mepiquat chloride at the start of inflorescence emergence did not increase seed yield, but paclobutrazol or paclobutrazol+daminozide did increase the seed yield. However, the method of action of these two treatments in increasing seed yield remains unclear. Niemeläinen (1987) suggested that the treatments probably gave a better seed set, as the inflorescence numbers per unit area and thousand seed weight were not significantly different from the control.

The application of plant growth regulators also produced inconsistent results in *Lotus spp*. White *et al*., (1987) reported that the application of daminozide or mepiquat chloride at the first-bloom stage of growth did not increase seed yield in *Lotus corniculatus* L. Supanjani (1991) also found that paclobutrazol, triapenthenol, chlormequat chloride and daminozide, which were individually applied at either the late vegetative stage (October) or early flowering stage (November), similarly did not increase seed yields of *Lotus corniculatus* L. On the other hand, Li and Hill (1989) reported that paclobutrazol applied at the reproductive node initiation stage or reproductive node visible stage, did increase seed yields of *Lotus corniculatus* L. through removing apical dominance and promoting branching, hence increasing inflorescence numbers. Seed yields of *Lotus uliginosus* Schkuhr. were also increased by paclobutrazol or daminozide application (Clifford and Hare, 1987; Tabora, 1991), and by chlormequat chloride application (Tabora and Hampton, 1992). While paclobutrazol and daminozide increased seed yield by increasing inflorescence numbers through promoting branching, the yield increases following chlormequat chloride application resulted from improvement in seed yield components, particularly enhanced pods per umbel and seeds per pod. Hampton *et al*., (1989) also reported that paclobutrazol increased seed yield in both *Lotus corniculatus* L. and *Lotus uliginosus* Schkuhr.

Mohamed (1981) found that attempts to influence inflorescence production of white clover by treatment with either chlormequat chloride or daminozide were unsuccessful. Similarly, Marshall and Hides (1986) reported that the application of paclobutrazol, chlormequat chloride or Cerone (2-chloroethyl phosphonic acid or ethephon) did not increase seed yield in white clover cv. Olwen, even though some rates of Cerone and chlormequat chloride increased inflorescence numbers per unit area at harvest. Yield was not increased because there were fewer florets per inflorescence in these treatments. However, following further glasshouse experiments, Marshall and Hides (1987) suggested that paclobutrazol was still a potentially useful plant growth regulator which could reduce the time period over which inflorescences appeared, and also increase the proportion of axillary buds per stolon and thereby stolon branching. This could lead to an increase in inflorescence production, or allow inflorescences to elevate above the leaf canopy because of the reduction in petiole height, and hence improve pollination by making inflorescences more accessible to pollinating insects. From further field experiments, Marshall and Hides (1989, 1991b) reported that paclobutrazol increased potential seed yield of white clover cvs. Olwen and Menna through increasing the number of inflorescences produced. Hampton (1991) also reported that paclobutrazol applied when first inflorescence buds become visible increased seed yield in four New Zealand white clover cultivars (Grasslands Kopu, Grasslands Pitau, Grasslands Huia and Grasslands Tahora) which differed in leaf size from large to small, but only in cvs. Grasslands Pitau and Grasslands Tahora when paclobutrazol was applied at inflorescence initiation. Seed yield increases were associated either with increased harvestable inflorescences, or increased seeds per inflorescence, but these responses differed with cultivar and application time. However, there was no clarification whether increased inflorescence production could be attributed to a greater proportion of reproductive nodes on each stolon, which could also influence the proportion of ripe inflorescences at harvest, or simply to increased stolon production per unit area. Nevertheless, Marshall and Hides (1991a,b) suggested that inflorescence increases were achieved by a significant increase in the number of

nodes per unit area and reproductive nodes per stolon, despite having previously reported (Marshall and Hides, 1987, 1989) that paclobutrazol did not affect the development of reproductive nodes along stolons.

In Belgium, paclobutrazol and triapenthenol have also been shown to increase seed yield in white clover cv. Merwi (Rijckaert, 1991). These increases were associated with a large reduction in the mass of vegetative crop growth, elevation of inflorescences above the canopy and better seed set per floret. However, the effects varied between years depending on climatic conditions. When paclobutrazol, triapenthenol, daminozide, Cerone (ethephon) and Folicur were individually applied to increase seed yield in white clover cv. Milkanova grown under the Danish environment, the first three plant growth regulators also inconsistently increased seed yield during the five years of trials (1986-1990), while Cerone and Folicur had no effect (Boelt, 1991). There was an indication that the paclobutrazol response was greatest in a poor season (adverse weather conditions) and less pronounced under favourable weather conditions for white clover seed production. This is presumably related to rainfall and the activation of paclobutrazol (Shearing and Batch, 1982; Hampton and Hebblethwaite, 1984b).

These apparently conflicting results lead to the conclusion that responses may differ depending on rate and time of application, cultivar, site and season. These aspects need further investigation, as do the type of plant growth regulators that are suitable for enhancing seed yield in herbage legumes, since the morphology and physiology of these species differs from that of cereals or grasses.

CHAPTER 3

EFFECT OF PLANT GROWTH REGULATORS ON THE GROWTH, DEVELOPMENT AND SEED YIELD OF A FIRST YEAR CROP OF WHITE CLOVER CV. GRASSLANDS PITAU

3.1. INTRODUCTION

The onset of reproductive growth generally marks the cessation of active vegetative growth in species with a determinate growth pattern. In white clover, however, the plant's indeterminate growth habit results in active vegetative growth continuing during reproductive development. Moreover, throughout the reproductive phase, a proportion of the stolons are insufficiently developed to produce inflorescences. Nevertheless, these stolons will still grow and compete with reproductive stolons. This partitioning of vegetative and reproductive growth complicates management decisions for white clover seed production (Clifford, 1987). Seed yield in white clover, in addition, 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, 1987c; Plate 3.1). This flowering pattern dictates that inflorescences appear over a period of time leading to wide variation in seed maturity and seed loss through sprouting or brackling (see section 2.3.2). Since seed yield in white clover depends largely on the number of ripe inflorescences per unit area (see section 2.3.1 and 2.3.3), manipulating this species to both increase and concentrate inflorescence production 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. and *Lotus* spp, through decreasing apical dominance, promoting branching and thereby increasing inflorescence production, and also improving

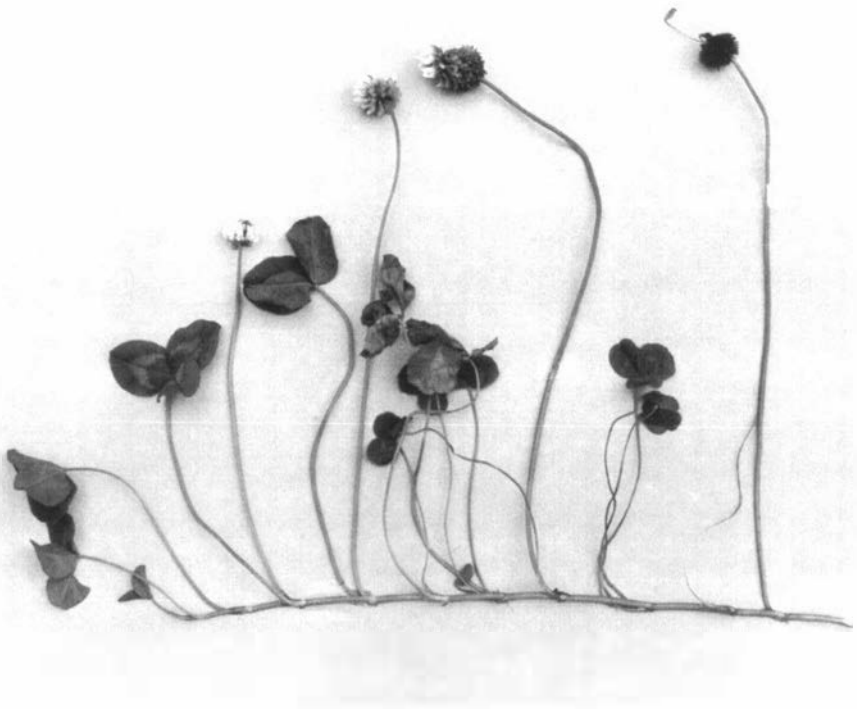


Plate 3.1. Inflorescence development along a main stolon.

other seed yield components (see section 2.4.2). Most plant growth regulators used are plant growth retardants such as paclobutrazol, triapenthenol, daminozide and chlormequat chloride (see section 2.4.1 for details of their mode of action, chemical composition and uses). Under the United Kingdom environment, plant growth regulators including paclobutrazol, chlormequat chloride, daminozide and ethephon have also been used to try and increase white clover seed yield (Mohamed, 1981; Marshall and Hides, 1986), but the results were unsuccessful. However, further field experiments recently reported by Marshall and Hides (1989, 1991b) showed that paclobutrazol increased the potential seed yield of white clover cvs. Menna and Olwen 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, under 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/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 ethephon 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.

The present experiment was conducted with the specific aims of:

1. Evaluating the potential effects of some plant growth regulators on seed yields of white clover planted under the New Zealand environment.
2. Studying the effects of different rates and the timing of plant growth regulator application on the growth, development and seed yield of a first year crop.

In this experiment, data collection up to harvest time was recorded by Dr J.G. Hampton.

3.2. MATERIALS AND METHODS

3.2.1. Experimental site, management and treatments

The experiment was carried out on the Frewin's block of the Pasture and Crop Research Unit, Massey University, Palmerston North (Plate 3.2) during the 1988/1989 growing season. The soil type was a Tokomaru silt loam, of moderate fertility. A full soil description is presented in Appendix 3.1A and 3.1B.

The cultivar used was Grasslands Pitau. The seed source was certified breeders seed (Grasslands No. C5890) harvested in the 1982/1983 season with a 78 % germination, supplied by AgResearch Grasslands, Palmerston North, New Zealand. A description of white clover cv. Grasslands Pitau is presented in Appendix 3.2. The seed was sown on 23-24 March 1988 at a sowing rate of 3 kg/ha and inter row spacing of 45 cm. 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 l/ha) was applied on 21 September 1988 to control weeds and to reduce vegetative growth. Paraquat is a very fast acting contact herbicide which destroys green plant tissue by a chemical and light interaction on chlorophyll (Anon., 1990). It is rainfast once dry on the plant, but inactivated on contact with the soil. Paraquat controls most fibrous rooted grasses and annual broadleaf weeds, but clovers recover quickly from its desiccation effects.

The experiment utilized a three replicate randomized complete block design. Plot size was 7 x 2 m with regularly cut pathways of 2 m between blocks and 1 m between plots. This experiment consisted of 14 treatments and one control (a treatment without plant growth regulator application). Treatments used were paclobutrazol at 0.5 and 1.0 kg a.i/ha (using Cultar which contains 250 g/l paclobutrazol in the form of a suspension concentrate), triapenthenol at 0.5 and 1.0 kg a.i/ha (using RSW-0411 which contains 700 g/kg triapenthenol in the form of a

Plate 3.2. The Frewin's block of the Pasture and Crop Research Unit, Massey University, Palmerston North, New Zealand.

water soluble granule), chlormequat chloride at 1.5 and 3.0 kg a.i/ha (using Cycocel which contains 750 g/l chlormequat chloride in the form of an aqueous concentrate) and daminozide at 2.0 and 4.0 kg a.i/ha (using Alar-85 which contains 850 g/kg daminozide in the form of a water soluble powder), applied during reproductive bud initiation (11 October 1988) and reproductive buds visible (8 November 1988) for paclobutrazol, triapenthenol and chlormequat chloride, and only at reproductive buds visible for daminozide. Reproductive development was determined by dissecting 10 growing points (stolon apices) under a microscope at 40X magnification every week starting in early September 1988. 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" (Figure 3.1), as opposed to the third youngest axil in the vegetative stage (Thomas, 1987c). When more than 75 % of growing points had initiated reproductive buds, plant growth regulators were applied as a reproductive bud initiation application. The second application time, reproductive buds visible, was determined when most buds had emerged from their surrounding stipular sheaths. For ease of reference, the time of application has been termed as October or November application. All plant growth regulators were applied with water at a volume equivalent to 500 l/ha by a knapsack sprayer with four fan nozzles held 25-30 cm above the herbage.

3.2.2. Plant measurements and statistical analysis

Growth analysis was conducted only for paclobutrazol 1.0 kg a.i/ha, triapenthenol 1.0 kg a.i/ha, chlormequat chloride 3.0 kg a.i/ha and daminozide 2.0 and 4.0 kg a.i/ha treatments. Samples were taken only at peak flowering (15 December 1988) from a quadrat of 0.125 m^2 per plot. All plant material was cut to ground level using an electric shearing machine. In the laboratory, each sample was cleaned of weeds and soil particles. The samples were weighed and weighed subsamples (approximately one third of a total sample) taken for growth analysis.

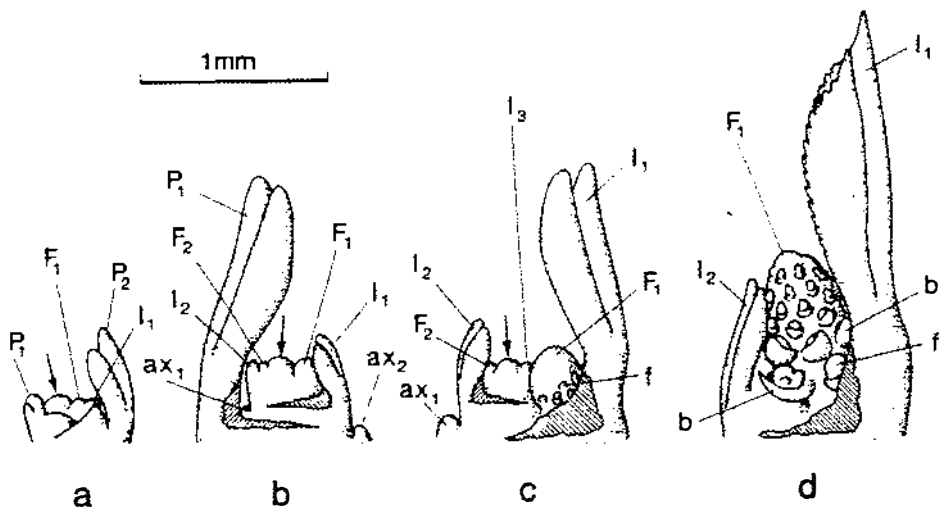


Figure 3.1. Early stages of development of inflorescence primordia at main stolon apices of white clover grown in continuous light (CL) at 23°C after pretreatment with warm short days. a, b, c and d show apical buds dissected out 6, 9, 11 and 13 days respectively after the start of CL. Hatched areas indicate tissues damaged by removal of stipules to expose the organs beneath. The youngest leaf primordium present at the start of CL treatment is labelled P_1 and the next youngest P_2; axillary buds subtended by these are labelled ax_1 and ax_2 respectively. New leaf primordia successively formed after the start of CL treatment are labelled I_1, I_2, and I_3; inflorescence primordia subtended by these are labelled F_1, F_2, and F_3 respectively. Bracts subtending floret primordia are labelleld 'b' and the floret primordia themselves 'f'. Arrow heads point to the meristematic dome at each stolon apex. (Thomas, 1987c).

The effect of treatments on dry matter composition was determined by dissecting the subsamples into three components, i.e. vegetative (stolons and leaves), reproductive (inflorescences and inflorescence buds) and dead material. From the subsamples, the number of reproductive nodes (nodes producing inflorescences), their composition (bud, white and brown inflorescences) and the number of vegetative nodes (nodes producing leaves and axillary buds) were counted. Peduncle length was measured from 10 randomly selected inflorescences/ plot. Petiole and leaf score were also measured from 10 leaves/plot selected at random. Leaf score measurement was conducted using a clover leaf area estimation chart (Williams *et al.*, 1964). All the fractions of the subsample and the remainder were then dried in an oven to constant dry weight (2 days at 80°C). Each subsample data set was then converted to an area value (i.e. inflorescences/m^2, vegetative nodes/m^2 or g/m^2).

The development of a white clover seed from pollination to ripeness occupies about 26 days, which may be reduced or extended by approximately 5 days in accordance with weather conditions (Hyde, 1950; Hyde *et al.*, 1959). Inflorescence counts were taken to enable estimation of peak flowering and thus physiological maturity for harvest timing. The number of white inflorescences were recorded weekly from within 0.5 x 0.5 m permanent quadrats established in each plot during the flowering period. Based on flowering pattern and weather conditions, harvesting was carried out on 16 January 1989. All plant material from within a quadrat of 0.5 m^2 from each plot was cut to ground level and placed in a paper bag. After allowing the bags of harvested material to dry indoors at ambient temperature, the inflorescences were collected, and then the vegetative parts of the harvested material were dried in an oven to obtain a constant dry weight.

Seed yield components, potential harvestable seed yield and actual seed yield were measured from the harvested inflorescences. Seed yield components included the number of inflorescences/unit area, the number of florets/inflorescence, the number of seeds/floret and thousand seed weight (TSW). The number of

inflorescences/unit area were counted directly from the harvested inflorescences. Twenty inflorescences/plot, which were selected randomly from the harvested inflorescences, were dissected to give the number of florets/inflorescence. The number of seeds/floret were counted from 100 florets/plot, which were selected randomly from a bulk of florets dissected from the harvested inflorescences. To count the number of seeds/floret, the 100 florets were placed on a Polaroid film type 55 and X-rayed using a Faxitron Hewlett-Packard X-ray machine model 43804 N with an exposure of 25 KVA for two minutes. TSW was determined by weighing 4 x 100 seeds/plot sampled from cleaned actual seed yield.

Potential harvestable seed yield/unit area was calculated from the seed yield components recorded at harvest according to the following formula:

$$\text{Potential harvestable seed yield} = P \times E \times N \times S$$

where P = the number of inflorescences/unit area
E = the number of florets/inflorescence
N = the number of seeds/floret
S = seed weight

To obtain actual seed yield/unit area, all inflorescences from each harvested quadrat were threshed by hand. Seeds were then separated from chaff by passing through different sized (0.610-1.270 mm) laboratory test sieves, and final cleaning was done using a vertical airblast seed blower (Burrows model No. 1836-4). The blower was set up with an airflow speed of 50 km/h and it was run for 3 minutes. There was no correction of seed moisture content (SMC) as it was assumed that SMC of all samples was in equilibrium after they had been stored for five months at ambient temperature.

In order to determine the quality of seed produced from each treatment, a germination test was conducted. Because the seed was hand-threshed, the levels of hard seed from both control and treated plants were high (88-93 %). To remove hardseededness, mechanical scarification using sand paper was conducted. A seed sample from each plot was placed between two pieces of sand paper and put on the top of a table. These seeds were then carefully scarified by gently moving circularly the top sand paper for one minute (Karen Hill, Seed Technology Centre, Massey University, pers. comm.). Four replicates of 50 scarified seeds were germinated using the top of paper method (ISTA, 1985). A moist towel was placed on a flat tray and covered by a sheet of white filter paper and small blotting pads (5 x 5 cm) were placed on top of this. The seeds were placed on the pads (50 seeds per pad) and covered to prevent desiccation. They were kept in a cabinet at a constant temperature of 20°C under continuous light. Seedling counting and assessment were carried out according to internationally recognized rules (ISTA, 1985).

Data for most characters were analyzed according to a randomized complete block design analysis by the use of analysis of variance. Treatment mean comparisons were performed using Fisher's LSD tests at a 5 % level of probability.

3.3. RESULTS

3.3.1. Meteorological conditions

In general, the trial period was warmer than average with the deviation of temperatures ranging from 1.1°C to 2.8°C and 0.5°C to 2.3°C for minimum and maximum temperatures respectively, except for maximum temperature in January 1989 (harvesting time) which was cooler than average (Appendix 3.3). The number of sunshine hours was below average in September and October but above average in November, December and January. Rainfall was below average during the flowering period (November and December) but higher in September, October and January (Appendix 3.3).

3.3.2. Effect of plant growth regulators on plant growth and development

3.3.2.1. Morphological characteristics

At peak flowering, plant growth regulators had no significant effect on leaf size (presented as leaf score in Table 3.1). On the other hand, petiole length was significantly reduced compared to the control by the application of paclobutrazol 1.0 kg a.i/ha in October and of daminozide 2.0 kg a.i/ha in November. It was apparent that these treatments also reduced peduncle length, although only the daminozide treatment was statistically significant. Therefore, the length differences between peduncle and petiole in both treatments compared to the control were not significantly different (Table 3.1). In the other treatments, both peduncle and petiole lengths were not significantly affected. Despite this fact, the application of triapenthenol 1.0 kg a.i/ha in November significantly increased the difference between peduncle and petiole lengths compared to the control, while the application of chlormequat chloride 3.0 kg a.i/ha in November resulted in the inflorescences remaining below the height of the leaf canopy.

3.3.2.2. Dry matter accumulation and distribution

Triapenthenol 1.0 kg a.i/ha applied in October significantly increased total dry matter accumulation at peak flowering, while other treatments did not differ from the control. The increase was attributed to the significant increase of dead material (Figure 3.2). However, in the paclobutrazol treatments there were significant differences in dry matter composition. Paclobutrazol 1.0 kg a.i/ha applied in October significantly increased reproductive dry matter and reduced dead dry matter, whereas paclobutrazol 1.0 kg a.i/ha applied in November significantly increased vegetative dry matter compared to the control.

Table 3.1. Effect of plant growth regulators on leaf score and peduncle and petiole lengths at peak flowering for a first year crop of white clover cv. Grasslands Pitau.

	Leaf score	Length (mm)		
		Petiole	Peduncle	Peduncle - Petiole
Control	19.9	202.5*abc*	244.2*ab*	41.7*b*[1)]
Paclobutrazol 1.0 kg Oct	20.0	153.1*de*	204.5*bc*	51.4*ab*
Paclobutrazol 1.0 kg Nov	19.7	165.4*cde*	224.1*abc*	58.7*ab*
Triapenthenol 1.0 kg Oct	20.2	215.7*ab*	262.7*a*	47.0*ab*
Triapenthenol 1.0 kg Nov	20.6	178.2*bcd*	255.0*a*	76.8*a*
Chlormequat chloride 3.0 kg Oct	20.2	177.7*bcd*	241.3*ab*	63.6*ab*
Chlormequat chloride 3.0 kg Nov	20.8	238.9*a*	220.4*abc*	- 18.5*c*
Daminozide 2.0 kg Nov	19.0	136.1*e*	183.9*a*	47.8*ab*
Daminozide 4.0 kg Nov	20.0	207.5*ab*	263.3*a*	55.8*ab*
LSD (P=0.05)	NS	39.8	45.7	34.4
CV	7	12	11	42

Note: 1. Values in the same column followed by the same letter are not significantly different according to Fisher's LSD test at P=0.05.

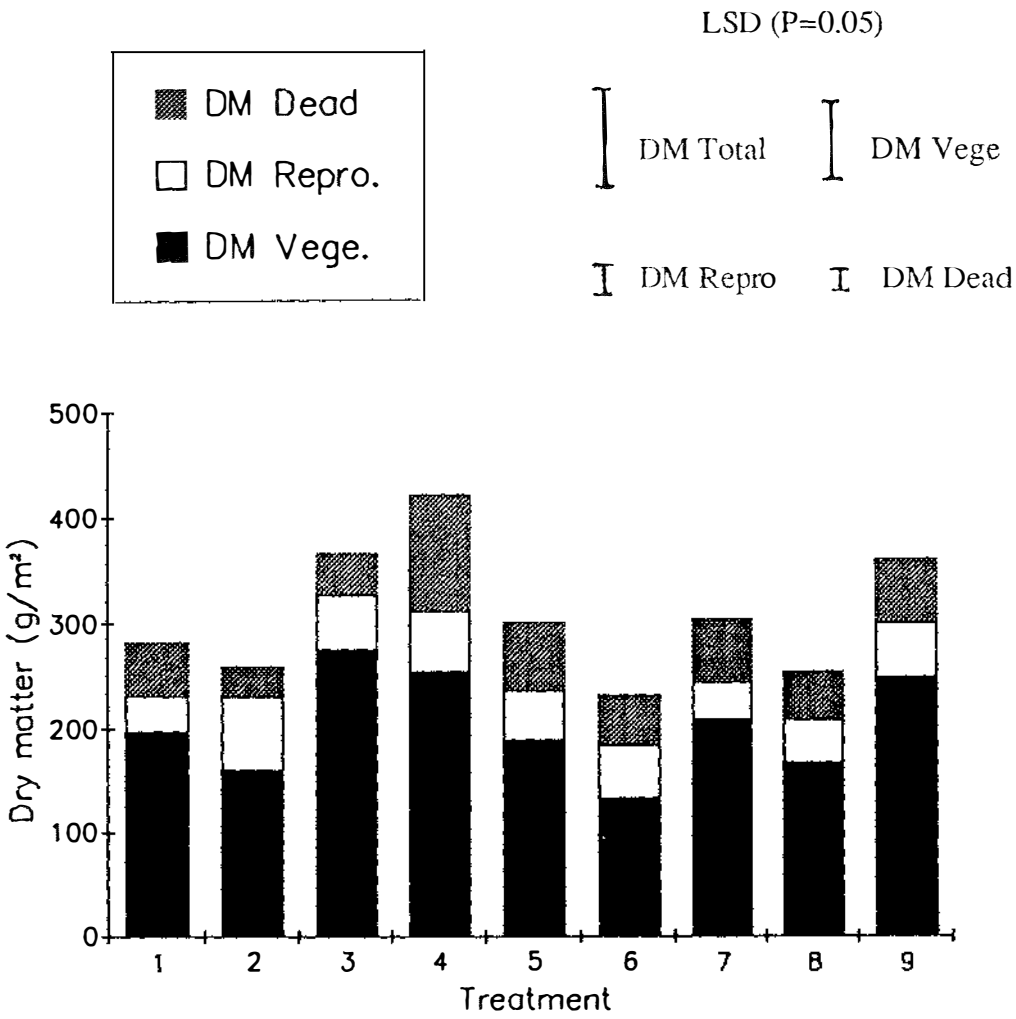


Figure 3.2. Effect of plant growth regulators on dry matter accumulation and distribution at peak flowering for a first year crop of white clover cv. Grasslands Pitau.

Treatments:

1. Control
2. Paclobutrazol 1.0 Oct
3. Paclobutrazol 1.0 Nov
4. Triapenthenol 1.0 Oct
5. Triapenthenol 1.0 Nov
6. Chlormequat chloride 3.0 Oct
7. Chlormequat chloride 3.0 Nov
8. Daminozide 2.0 Nov
9. Daminozide 4.0 Nov

3.3.2.3. Vegetative and reproductive nodes

Paclobutrazol treated plants produced more vegetative nodes than control plants at peak flowering (Figure 3.3), although the difference was significant only for paclobutrazol 1.0 kg a.i/ha applied in November. In the other treatments, there were no significant differences in vegetative nodes produced compared to the control. There was a tendency for all treated plants to show a higher total number of reproductive nodes produced per unit area at peak flowering than control plants, although the differences were not significant (Figure 3.3 and Table 3.2).

3.3.2.4. Composition of reproductive nodes

Although plant growth regulators had no significant effect on the total number of reproductive nodes, some differences were observed in the composition of reproductive nodes at peak flowering (Table 3.2). Despite the fact that there were no significant differences in the number of brown inflorescences and inflorescence buds, the number of white inflorescences was increased significantly by the application of paclobutrazol 1.0 kg a.i/ha in November. On the other hand, the other treatments, except the November chlormequat chloride 3.0 kg a.i/ha treatment, tended to increase the number of brown inflorescences, but the increases were not statistically significant. The number of white inflorescences and inflorescence buds was not significantly affected by these treatments, except that daminozide 2.0 kg a.i/ha applied in November significantly increased the number of inflorescence buds (Table 3.2).

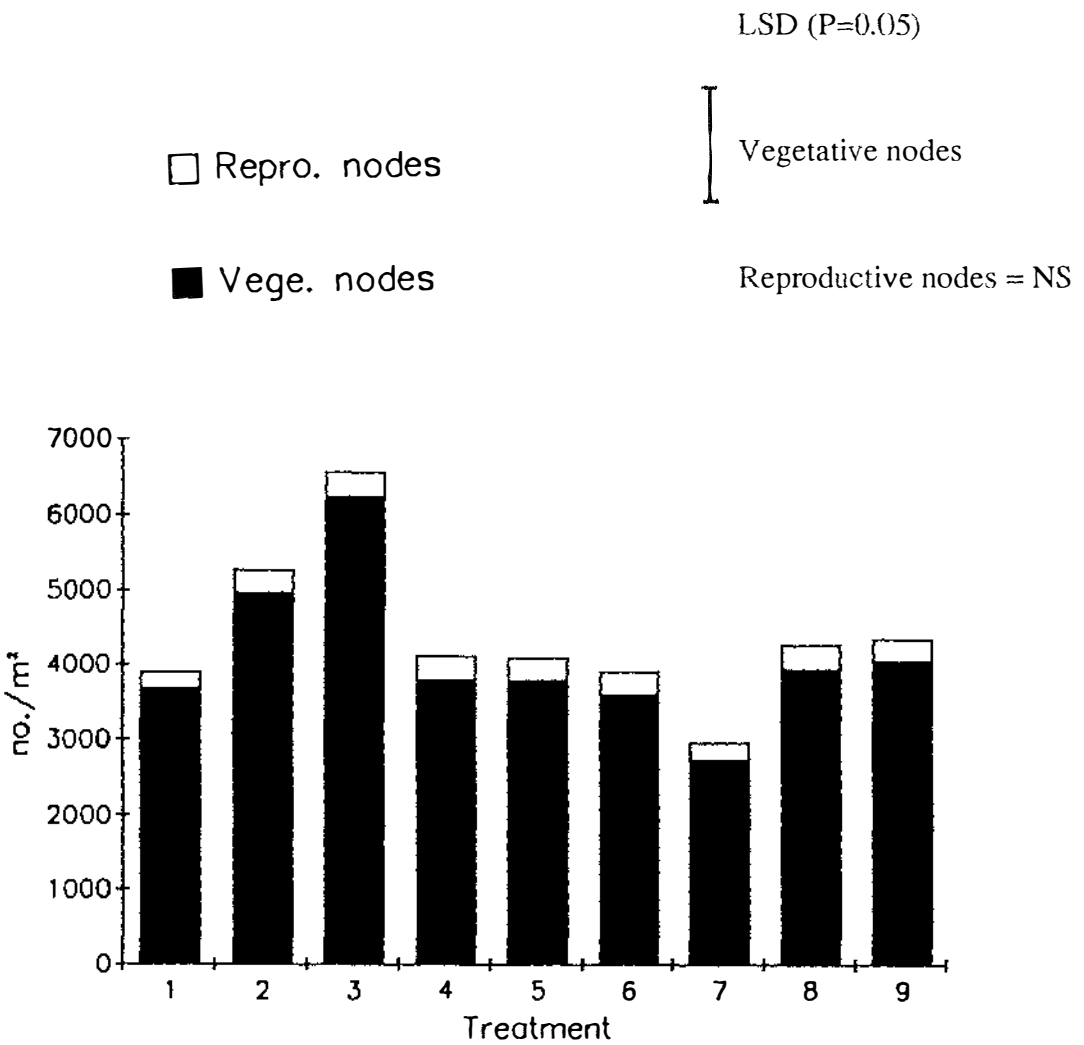


Figure 3.3. Effect of plant growth regulators on vegetative and reproductive node numbers at peak flowering in white clover cv. Grasslands Pitau.

Treatments:

1. Control
2. Paclobutrazol 1.0 Oct
3. Paclobutrazol 1.0 Nov
4. Triapenthenol 1.0 Oct
5. Triapenthenol 1.0 Nov
6. Chlormequat chloride 3.0 Oct
7. Chlormequat chloride 3.0 Nov
8. Daminozide 2.0 Nov
9. Daminozide 4.0 Nov

Table 3.2. Effect of plant growth regulators on reproductive node composition at peak flowering for a first year crop of white clover cv. Grasslands Pitau.

	Inflorescence numbers/m^2			
	Bud	White	Brown	Total
Control	33*b*	89*b*[1)]	134	256
Paclobutrazol 1.0 kg Oct	45*ab*	110*ab*	185	340
Paclobutrazol 1.0 kg Nov	59*ab*	177*a*	118	354
Triapenthenol 1.0 kg Oct	63*ab*	126*ab*	167	356
Triapenthenol 1.0 kg Nov	61*ab*	80*b*	201	342
Chlormequat chloride 3.0 kg Oct	21*b*	148*ab*	174	343
Chlormequat chloride 3.0 kg Nov	51*ab*	88*b*	128	267
Daminozide 2.0 kg Nov	91*a*	105*ab*	167	363
Daminozide 4.0 kg Nov	71*ab*	79*b*	180	330
LSD (P=0.05)	53	79	NS	NS
CV	56	41	42	27

Note : 1. Values in the same column followed by the same letter are not significantly different according to Fisher's LSD test at P=0.05.

3.3.3. Effect of plant growth regulators on flowering pattern

The effect of paclobutrazol on flowering pattern was influenced by the rate and the timing of application. No difference was found in the flowering pattern of the plants treated with paclobutrazol 0.5 kg a.i/ha at reproductive initiation (October) compared to the control, while the application of paclobutrazol 0.5 kg a.i/ha at reproductive buds visible (November) increased the number of inflorescences and concentrated flowering pattern with only one peak flowering. The other two paclobutrazol treatments (1.0 kg a.i/ha applied in either October or November) resulted in two flowering peaks, but an increased flower number (Figure 3.4). However, November application advanced the second flowering peak by about one week.

The rate of triapenthenol application strongly affected flowering pattern. Low rates greatly increased the number of inflorescences at the first flowering peak, while high rates resulted in a flowering pattern similar to the control (Figure 3.5). However, the high rate November application increased the number of inflorescences and advanced the second flowering peak by about one week, while the high rate October application did not differ from the control.

Chlormequat chloride 3.0 kg a.i/ha applied in either October or November increased the number of inflorescences at the second flowering peak and advanced it by about one week compared to the control. Low rate chlormequat chloride applications showed no significant effect on flowering pattern whether applied in October or November (Figure 3.6).

Low rate daminozide application increased the number of inflorescences and advanced the second flowering peak by about one week compared to the control (Figure 3.7), while the high rate daminozide application did not differ from the control.

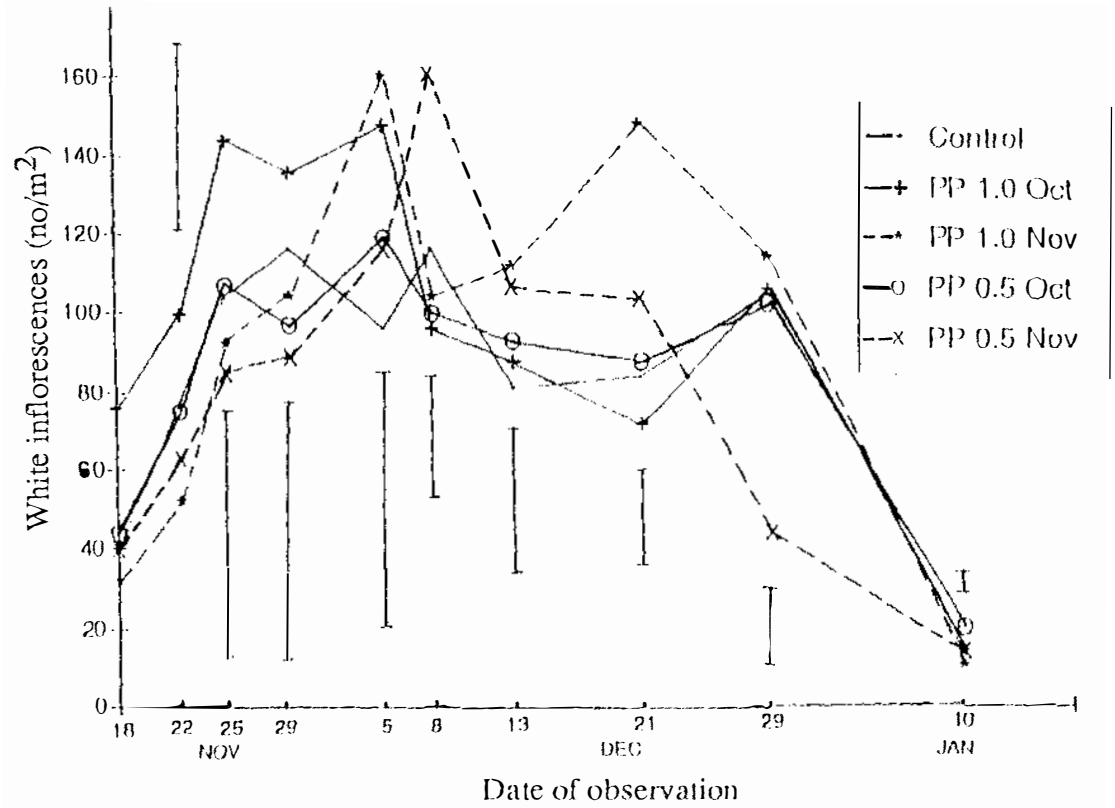


Figure 3.4. Flowering pattern in paclobutrazol treatments.

(Vertical bars are LSD at P = 0.05, same annotations for all following figures)

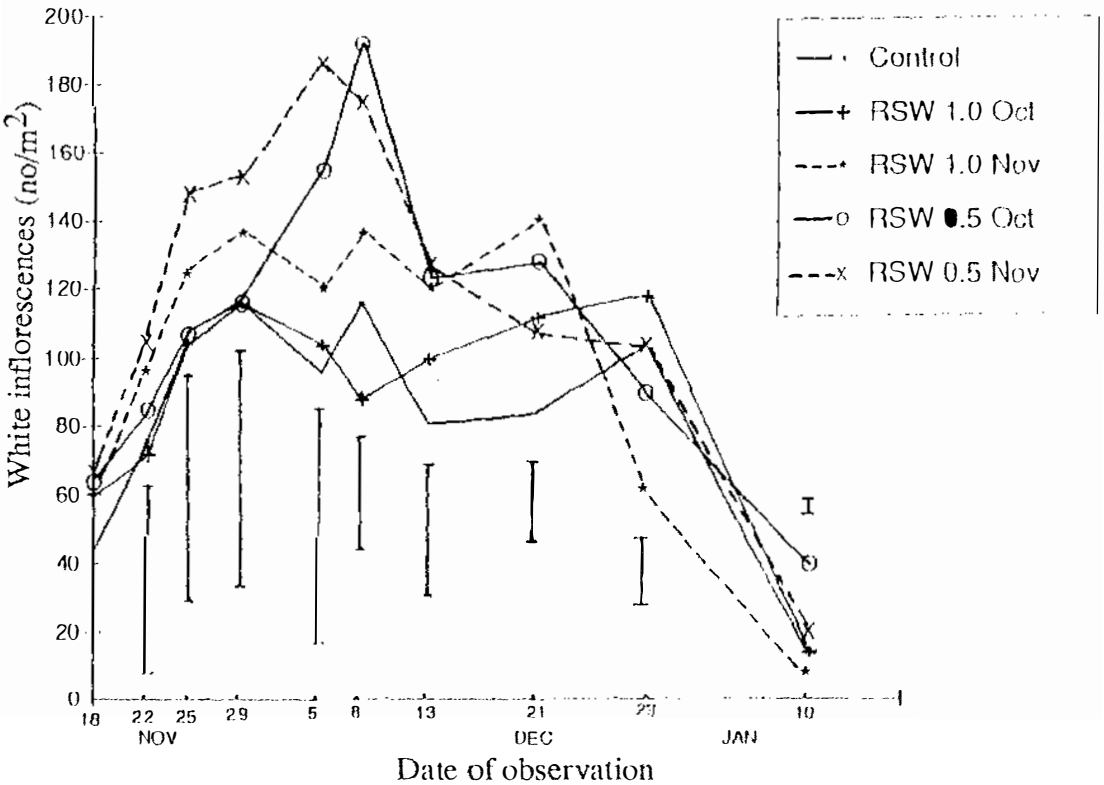


Figure 3.5. Flowering pattern in triapenthenol treatments.

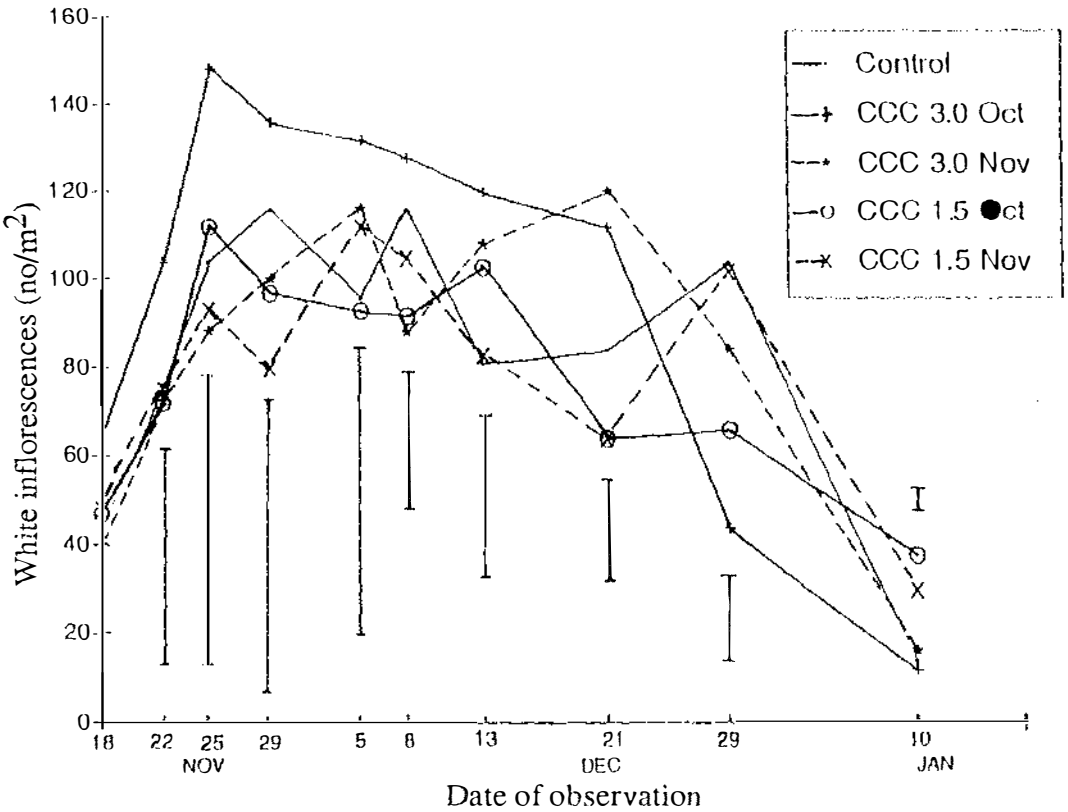


Figure 3.6. Flowering pattern in chlormequat chloride treatments.

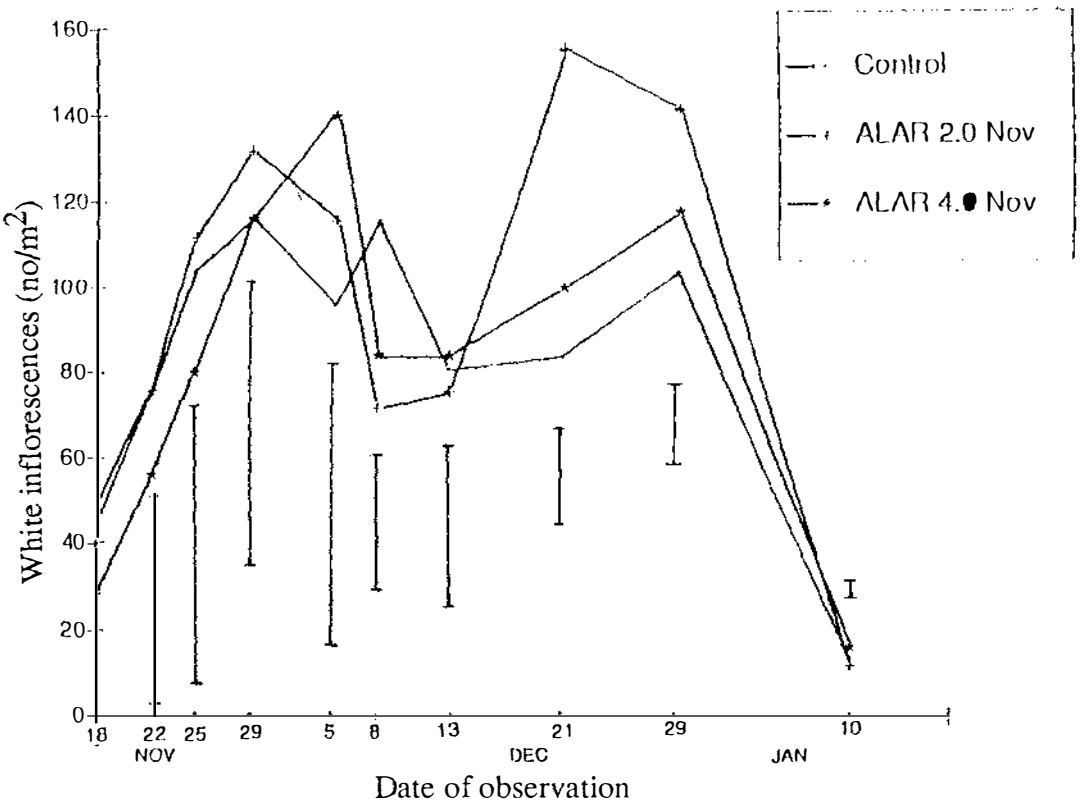


Figure 3.7. Flowering pattern in daminozide treatments.

3.3.4. Effect of plant growth regulators on seed yield components, seed yield and seed quality

The seed yield components at harvest are presented in Table 3.3. The number of inflorescences/m^2 at the time of harvest was significantly increased by the application of paclobutrazol 1.0 kg a.i/ha in either October or November, whereas other differences were not statistically significant. Plant growth regulators did not significantly either increase or decrease the number of florets/inflorescence or the number of seeds/floret compared to the control. However, there was a tendency for plant growth regulators to reduce seed weight, although only all triapenthenol treatments, chlormequat chloride 1.5 kg a.i/ha applied in October and daminozide 2.0 kg a.i/ha applied in November differed significantly from the control.

The application of paclobutrazol 1.0 kg a.i/ha in October significantly increased potential harvestable seed yield over the control by 71 %, but other differences were not significant (Table 3.4). However, there was also a tendency for paclobutrazol, triapenthenol and chlormequat chloride November applications to increase potential harvestable seed yield (Table 3.4). Similarly, although actual seed yield was increased by 25-26 % over the control by the application of paclobutrazol 1.0 kg a.i/ha in either October or November or daminozide 4.0 kg a.i/ha in November, there were no statistically significant increases.

All plant growth regulator treatments reduced vegetative dry matter at harvest compared to the control (Table 3.4). However, only the reductions following the applications of paclobutrazol 0.5 and 1.0 kg a.i/ha in November, triapenthenol 0.5 kg a.i/ha in October and daminozide 2.0 kg a.i/ha in November were statistically significant. Nevertheless, the dry matter harvested was still very bulky compared to the seed harvested, and hence harvest indexes for all treatments did not differ from harvest index for the control (Table 3.4).

There were no effects of the plant growth regulators on seed quality in terms of germination capability. Seed from all treatments showed high levels of germination, i.e. $\geq$ 90% (Appendix 3.4).

Table 3.3. Effect of plant growth regulators on seed yield components at harvest for a first year crop of white clover cv. Grasslands Pitau.

	Inflore-scences/m^2	Florets/ Inflore-scence	Seeds/ Floret	TSW (g)
Control	324*c*	68	4.2	0.635*a*[1)]
Paclobutrazol 0.5 kg Oct	418*abc*	64	4.1	0.629*ab*
Paclobutrazol 1.0 kg Oct	563*a*	66	4.3	0.623*ab*
Paclobutrazol 0.5 kg Nov	481*abc*	62	3.9	0.616*abc*
Paclobutrazol 1.0 kg Nov	551*ab*	64	4.3	0.618*abc*
Triapenthenol 0.5 kg Oct	361*c*	68	4.1	0.605*bcd*
Triapenthenol 1.0 kg Oct	367*bc*	66	4.2	0.586*d*
Triapenthenol 0.5 kg Nov	371*bc*	71	4.5	0.603*bcd*
Triapenthenol 1.0 kg Nov	456*abc*	65	3.8	0.604*bcd*
Chlormequat chloride 1.5 kg Oct	400*abc*	73	4.1	0.604*bcd*
Chlormequat chloride 3.0 kg Oct	352*c*	64	3.9	0.615*abc*
Chlormequat chloride 1.5 kg Nov	375*bc*	71	4.2	0.616*abc*
Chlormequat chloride 3.0 kg Nov	423*abc*	65	4.1	0.610*abcd*
Daminozide 2.0 kg Nov	435*abc*	65	3.9	0.592*cd*
Daminozide 4.0 kg Nov	345*c*	73	4.4	0.610*abcd*
LSD (P=0.05)	185	NS	NS	0.028
CV (%)	27	7	10	3

Note : 1. Values in the same column followed by the same letter are not significantly different according to Fisher's LSD test at P=0.05.

Table 3.4. Effect of plant growth regulators on vegetative dry matter, potential harvestable seed yield (PHSY), actual seed yield (ASY) and harvest index.

Treatments	Vegetative dry matter (g/m^2)	Reduction/ increment (%)	PHSY (g/m^2)	Reduction/ increment (%)	ASY (g/m^2)	Reduction/[1] increment (%)	Harvest index[2]
Control	392.3*a*		59.3*bc*		39.4		9
Paclobutrazol 0.5 kg Oct	350.9*ab*	- 11	69.4*abc*	17	34.5	- 12	9
Paclobutrazol 1.0 kg Oct	354.1*ab*	- 10	101.3*a*	71	49.1	25	12
Paclobutrazol 0.5 kg Nov	296.5*cd*	- 24	72.7*abc*	23	46.8	19	13
Paclobutrazol 1.0 kg Nov	339.5*bc*	- 14	92.8*ab*	57	49.8	26	13
Triapenthenol 0.5 kg Oct	338.0*bc*	- 14	60.9*bc*	3	36.0	- 9	10
Triapenthenol 1.0 kg Oct	351.7*ab*	- 10	58.5*bc*	- 1	35.0	- 11	9
Triapenthenol 0.5 kg Nov	381.9*ab*	- 3	73.1*abc*	23	39.8	1	9
Triapenthenol 1.0 kg Nov	350.4*ab*	- 11	68.8*abc*	16	39.8	1	10
Chlormequat chloride 1.5 kg Oct	382.9*ab*	- 2	70.7*abc*	19	38.8	- 2	9
Chlormequat chloride 3.0 kg Oct	353.7*ab*	- 10	53.4*c*	- 10	27.9	- 29	7
Chlormequat chloride 1.5 kg Nov	362.1*ab*	- 8	68.2*abc*	15	43.8	11	10
Chlormequat chloride 3.0 kg Nov	361.2*ab*	- 8	70.0*abc*	18	40.1	2	10
Daminozide 2.0 kg Nov	274.6*d*	- 30	64.3*abc*	8	35.5	- 10	12
Daminozide 4.0 kg Nov	359.5*ab*	- 8	67.6*abc*	14	49.3	25	12
LSD (P=0.05)	50.5		37.7		NS		NS
CV (%)	9		32		41		35

Note: 1. The percentage reduction or increment as compared to the control.
2. Harvest index = actual seed yield (0% SMC) : total dry matter (vegetative + seed) x 100 %.

3.4. DISCUSSION

Petiole length measured at peak flowering was effectively retarded by paclobutrazol and daminozide (Table 3.1), which led to a reduction in leaf canopy height. Provided peduncle length was not decreased, inflorescences would be elevated through the leaf canopy, resulting in a more favorable micro-environment for pollination as suggested by Marshall and Hides (1986). However, in this trial, paclobutrazol and daminozide also reduced the length of the peduncle (Table 3.1). As a consequence, the length differences between peducle and petiole in both paclobutrazol and daminozide treated plants compared to the untreated plants were not significantly different (Table 3.1). This resulted in no improvement of pollination as indicated by the fact there were no significant increases in the number of seeds/floret at harvest following paclobutrazol or daminozide applications (Table 3.3). A likely advantage of this retardation process, particularly in peduncle length, might be that the shorter and more compact peduncles of treated plants would not lodge during seed development, while the tall peduncles of untreated plants tend to fall under the canopy as seed weight increased. This lodging process becomes more severe in wet weather and results in seed yield losses (Mohamed, 1981; Evan *et al.*, 1986). The number of inflorescences at harvest was increased by paclobutrazol (Table 3.3). However, whether the increase was a result of the prevention of inflorescence lodging and subsequent death, or an increase in the production of inflorescences (Table 3.2) or both following paclobutrazol application could not be determined because observations on inflorescence lodging were not made in this trial. This matter needs clarification.

Triapenthenol and chlormequat chloride did not demonstrate any retardation effects on petiole length when measurements were made at peak flowering (Table 3.1), an effect also demonstrated in *Trifolium pratense* L. (Niemeläinen, 1987). On the other hand, Supanjani (1991) recently reported that both triapenthenol and chlormequat chloride reduced shoot height of *Lotus corniculatus* L. following application at the time when the plant was growing vigorously before flowering, but

that this retardation was not carried over to peak flowering time. Irving and Pallesen (1989) also reported that triapenthenol reduced shoot growth of apple trees, although the effect was shortlived compared to paclobutrazol. Whether triapenthenol and chlormequat chloride have a retardation effect at an early stage of white clover growth needs to be further clarified.

Despite the fact that paclobutrazol and daminozide reduced plant growth, in general, there was no significant reduction of total plant dry matter measured at peak flowering (Figure 3.2). In fact, triapenthenol 1.0 kg a.i/ha applied in October significantly increased total plant dry matter. This suggests that these plant growth regulators, particularly paclobutrazol and daminozide, do not reduce plant assimilation productivity but simply alter plant growth pattern. All plant growth regulators used in this trial are plant growth retardants and basically reduce plant growth by inhibiting gibberellin biosynthesis, despite their different modes of action (Hedden, 1990). Their important physiological effect is on cell elongation. Therefore, these plant growth regulators retard growth only, and the developmental sequence of the plant continues, although new plant organs develop in miniature size (Kaufmann, 1990). In the case of paclobutrazol, it seems likely that the suppression of apical dominance following application results in increased branching, as indicated by the increases in node numbers per unit area (Figure 3.3). Marshall and Hides (1991a) also reported that paclobutrazol increased the number of nodes and growing points. This might also lead to increased inflorescence numbers as reflected in dry matter partitioning (Figure 3.2) (the effect of paclobutrazol on inflorescence production will be discussed further separately). These increased numbers of branches and reproductive organs were significant contributors to the total dry matter. On the other hand, the results are more difficult to explain in the case of triapenthenol, chlormequat chloride and daminozide, since node numbers were not increased by these plant growth regulators (Figure 3.3). For triapenthenol and chlormequat chloride, the possible explanation might be that the plants recovered from the effect of chemical application in blocking gibberellin biosynthesis (Hedden, 1990) and 'caught up' to the growth of untreated plants by peak flowering,

and were even more vigorous in triapenthenol treated plants. In daminozide treated plants, despite the retardation of petiole and peduncle, leaf size remained unchanged (Table 3.1). Therefore, its seemed likely that leaf photosynthesis efficiency of the plant was not changed, although this was not measured. In addition, gibberellin effects on photosynthesis have ranged from enhancement to no effect or depression (Treharne, 1982). Thus, the reduction of gibberellin levels by daminozide might have had no effect on photosynthesis activity. Maintaining assimilate supplies during flowering is very important to support reproductive growth and development for seed production. Therefore, these plant growth regulators had no detrimental effects on the growth and development of the white clover seed crop.

In this trial, the application of paclobutrazol, triapenthenol, chlormequat chloride and daminozide failed to reduce the flowering duration of white clover, which extended over two months. In the case of paclobutrazol, the results were in contrast with those found in *Lotus corniculatus* L., where paclobutrazol reduced the flowering duration (Hampton et al., 1989). This variation was possibly due to generic differences. However, these plant growth regulators had the potential to increase flowering intensity (Figures 3.4, 3.5, 3.6 and 3.7). Unfortunately the potential of triapenthenol, chlormequat chloride and daminozide was not reflected in increased inflorescences and seed yield at harvest. This might be because harvest time was incorrect in relation to the time of flowering intensity. As two flowering peaks occurred during the 1988/1989 growing season, the harvest time was decided at 30 days from the date between the two peaks, i.e. 15 December 1988, for all treatments. As a consequence, harvest might have been conducted too late for treatments which produced an early flowering intensity (low rate of triapenthenol) or too early for treatments which produced a late flowering intensity (high rate of triapenthenol and chlormequat chloride, and low rate of daminozide). Some of the early inflorescences would have shattered and shed seed in the former, and some of the late inflorescences were unripe in the latter. On the other hand, the high rate paclobutrazol application increased the intensity of both flowering peaks (Figure

3.4). Thus, the losses could mathematically be offset by the increased production of second peak inflorescences which subsequently resulted in a significantly increased number of inflorescences/m^2 at harvest (Table 3.3).

In other herbage legumes, e.g. *Trifolium pratense* L. (Niemeläinen, 1987) and *Lotus corniculatus* L. (Supanjani, 1991) triapenthenol also did not increase seed yield. However, Rijckaert (1991) reported that triapenthenol increased the seed yield of white clover cv. Merwi. In this trial, triapenthenol increased potential seed yield by up to 23%, but this was not reflected in increased actual seed yield (Table 3.4). This difference might have resulted from shedding, immature inflorescences or both, but as triapenthenol reduced seed weight significantly (Table 3.3), this suggests that harvest may have been too early in triapenthenol treated plants and therefore seeds had not fully matured. Besides incorrect harvest timing in relation to the time of flowering intensity, there is a possibility that triapenthenol delays crop maturity, as has been reported to occur in ryegrass (Wiltshire and Hebblethwaite, 1990). However, whether this did occur in white clover is not known. This matter needs to be further investigated.

The results following chlormequat chloride application confirmed those reported by Mohamed (1981) and Marshall and Hides (1986) who similarly showed that chlormequat chloride was unsuccessful in increasing seed yield. Similar to triapenthenol, chlormequat chloride increased potential seed yield by up to 19% (Table 3.4). However, seed weight tended to be reduced by chlormequat chloride application, while the numbers of inflorescences, florets/inflorescence and seeds/floret at harvest were unaffected (Table 3.3).

White clover seemed to be more responsive to the low rate of daminozide application which resulted in a higher number of inflorescences produced compared to untreated plants and high rate daminozide treated plants, although this difference was not statistically significant. However, this was offset by a significant reduction of seed weight (Table 3.3). Other components (florets/inflorescence and seeds/floret

were unaffected (Table 3.3). As a result, there was no improvement in seed yield. In addition, both low and high rate daminozide applications increased potential seed yield by less than 15 %. Mohamed (1981) also reported that daminozide was unsuccessful in increasing white clover seed yield in the U.K. Daminozide also did not increase seed yield in *Lotus corniculatus* L. (White *et al.*, 1987; Supanjani, 1991). However, daminozide did increase seed yields in *Lotus uliginosus* Schkuhr., although this did not occur at all rates or times of application (Clifford and Hare, 1987; Tabora, 1991). Similarly, many investigators have reported that seed yields in *Trifolium pratense* L. were significantly increased by daminozide (Puri and Laidlaw, 1983; Jakesova and Svetlik, 1986; Trofimova *et al.*, 1987; Rybak and Walczak, 1988; Christie and Choo, 1990). These differences in different crops suggest that daminozide is species specific and the effects of daminozide may be influenced by rate and time of application, site and season (see section 2.4.2). However, human health concern about possible carcinogenic effects of daminozide (Davis and Andersen, 1989), strongly suggest that the use of daminozide is unlikely to be continued.

Paclobutrazol was the only plant growth regulator considered to have potential benefits for white clover seed production. Seed yield was potentially increased by paclobutrazol through a significant increase in the number of inflorescences produced. This result confirms the findings of Marshall and Hides (1989, 1991b) and Hampton (1991). While Marshall and Hides (1989, 1991b) did not report the effect of paclobutrazol on actual seed yield harvested, in this trial paclobutrazol also increased actual seed yield by 19-26% over the control, but this was not statistically significant. There were two possible explanations for this. Firstly, the variation between plots was high (CV=41% for actual seed yield). This may have been because some plots which were subsequently found to be situated over a former water course dried out more rapidly than other plots as a result of low rainfall during the flowering and seed development stages (November and December 1988). Secondly, despite the fact that actual seed yield of paclobutrazol treated plants was higher than the control, it was obvious that there were big losses of seed prior to

harvest (Table 3.4). The degree of losses was affected by paclobutrazol application, i.e. seed loss in control plants was 34 %, while seed losses in paclobutrazol treated plants ranged from 36 % to 52 %. Furthermore, the reduction of vegetative dry matter at harvest caused by paclobutrazol application did not have any advantage for improved seed yield recovery, as the seed yield increase was so small relative to the mass of dry matter present, i.e. no improvement in harvest index (Table 3.4). This suggests that it is harvest timing which is crucial for high seed recovery. In addition, it was not known whether the duration of inflorescence growth and development from bud to anthesis and anthesis to ripening was affected by paclobutrazol application.

The mechanism of increased inflorescence production by paclobutrazol application was not clear from this trial. However, there are two possibilities. Firstly, increased inflorescence production could be attributed to a greater proportion of reproductive nodes on each stolon resulting from direct initiation by paclobutrazol. Secondly, paclobutrazol simply increased stolon production and subsequently gave more sites for inflorescence production. Under glasshouse conditions, Marshall and Hides (1987, 1989) reported that paclobutrazol did not increase total nodes and inflorescences per stolon, but increased the total axillary buds formed along stolons. When these axillary buds grow as stolons, the total number of nodes consequently will increase. In this trial, paclobutrazol increased the number of nodes per unit area (Figure 3.3). This suggests that paclobutrazol increases branching and in turn increases inflorescence production. Nevertheless, the time needed for axillary buds to become independent stolons under field conditions following paclobutrazol application, and whether these stolons subsequently became fertile is not known and still remaines to be investigated. In addition, the possibility that paclobutrazol enhanced inflorescence initiation in conjunction with an environmental stimulus in the field could not be overruled.

Based on the results from the present experiment and studies of Marshall and Hides (1989, 1991b), the application of paclobutrazol at 1.0 kg a.i/ha was considered to be an acceptable rate. However, it is still difficult to recommend an optimum application time. The decision to apply paclobutrazol using calendar dates is not recommended as crop age, crop management and weather conditions may all modify plant growth and development patterns. Therefore, application based on the growth and development stages of the plant is more suitable. In terms of potential seed yield, application in early October, when reproductive buds were still within growing points or initiating, gave slightly better results than application in early November when reproductive buds had emerged, although statistically this difference was not significant. Marshall and Hides (1989) found that paclobutrazol applied when growing points became reproductive (middle of March in UK) gave the greatest response. However, as initiation can still be taking place when white clover is already flowering, particularly in cultivars from high latitudes (Thomas, 1987c), this matter still needs to be clarified further.

Regardless of the effect of treatments, the average actual seed yield from this experiment exceeded the New Zealand average of 300 kg/ha (Appendix 3.2). This implies that the 1988/1989 growing season in Manawatu, which is not a traditional white clover seed growing area, was reasonable for seed production. Weather conditions were warm and sunny during the flowering period, although this time was slightly drier than normal as rainfall was below average and caused some plots to dry out (Appendix 3.3). Rijckaert (1991) reported that paclobutrazol response was greatest when weather conditions were unfavourable for white clover seed production, while the effect was less pronounced in a good season. However, although actual seed yield increases were not statistically significant, results from this trial indicated that paclobutrazol showed potential to increase seed yield under favourable conditions for white clover seed production, a situation which would be worth further investigation.

CHAPTER 4

EFFECT OF PLANT GROWTH REGULATORS ON THE GROWTH, DEVELOPMENT AND SEED YIELD OF A SECOND YEAR CROP OF WHITE CLOVER CV. GRASSLANDS PITAU

4.1. INTRODUCTION

Seed yield potential in white clover is generally low for a second year crop (Evans *et al.*, 1986; Van Bockstaele and Rijckaert, 1988). These authors reported that seed yield reduction in a second year crop was associated with a drastic reduction in the number of inflorescences. Smaller inflorescences were also formed and a lower seed weight was recorded, resulting in a much lower seed yield per inflorescence. Under the New Zealand certification scheme (Anon., 1988), white clover seed can be harvested for up to four harvest seasons. Therefore, seed growers also want good yields from their second and subsequent year crops. New Zealand growers have adopted management practices suggested by Clifford (1987) to increase white clover seed yield. However, those management practices are mostly suitable for a one harvest only commercial crop system. Reports on research specifically intended to optimize production from a second year white clover seed crop are limited. For example, the most recent report on the effect of closing date on potential seed yield from second year crops of white clover cvs. Grasslands Huia and Grasslands Pitau was published more than 10 years ago (Clifford, 1979). The use of plant growth regulators to increase the number of inflorescences and improve other yield components for a second year crop is an area for possible research, as suggested by Van Bockstaele and Rijckaert (1988).

The 1988/1989 trial showed that some plant growth regulators had potential for increasing seed yield of a first year white clover seed crop, through increasing the number of inflorescences produced per unit area. In a preliminary trial, positive

effects of plant growth regulators for increasing inflorescences and thereby seed yield in a second year white clover seed crop have also been reported (Hampton, 1991). However, the results from both trials did not clarify whether the increase in inflorescence production could be attributed to a greater proportion of reproductive nodes on each stolon, which would also influence the proportion of ripe inflorescences at harvest, or simply to increased stolon production per unit area, hence providing more sites for floral expression.

In 1989/1990 a further trial examined the effect of plant growth regulators and time of application on the growth, development and seed yield of a second year white clover seed crop. The possible ways by which inflorescences may be increased were also investigated.

4.2. MATERIALS AND METHODS

4.2.1. Experimental site, management and treatments

The trial was conducted in the 1989/1990 growing season. The crop of white clover cv. Grasslands Pitau described in section 3.2.1 was utilized. Based on a soil test result (Appendix 3.1), 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 (equal to 50 kg KCl/ha) to remedy this deficiency. No irrigation was applied. Grazing was stopped on 26 August 1989. To control weeds, herbicides were applied on three occasions, i.e. 2,4-DB at a rate of 1.6 kg a.i/ha or 4 l 2,4-DB/ha on 4 September 1989, MCPB at a rate of 1.6 kg a.i/ha or 4 l MCPB/ha and Fusilade (fluazifop-P-butyl) at a rate of 0.25 kg a.i/ha or 2 l Fusilade/ha on 4 October 1989, and 2,4-DB at a rate of 2.8 kg a.i/ha or 7 l 2,4-DB/ha on 20 October 1989. 2,4-DB herbicide is a selective herbicide which moves and acts in the plant in a similar fashion to 2,4-D, i.e. it is absorbed mainly through leaves and stems and a little through roots, and moves readily in the plant to accumulate in actively growing parts of the plant to cause interference in cell division and enlargement (Anon.,

1990). MCPB, a selective herbicide for use in white clover to control broadleaf weeds (Anon., 1990), is absorbed mainly through leaves and readily translocates in the plant with nutrient movement. Susceptible plants convert MCPB to MCPA which interferes with cell division and enlargement. Fusilade (fluazifop-P-butyl) is a selective systemic post-emergence herbicide for the control of most annual and perennial grasses in broadleaf crops (Anon., 1990). Following foliar uptake it moves upwards and downwards in the plant to occupy roots, stolons, rhizomes, stems and leaves, and growth ceases almost immediately.

The experiment utilized a three replicate randomized complete block design. Plots were set out avoiding areas used in the previous year for paclobutrazol treatments, as paclobutrazol is persistent in the soil (Lever, 1986) and hence has a residual activity (Hampton, 1988b). Plot size was 7 x 2 m with regularly cut pathways of 2 m between blocks and 1 m between plots. This experiment consisted of nine treatments and one control. Based on the results of the 1988/1989 experiment, paclobutrazol, triapenthenol and chlormequat chloride were selected for further evaluation, but only one rate was used, i.e. 1.0 kg a.i/ha for paclobutrazol and triapenthenol, and 3.0 kg a.i/ha for chlormequat chloride. As cv. Grasslands Pitau is an early initiating cultivar (Thomas, 1980a; Clifford, 1987), three different times of application were examined in this experiment: early reproductive initiation (13 September 1989), during reproductive initiation (10 October 1989) and reproductive buds visible/early flowering (8 November 1989). Starting in early September, reproductive development was monitored weekly by dissecting ten growing points (stolon apices) and examining them under a microscope at 40X magnification. A definition and indication of reproductive initiation is presented in section 3.2.1. Early reproductive initiation was defined as being when less than 25 % of the growing points had initiated, while reproductive initiation was defined as being when more than 75 % of growing points had initiated. Reproductive buds visible/early flowering was defined as being when most buds had emerged from their surrounding stipular sheaths and a few buds had developed into white

inflorescences. For ease of reference, the time of application has been termed as September, October or November application. Plant growth regulators were applied using a similar knapsack sprayer to that described in section 3.2.1.

4.2.2. Plant measurements and statistical analysis

Growth analysis was carried out using techniques described in the previous experiment (section 3.2.2). Samples from a quadrat of 0.15 m^2 per plot were taken at four different times, i.e. during reproductive initiation (6 October 1989), reproductive buds visible/early flowering (7 November 1989), peak flowering (7 December 1989) and harvest (3 January 1990). At reproductive initiation, the analysis was conducted only for samples from the control and September application plots, at reproductive buds visible for the control, September and October treatments, and samples from all plots were analyzed at peak flowering and harvest. In this trial, dry matter composition was determined by dissecting subsamples into four components: leaf, stolon, reproductive (inflorescence and inflorescence bud) and dead material. The number of vegetative nodes was also measured from the subsamples. The number of reproductive nodes and their composition (bud, white and brown inflorescences) were counted only at peak flowering. The number of growing points from the subsamples was also recorded at peak flowering and harvest to determine the number of stolons produced per unit area. Leaf score (Williams *et al.*, 1964) and petiole length was measured from 20 leaves/plot selected at random. Peduncle length was also measured from 20 inflorescences/plot randomly selected.

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 marked by placing bamboo canes to ease the stolon collection. Subsequently, measurements were made of the number of vegetative and reproductive nodes, axillary buds and stolon length.

Similarly to the previous investigation (section 3.2.2), peak flowering was determined by counting the number of white inflorescences within a permanent quadrat of 0.5 x 0.5 m established in each plot at a regular interval of 5 days. Harvesting was conducted on 4 January 1990 (30 days after peak flowering).

Both actual and potential harvestable seed yields, seed yield components and harvest indexes were measured as described for the 1988/1989 experiment (see section 3.2.2) except that harvested material was obtained from two quadrats of 0.5 m^2 from each plot, the number of inflorescences produced were separated into two groups (ripe and unripe inflorescences), and floret numbers were counted from 30 inflorescences/plot selected randomly from the field. Inflorescences were considered to be fully ripe when the colour of their florets and pedicels had turned brown, while inflorescences with light brown and/or white florets and green pedicels were classified as unripe inflorescences (Plate 4.1). Only ripe inflorescences were used to calculate potential harvestable seed yield. Actual seed yield and thousand seed weight were expressed at 10 % seed moisture content.

Seed quality was determined by the germination testing of 4 x 50 scarified seeds using the top of paper method (ISTA, 1985) at 20°C (see section 3.2.2 for details).

Data collected in this experiment were analyzed in the same way as in the previous experiment, by the use of analysis of variance and Fisher's LSD test at P=0.05.

Plate 4.1. White clover inflorescences showing different ripeness.
Left = ripe inflorescences Right= unripe inflorescences

4.3 RESULTS

4.3.1. Meteorological conditions

The early trial period (September to November) was warmer than average with deviations of temperature ranging from 1.3°C to 2.2°C and 1.4°C to 1.8°C for minimum and maximum temperatures respectively. However, temperatures during peak flowering (December) and harvest (January) were generally cooler than average (Appendix 3.3). The number of sunshine hours were above average in September and November, but lower in October, December and January (Appendix 3.3). Rainfall was below average during early growth (September) and flowering (November and December), but higher in October and January (Appendix 3.3).

4.3.2. Effect of plant growth regulators on plant growth and development

4.3.2.1. Morphological characteristics

Petiole length and, to a lesser extent, leaf size were characters sensitively affected by two plant growth regulators. Regardless of application time, paclobutrazol significantly reduced leaf size (presented as leaf score in Table 4.1) compared to the control at reproductive initiation (October) and harvest (January), but not during flowering (November and December). Triapenthenol and chlormequat chloride did not affect leaf size (Table 4.1 and Plate 4.2). At reproductive initiation (± three weeks following the September application), petiole length was significantly reduced by both paclobutrazol and triapenthenol, while chlormequat chloride did not differ from the control (Table 4.2). This reduction in petiole length resulted in a reduced plant height (Plate 4.3). During subsequent growth stages, only paclobutrazol treatments consistently significantly retarded petiole length (for three months), while the retardation effect of triapenthenol applied in September was not carried over (Table 4.2 and Plate 4.4). The application of triapenthenol in October and November did not affect petiole length. Similarly, chlormequat chloride treated plants did not differ from the control.

Table 4.1. Effect of plant growth regulators on leaf score for a second year crop of white clover cv. Grasslands Pitau at reproductive initiation (October), reproductive buds visible/early flowering (November), peak flowering (December) and harvest (January).

Treatments	October	November	December	January
Control	20.27*a*[1)]	21.30*ab*	20.77	20.30*a*
Paclobutrazol Sept	18.37*b*	20.23*b*	19.73	19.13*bcd*
Paclobutrazol Oct	-	20.30*b*	20.07	18.83*cd*
Paclobutrazol Nov	-	-	20.03	18.67*d*
Triapenthenol Sept	18.93*ab*	21.30*ab*	20.83	20.17*a*
Triapenthenol Oct	-	21.60*a*	20.97	19.67*abc*
Triapenthenol Nov	-	-	20.93	19.70*abc*
Chlormequat chloride Sept	20.20*a*	21.83*a*	20.70	19.67*abc*
Chlormequat chloride Oct	-	21.70*a*	20.60	19.97*ab*
Chlormequat chloride Nov	-	-	21.13	20.23*a*
LSD (P=0.05)	1.40	1.09	NS	0.98
CV (%)	3.6	2.9	3.2	2.9

Note: 1. Values in the same column followed by the same letter are not significantly different according to Fisher's LSD test at P=0.05.

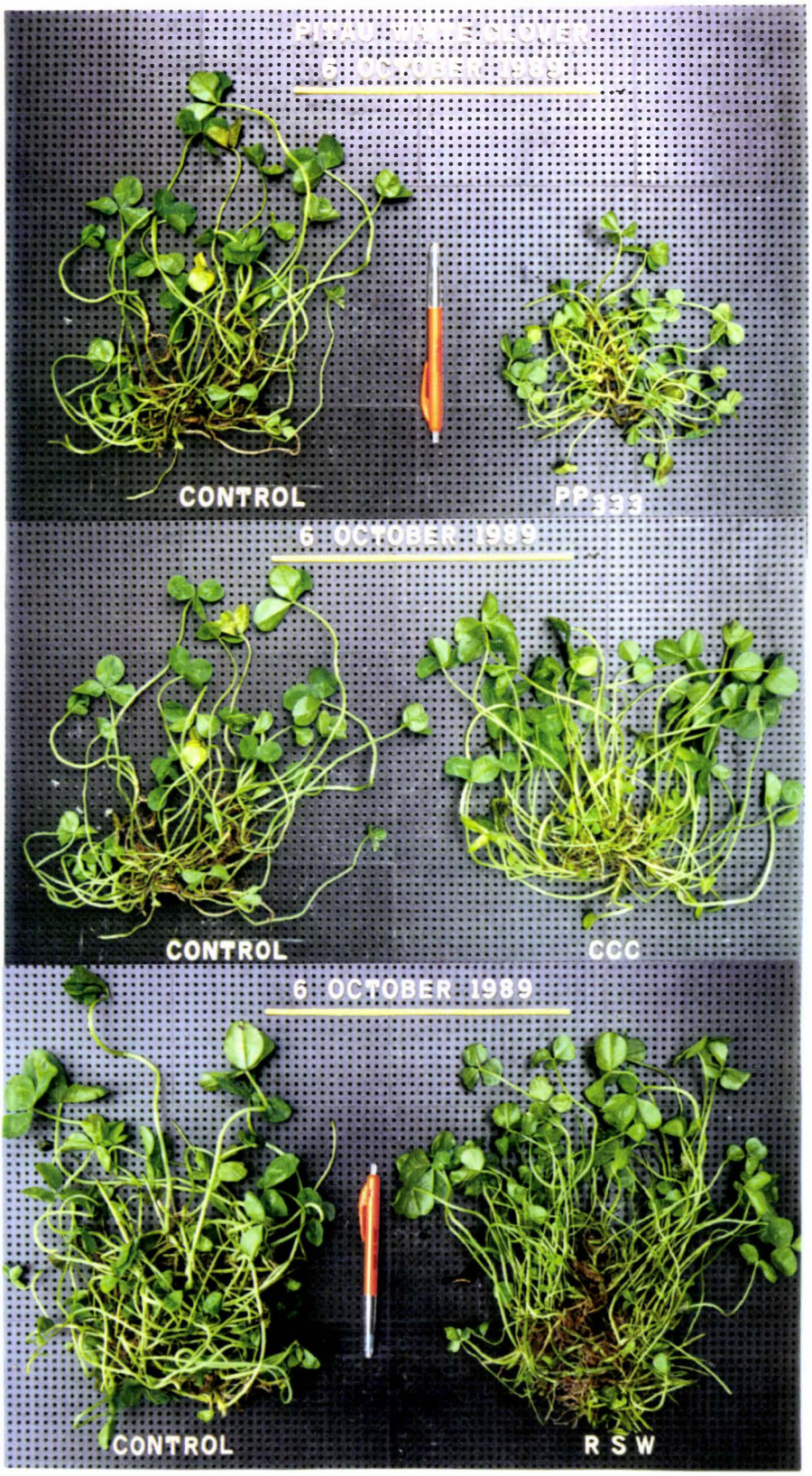


Plate 4.2. White clover plants at 23 days after plant growth regulator application.

Table 4.2. Effect of plant growth regulators on petiole and peduncle lengths for a second year crop of white clover cv. Grasslands Pitau at reproductive initiation (October), reproductive buds visible/early flowering (November), peak flowering (December) and harvest (January).

Treatments	Petiole Length (mm)				Peduncle Length (mm)		Length Differences (mm)	
	Oct	Nov	Dec	Jan	Dec	Jan	Dec	Jan
Control	161.6*a*[1)]	245.0*a*	209.4*a*	208.4*a*	251.8	268.4	42.4	60.0
Paclobutrazol Sept	107.5*b*	195.5*b*	167.6*bc*	185.4*ab*	221.3	253.0	53.7	67.6
Paclobutrazol Oct	-	171.3*b*	170.3*bc*	157.9*b*	232.7	234.7	62.4	76.8
Paclobutrazol Nov	-	-	177.3*abc*	157.8*b*	222.3	235.8	45.0	78.0
Triapenthenol Sept	127.1*b*	252.9*a*	194.9*abc*	197.4*ab*	244.6	262.4	49.7	65.0
Triapenthenol Oct	-	250.4*a*	208.1*ab*	189.2*ab*	256.8	243.6	48.7	54.4
Triapenthenol Nov	-	-	207.8*ab*	185.9*ab*	273.5	249.9	65.7	64.0
Chlormequat chloride Sept	154.2*a*	245.6*a*	214.3*a*	190.2*ab*	259.3	262.8	45.0	72.6
Chlormequat chloride Oct	-	256.7*a*	200.6*abc*	185.8*ab*	242.2	241.9	41.6	56.1
Chlormequat chloride Nov	-	-	208.1*ab*	211.8*a*	261.4	278.7	53.3	66.9
LSD (P=0.05)	2 6.5	3 9.4	3 7.9	4 6.0	NS	NS	NS	NS
CV (%)	10	10	11	14	8	10	22	21

Note: 1. Values in the same column followed by the same letter are not significantly different according to Fisher's LSD test at P=0.05.

Plate 4.3. Second year white clover plants, showing the effect of paclobutrazol on plant height (1 month after application).

Plate 4.4. White clover plants at 55 days after plant growth regulator application.

All three plant growth regulators had no significant effect on peduncle length compared to the control. Despite there being a reduction of petiole length following paclobutrazol application, there were no significant increases in the length differences between peduncle and petiole (Table 4.2). Inflorescences from both control and treated plants were elevated above the leaf canopy at peak flowering and at harvest (Table 4.2).

4.3.2.2. Dry matter accumulation and distribution

From the time of reproductive initiation to harvest, plant growth regulators generally did not affect total dry matter accumulation, except that chlormequat chloride applied in October significantly increased total dry matter at peak flowering. Therefore, there were similar growth patterns for both control and treated plants, i.e. the plants grew rapidly, beginning at reproductive initiation and throughout flowering (October to December), and when the plants reached peak flowering, the growth started to level off or decline (Figure 4.1). However, there were significant differences in dry matter distribution (Appendix 4.1), the differences being influenced by growth stages, type of plant growth regulator and application time. Despite the fact that there were no changes in stolon dry matter, paclobutrazol significantly decreased leaf dry matter at reproductive initiation, while other treatments did not differ from the control. At early flowering, leaf, stolon and reproductive dry matter were not affected by plant growth regulators, but dead dry matter was significantly increased by chlormequat chloride applied in September. At peak flowering, triapenthenol applied in either October or November significantly increased reproductive dry matter compared to the control, despite there being no changes in vegetative and dead dry matter, whereas other treatments did not differ. At harvest, paclobutrazol applied in September or November significantly increased stolon dry matter, but reproductive dry matter was significantly decreased by the September application. Other treatments showed no significant effects. Despite those differences, however, there was a similar dry matter distribution pattern over growth stages in both control and treated plants (Appendix 4.1). Leaf dry matter was

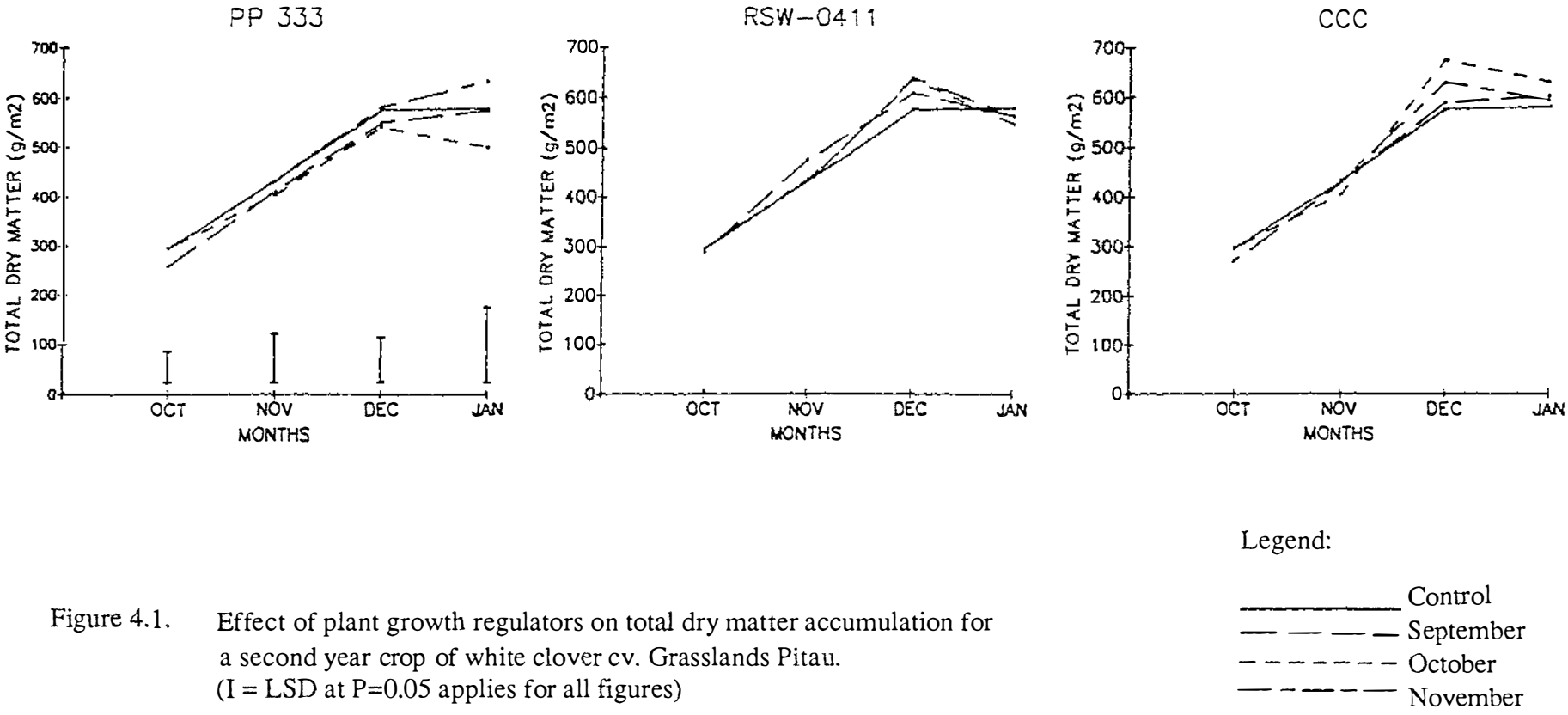


Figure 4.1. Effect of plant growth regulators on total dry matter accumulation for a second year crop of white clover cv. Grasslands Pitau.
(I = LSD at P=0.05 applies for all figures)

increased rapidly, beginning at reproductive initiation, leveled off at peak flowering and then declined at harvest. Stolon dry matter was also increased steadily from reproductive initiation up to flowering, and leveled off or declined at harvest, except for paclobutrazol applied in September and November which continued to increase. On the other hand, reproductive dry matter continued to increase up to harvest.

4.3.2.3. Vegetative nodes and growing points

The pattern of vegetative node development following plant growth regulator application is presented in Figure 4.2. Paclobutrazol, triapenthenol and chlormequat chloride applied in September significantly reduced vegetative node development at reproductive initiation (October). When flowering started (November), the general trend was for a decline in vegetative node development per unit area. However, the paclobutrazol September application significantly increased the number of vegetative nodes compared to the control, while other treatments did not differ. On the other hand, paclobutrazol applied in November significantly increased vegetative nodes at peak flowering (December), whereas other treatments did not differ. Although paclobutrazol applied either in September or November increased vegetative nodes by 33 % over the control, there were no statistically significant increases at harvest.

Although there were some increases, plant growth regulators did not significantly affect the number of growing points produced per unit area at peak flowering (Table 4.3). However, paclobutrazol applied in either September or November significantly increased the number of growing points at harvest compared to the control.

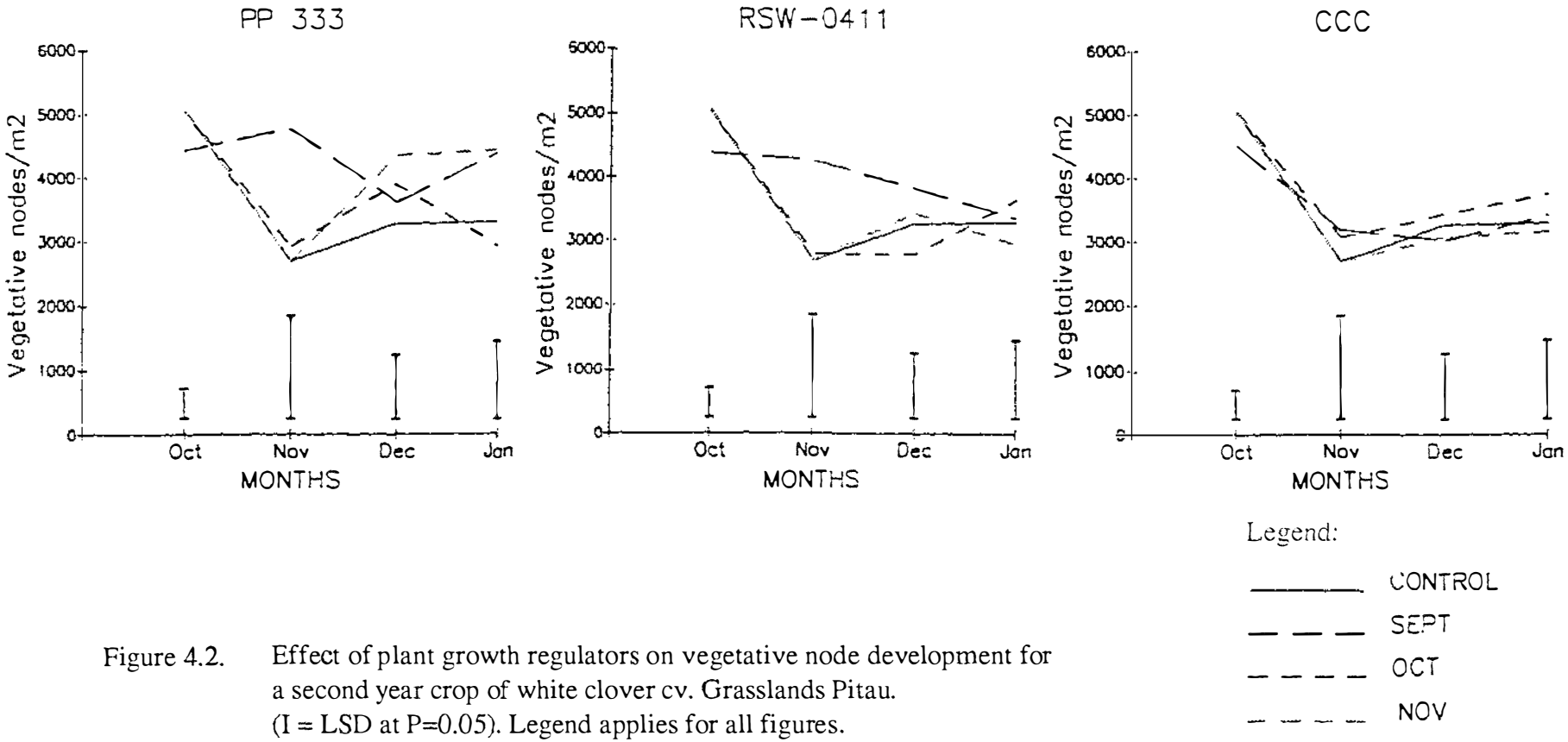


Figure 4.2. Effect of plant growth regulators on vegetative node development for a second year crop of white clover cv. Grasslands Pitau. (I = LSD at P=0.05). Legend applies for all figures.

Table 4.3. Effect of plant growth regulators on growing points (stolon apices) for a second year crop of white clover cv. Grasslands Pitau.

Treatments	Growing points (no/m^2) Peak flowering	Harvest
Control	1255	1242*b*[1)]
Paclobutrazol Sept	1511	1871*a*
Paclobutrazol Oct	1484	1233*b*
Paclobutrazol Nov	1469	1900*a*
Triapenthenol Sept	1616	1405*ab*
Triapenthenol Oct	1275	1489*ab*
Triapenthenol Nov	1398	1169*b*
Chlormequat chloride Sept	1351	1493*ab*
Chlormequat chloride Oct	1447	1389*ab*
Chlormequat chloride Nov	1249	1309*b*
LSD (P=0.05)	NS	529
CV (%)	18	21

Note: 1. Values in the same column followed by the same letter are not significantly different according to Fisher's LSD test at P=0.05.

4.3.2.4. Reproductive nodes and their composition

Although there was a tendency for all treated plants to show a greater total number of reproductive nodes per unit area than the control at peak flowering, only triapenthenol applied in November differed significantly (Table 4.4). This significant increase was attributed to the significant increase of white inflorescences. Despite there being no changes in the number of buds, brown inflorescences and total reproductive nodes, paclobutrazol applied in September also significantly increased the number of white inflorescences. The rest of the treatments did not affect the composition of reproductive nodes.

4.3.2.5. Growth and development of main stolons

Visually, stolon length appeared to be shortened by paclobutrazol, particularly the September application (Plate 4.5), but there were no significant differences in stolon length following plant growth regulator application when measured at harvest (Table 4.5). The stolon length of a second year crop was relatively short, between 7.4-14.5 cm. The development of vegetative nodes along stolons was significantly reduced by paclobutrazol and triapenthenol applied in September, while other treatments did not affect the development. On the other hand, 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 4.5). The number of axillary buds along stolons was significantly increased by the application of paclobutrazol in November compared to the control, while other treatments were not significantly different (Table 4.5).

Table 4.4. Effect of plant growth regulators on reproductive nodes and their composition at peak flowering for a second year crop of white clover cv. Grasslands Pitau

Treatments	Buds	Reproductive nodes/m^2 White inflorescences	Brown inflorescences	Total
Control	292	244*c*[1)]	206	742*b*
Paclobutrazol Sept	223	454*ab*	230	907*ab*
Paclobutrazol Oct	247	325*c*	217	789*b*
Paclobutrazol Nov	402	267*c*	142	811*b*
Triapenthenol Sept	408	352*bc*	171	931*ab*
Triapenthenol Oct	324	363*bc*	206	893*ab*
Triapenthenol Nov	303	517*a*	293	1113*a*
Chlormequat chloride Sept	251	323*c*	222	796*b*
Chlormequat chloride Oct	320	315*c*	134	769*b*
Chlormequat chloride Nov	357	268*c*	175	800*b*
LSD (P=0.05)	NS	125	NS	234
CV (%)	25	21	33	16

Note: 1. Values in the same column followed by the same letter are not significantly different according to Fisher's LSD test at P=0.05.

Plate 4.5. Effect of paclobutrazol on main stolon length (Photographed on 7 December 1989).

Table 4.5. Effect of plant growth regulators on the growth and development of a second year crop's main stolons

Treatments	Vegetative nodes (no/stolon)	Reproductive nodes (no/stolon)	Axillary buds (no/stolon)	Stolon length (mm)
Control	9.9*a*[1)]	1.1*b*	1.1*b*	110.7*abcd*
Paclobutrazol Sept	8.5*b*	1.2*ab*	1.1*b*	74.3*d*
Paclobutrazol Oct	9.7*a*	1.3*ab*	1.5*ab*	98.9*bcd*
Paclobutrazol Nov	10.1*a*	1.6*a*	2.2*a*	116.7*abc*
Triapenthenol Sept	8.4*b*	1.3*ab*	1.4*ab*	90.6*cd*
Triapenthenol Oct	9.4*ab*	1.4*ab*	1.6*ab*	117.9*abc*
Triapenthenol Nov	10.1*a*	1.7*a*	2.0*ab*	143.5*a*
Chlormequat chloride Sept	9.7*a*	1.5*ab*	1.9*ab*	131.7*ab*
Chlormequat chloride Oct	9.5*ab*	1.3*ab*	1.2*b*	132.8*ab*
Chlormequat chloride Nov	9.3*ab*	1.5*ab*	1.6*ab*	119.3*abc*
LSD (P=0.05)	1.2	0.4	0.9	39.8
CV (%)	8	19	35	20

Note: 1. Values in the same column followed by the same letter are not significantly different according to Fisher's LSD test at P=0.05.

4.3.3. Effect of plant growth regulators on flowering pattern

In this second year crop, only one distinct peak was found during the flowering season of about two months (Figure 4.3). In all treatments this peak was well defined and occurred on the same date (5 December 1989). During the rapid increase of inflorescences to reach peak flowering (from 25 November to 5 December), which coincided with the mid season flowering, paclobutrazol tended to increase the number of inflorescences, although only the October application was significant. Triapenthenol applied in November also significantly increased the number of white inflorescences at the same period, while the September and October applications did not differ from the control. All chlormequat chloride treatments had no effects on flowering pattern.

4.3.4. Effect of plant growth regulators on seed yield components, seed yield and seed quality

The seed yield components at harvest are presented in Table 4.6. The total number of inflorescences per unit area was significantly increased by the application of paclobutrazol in November compared to the control, while other treatments showed no significant effects. However, the increase was mainly attributed to the significant increase of unripe inflorescences. Ripe inflorescences were increased by 23 % over the control, but this difference was not statistically significant. The percentage of unripe inflorescences from all treated plants were not significantly different from the control at harvest, with the percentages ranging from 26 % to 34 % (Table 4.6). The conditions of the plants at harvest can be seen in Plate 4.6. Plant growth regulators did not significantly affect the number of florets per inflorescence or the number of seeds per floret. However, thousand seed weight was significantly reduced by all paclobutrazol treatments and triapenthenol applied in October, while other treatments did not differ from the control.

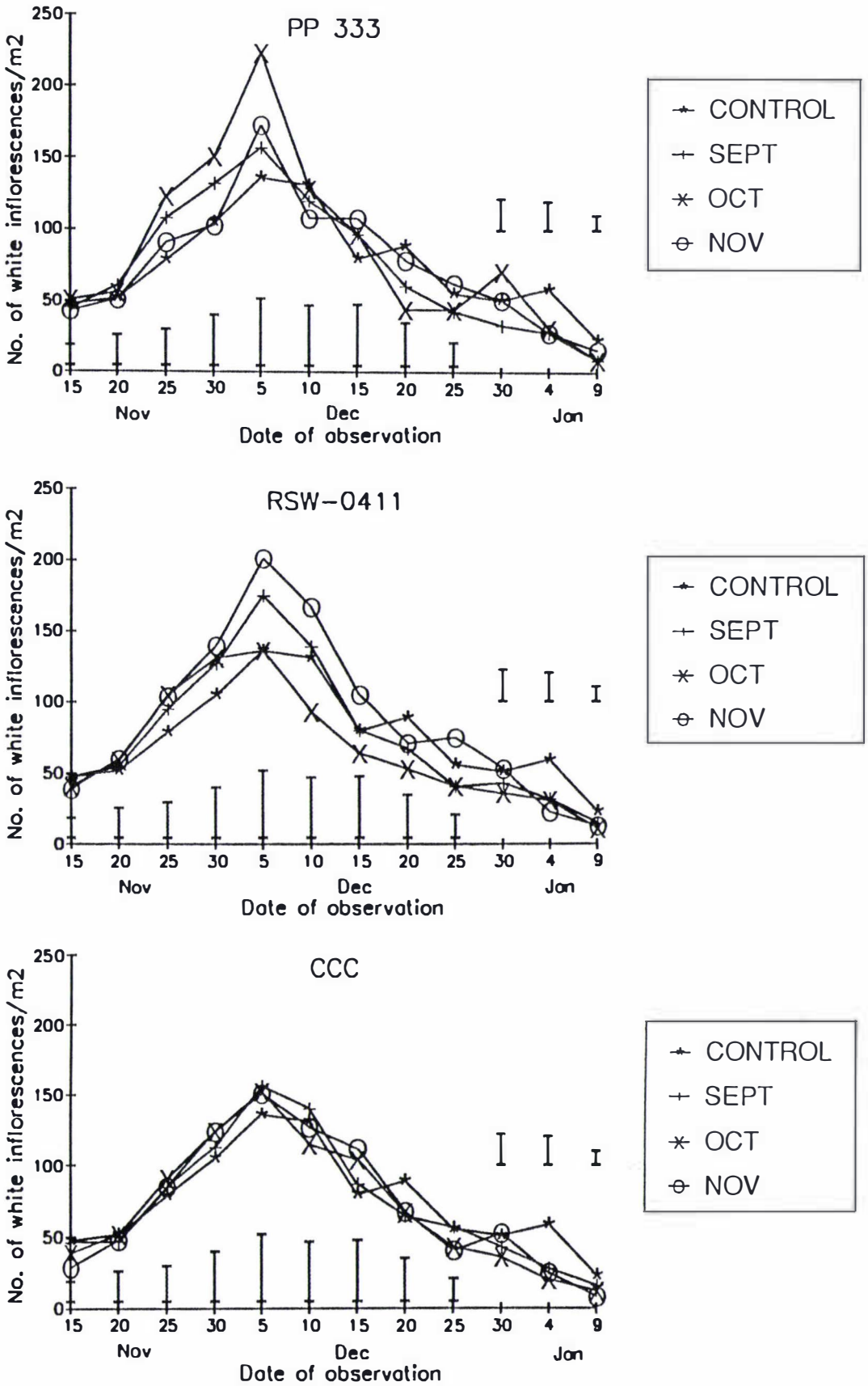


Figure 4.3. Effect of plant growth regulators on the flowering pattern of white clover cv. Grasslands Pitau.

Table 4.6. Effect of plant growth regulators on seed yield components for a second year crop of white clover cv. Grasslands Pitau.

Treatments	TSW (g)	Seeds/ Floret	Florets/ Inflore-scence	Inflorescences/m^2 Total	Ripe	Unripe no	Unripe %[1)]
Control	0.67*a*[2)]	3.9	69	588*b*	410	178*bc*	30
Paclobutrazol Sept	0.60*c*	3.9	65	671*ab*	449	222*ab*	33
Paclobutrazol Oct	0.62*bc*	4.1	64	650*ab*	456	194*abc*	30
Paclobutrazol Nov	0.61*bc*	4.0	66	750*a*	505	245*a*	33
Triapenthenol Sept	0.64*ab*	4.0	72	563*b*	373	190*bc*	34
Triapenthenol Oct	0.63*bc*	4.2	68	618*b*	460	158*c*	26
Triapenthenol Nov	0.64*ab*	4.2	65	673*ab*	448	225*ab*	34
Chlormequat chloride Sept	0.64*ab*	4.0	68	581*b*	421	160*c*	28
Chlormequat chloride Oct	0.67*a*	3.9	72	645*ab*	470	175*bc*	27
Chlormequat chloride Nov	0.64*ab*	4.1	73	579*b*	369	210*abc*	36
LSD (P=0.05)	0.03	NS	NS	119	NS	54	NS
CV (%)	3	5	6	11	14	16	16

Note: 1. Unripe inflorescences compared to total inflorescences
2. Values in the same column followed by the same letter are not significantly different according to Fisher's LSD test at P=0.05.

Plate 4.6. Plant's condition at harvest (Photographed on 3 January 1990, one day before harvest).

In this trial, both potential harvestable seed yield and actual seed yield were not affected by the application of plant growth regulators. Vegetative dry matter at harvest also did not differ significantly. Consequently, the harvest indices of both control and treated plants were not significantly different (Table 4.7). Seed losses at harvest were relatively high, ranging from 54 % to 65 %. Triapenthenol and chlormequat chloride applied in October, and paclobutrazol applied in November had greater losses than the control (Table 4.7).

As seed processing was done manually, the seed harvested from both control and treated plants showed a high level of hard seed, ranging from 88 % to 94 %. However, the seed from all treatments had high germination, i.e. more than 90 %, following scarification, and was significantly greater than that of the control (Appendix 4.2).

Table 4.7. Effect of plant growth regulators on potential harvestable seed yield, actual seed yield, seed loss and harvest index for a second year crop of white clover cv. Grasslands Pitau.

Treatments	Potential harvestable seed yield (g/m^2)	Actual seed yield (g/m^2)	Loss (%)	Vegetative dry matter (g/m^2)	HI[1)]
Control	73.4	33.5	54.3*d*[2)]	546.6	5.9
Paclobutrazol Sept	68.6	28.7	58.3*bcd*	546.7	5.2
Paclobutrazol Oct	72.6	33.4	54.3*d*	467.9	6.9
Paclobutrazol Nov	82.5	31.6	61.3*abc*	602.5	5.0
Triapenthenol Sept	69.1	30.9	54.7*cd*	532.4	5.5
Triapenthenol Oct	83.3	28.6	65.3*a*	519.0	5.3
Triapenthenol Nov	78.5	34.1	56.3*bcd*	530.3	6.1
Chlormequat chloride Sept	73.8	32.0	56.7*bcd*	569.7	5.3
Chlormequat chloride Oct	87.0	32.7	62.3*ab*	595.1	5.2
Chlormequat chloride Nov	70.5	31.1	56.3*bcd*	562.2	5.3
Mean	75.9	31.7	58.0	547.2	5.6
LSD (P=0.05)	NS	NS	6.9	NS	NS
CV (%)	18	17	7	16	20

Note: 1. $HI = \frac{\text{actual seed yield at 0 \% SMC}}{\text{total dry matter (vegetative + seed)}} \times 100\ \%$

2. Values in the same column followed by the same letter are not significantly different according to Fisher's LSD test at P=0.05.

4.4. DISCUSSION

In terms of growth retardation, white clover responded well to the application of paclobutrazol and, to a lesser extent, triapenthenol, but not chlormequat chloride. Petiole length was effectively retarded by paclobutrazol and triapenthenol (Table 4.2). However, triapenthenol was effective only when applied at an early growth stage (September), and not when applied later. The retardation effects lasted about three months for paclobutrazol, but were shortlived for triapenthenol (less than one month). In apple trees, Irving and Pallesen (1989) reported that paclobutrazol reduced growth for up to 82 days, while the effects of triapenthenol lasted between 27-40 days. This might explain why, in the previous experiment when petiole length was measured at peak flowering (Chapter 3), triapenthenol did not affect petiole length.

As in the previous experiment, chlormequat chloride did not produce any retardation effects in white clover. The results confirm those reported by Marshall and Hides (1986) and in other herbage legumes, e.g. *Trifolium pratense* L. (Niemeläinen, 1987) and *Lotus uliginosus* Schkuhr. (Tabora and Hampton, 1992), although chlormequat chloride has been shown to result in some early vegetative growth retardation in *Lotus corniculatus* L. (Supanjani, 1991). However, in wheat (Jung, 1984; Kust, 1986) and rice (Moody, 1986), chlormequat chloride is able to effectively shorten stems and prevent stem break or lodging, and thereby increase seed yield, although it is less effective in other cereals such as barley, oats and rye (Koranteng and Matthews, 1982; Woolley, 1982; Jung, 1984). Its response also varies in grasses, as it shortens stems in *Bromus* and *Dactylis* (J.G. Hampton, pers. comm.) but is not effective in reducing plant height and lodging in perennial ryegrass (Hampton, 1986) and Yorkshire fog (*Holcus lanatus* L.) (Tolentino, 1990; Hampton *et al.*, 1992)). Dicks (1980) suggested that chlormequat chloride might act by inhibiting gibberellin synthesis and also its conversion, the latter particularly at lower rates. Thus the rate of chlormequat chloride used in the present experiment (3 kg a.i./ha) might be still too low to reduce growth of white clover. Chlormequat

chloride actually increased plant growth, as shown by the increase of total dry matter accumulation at peak flowering, although this effect only occurred following the October application (Figure 4.1).

The previous and present results confirm that paclobutrazol is a plant growth regulator which consistently retards petiole length of white clover (Marshall and Hides, 1986, 1989, 1991a; Hampton, 1991; Rijckaert, 1991). However, in this experiment, despite there being no changes in the peduncle length, the reduction of petiole length following paclobutrazol application did not provide any advantages, as inflorescences from both the control and treated plants were elevated above the leaf canopy (Table 4.2). In addition, there were no significant increases in the number of seeds per floret at harvest following paclobutrazol treatments (Table 4.6) which might have been expected if better pollination had occurred. Regardless of application time, paclobutrazol also reduced leaf size, but only at early (during reproductive initiation) and late (at harvest) growth stages (Table 4.1). Hampton (1991) also reported that paclobutrazol applied in October did not reduce leaf area of cv. Grasslands Pitau white clover when measured at peak flowering. In this present second year crop, leaves were developed from young regrown stolons at an early growth stage and mostly from new young branches at a late growth stage, while at a middle (during the flowering period) growth stage, leaves were developed from mature stolons as indicated by the fact that the average size of leaves was generally larger than the average size of leaves at early and late growth stages (Table 4.1). This suggests that young regrown and newly formed stolons of cv. Grasslands Pitau might be more sensitive to paclobutrazol than mature stolons, with the result that leaf size was reduced in the former but not the latter.

Despite the fact there were changes in morphological characteristics following plant growth regulator applications, (particularly paclobutrazol), total dry matter generally was not affected (Figure 4.1). The results were similar to those in the previous experiment (Chapter 3). This again indicates that plant growth regulators alter plant growth, (especially paclobutrazol), without necessarily reducing plant

assimilate production, although alterations were different depending on growth stage, type of plant growth regulator and application time (Appendix 4.1). Despite these differences, both control and treated plants had a similar growth pattern. During the flowering and seed development stages, plants favoured assimilate partitioning to reproductive parts at the expense of vegetative parts as indicated by the fact that vegetative dry matter stopped increasing and declined at peak flowering and harvest respectively, while reproductive dry matter was increasing at these stages (Appendix 4.1). This behaviour was partly because it is impossible for a node to form an inflorescence and an axillary bud, which subsequently develops into a lateral stolon (Thomas, 1987c). Secondly, there is a possibility that while new vegetative parts were growing and developing, older vegetative parts started to die off during the reproductive stage, and that the number of dead vegetative parts were higher than the number of new vegetative parts.

Paclobutrazol applied in September and November produced a higher number of stolons as indicated by a significantly increased number of growing points (Table 4.3) and stolon dry matter at harvest (Appendix 4.1). However, the October application failed to do so. The reason for this is not clear. One explanation might be that the effects of paclobutrazol were influenced by light intensity. The number of sunshine hours in September and November was higher than average, while it was lower in October (Appendix 3.3). This suggest that paclobutrazol might more effectively suppress apical dominance, and hence the development of stolons, under sunny conditions than dull weather, or that stolon formation was inhibited by low light intensity (Beinhart, 1963). Another possibility is that October was the time when white clover started to flower, which naturally reduced the production of vegetative nodes (Figure 4.2). Thus, the application of paclobutrazol at this time perhaps had no effect on vegetative development.

Reproductive nodes along stolons (Table 4.5) and stolon numbers (Table 4.3) were increased by paclobutrazol applied in November which subsequently resulted in significantly greater inflorescence numbers at harvest (Table 4.6). This agrees with the findings of Marshall and Hides (1991a,b). On the other hand, the October application failed to do so, although it increased inflorescences during peak flowering (Figure 4.3). In the case of the November application, the findings might explain the mechanism of increased inflorescences. The increases perhaps were caused by paclobutrazol being directly involved at a hormonal level to enhance reproductive initiation, as indicated by a higher number of reproductive nodes along stolons, or by increasing stolon numbers, hence providing more sites for inflorescence production. This latter theory was proposed by Marshall and Hides (1986). In the case of the former, however, Cohen and Dovrat (1976) reported that inflorescences of white clover cv. Tamar (a low latitude cultivar) were increased by the application of GA_3, and Thomas (1987c) also found similar increases in clones of several high latitude cultivars following GA_3 application. Thus, the effect of paclobutrazol in this experiment was in contrast with these findings, since paclobutrazol apparently interferes with gibberellin biosynthesis, resulting in reduced gibberellin levels within treated plants (Schott *et al.*, 1984; Burden *et al.*, 1987; Hedden, 1990). Roberts *et al.*, (1991) reported that a decline in gibberellins (primarily GA_1 and GA_3) occurred prior to flower bud initiation in *Boronia megastigma* Nees. plants. These conflicting results suggest that plant hormones other than gibberellins might be involved in inflorescence initiation. The increase in inflorescence numbers during peak flowering following the October application is difficult to explain. Since this treatment did not affect stolon numbers or the development of inflorescences along stolons, one possible explanation might be that the October application increased the number of stolons bearing reproductive nodes (fertile stolons). In *Lotus uliginosus* Schkuhr., Clifford and Hare (1987) and Tabora (1991) reported that paclobutrazol increased the number of reproductive shoots. However, Supanjani (1991) found no differences in *Lotus corniculatus* L. In addition, the September application increased the number of growing points, but not

the number of inflorescences. Further investigations are needed to clarify the possible mechanism(s) of increased inflorescence production following paclobutrazol treatment.

Triapenthenol effectively altered plant development when applied at an early growth stage, (September), by reducing vegetative development per unit area and along stolons (Figure 4.2 and Table 4.5). However, the effects had no advantage as the number of reproductive nodes were not affected (Table 4.5). On the other hand, the November application increased the number of inflorescences at peak flowering significantly (Table 4.4 and Figure 4.3). This increase was probably caused by a significant increase in reproductive nodes along stolons (Table 4.5) and/or increased reproductive stolons (as discussed earlier), as the treatment did not affect the number of growing points (Table 4.3). This also perhaps explains why reproductive dry matter was significantly increased by triapenthenol at peak flowering, despite there being no changes in vegetative dry matter (Appendix 4.1). Triapenthenol seems likely to be involved directly in reproductive initiation at a hormonal level. However, this matter is still uncertain, as there are reports indicating that GA_3 is involved in reproductive initiation in white clover (Cohen and Dovrat, 1976; Thomas, 1987c), while triapenthenol interferes with gibberellin biosynthesis and hence reduces gibberellin levels within treated plants (Lürssen and Reiser, 1985; Hedden, 1990). Thus, like the results following paclobutrazol November application discussed previously, this matter needs to be further investigated.

Chlormequat chloride is not likely to be a plant growth regulator that could be used to manipulate white clover growth and development. In the absence of lodging, Koranteng and Matthews (1982) reported that seed yields in barley were increased following chlormequat chloride application due to mainly increased tillering and tiller survival. Increased tillering is presumably due to a reduction in apical dominance by chlormequat chloride (Jung, 1984). However, the development of stolons and also nodes along stolons (sites for inflorescence production) in white clover was not affected by chlormequat chloride (Tables 4.3 and 4.5). It also did not

increase the development of shoots in *Lotus corniculatus* L. (Supanjani, 1991) and *Lotus uliginosus* Schkuhr. (Tabora and Hampton, 1992). As a result, it is not surprising that the present study and studies by Mohamed (1981), Marshall and Hides (1986) and Boelt (1991) produced results which showed that chlormequat chloride had no effect on white clover seed yield. In other herbage legumes, e.g. *Trifolium pratense* L. (Niemeläinen, 1987) and *Lotus corniculatus* L. (Supanjani, 1991), chlormequat chloride similarly did not improve seed yield. However, Tabora and Hampton (1992) reported increased seed yields in *Lotus uliginosus* Schkuhr. through enhanced pods per umbel and seeds per pod. Chlormequat chloride also increased seed yield in *Lolium perenne* L through increased seeds/spikelet (Hampton, 1986), while in cereals, increases in seed yield mainly by preventing lodging and increasing the number of fertile tillers have been reported (Koranteng and Matthews, 1982; Jung, 1984; Kust, 1986). This inconsistency of chlormequat chloride effects in different crops suggests that it may be species specific.

In 1989/90, plant growth regulators again had no effects on the flowering duration of white clover (Figure 4.3). The flowering duration of this second year crop lasted about two months, which was similar to the first year crop in the previous experiment. However, there were differences in the number of flowering peaks. Only one peak occurred in the second year crop, while there were two peaks in the previous first year crop. These differences might be because of weather conditions. The peak flowering of the second year crop and the first flowering peak of the first year crop occurred in the first week of December, while the second flowering peak in the first year crop occurred in late December. Temperature was warmer than average in December 1988 but cooler in December 1989 (Appendix 3.3). Higher temperatures in long days are found to favour inflorescence initiation in cultivars showing a strong long day response, e.g. Ladino (Ridley and Laude, 1968) and Grasslands Huia (Thomas, 1980a). As cv. Grasslands Pitau was bred by crossing cv. Grasslands Huia and a Spanish ecotype (Appendix 3.2), it seems possible that cv. Grasslands Pitau produced a second flowering peak in the 1988/1989 trial in response to higher temperatures in long days in December 1988.

In addition, the number of sunshine hours was also higher than average in December 1988, but lower in December 1989 (Appendix 3.3). The higher number of sunshine hours may also have contributed to a production of a second flowering peak in the 1988/1989 trial, as high light intensity in sunny days is a condition which is favourable for inflorescence production (Thomas, 1987c).

The one well defined peak flowering in this trial was useful for calculating harvest date. Based on the time frame of the white clover seed development process, i.e. 26 ± 5 days (Hyde, 1950; Hyde *et al.*, 1959), harvest was conducted 30 days after peak flowering. However, the results were disappointing. Despite inflorescence numbers at peak flowering being increased by the paclobutrazol October and triapenthenol November applications (Table 4.4), the total number of inflorescences harvested was not significantly increased, with the exception of paclobutrazol applied in November (Table 4.6). In contrast, Hampton (1991) reported that an October application of paclobutrazol significantly increased the number of harvested inflorescences for cv. Grasslands Pitau, while the November application failed to do so. Marshall and Hides (1986) have also reported no increase in inflorescences following paclobutrazol application in cv. Olwen, and Rijckaert (1991) reported that paclobutrazol caused a reduction of inflorescences in cv. Merwi. The conflicting results in this experiment might have been caused by inflorescence shattering (Plate 4.7). Nevertheless, all paclobutrazol treatments produced a higher total number of harvested inflorescences than the control, although only the November application was significant (Table 4.6). However, almost one third of the inflorescences harvested were unripe (Table 4.6). This suggests that the number of inflorescences shattering after the ripening stage were less than the number of late inflorescences produced, but that harvesting 30 days after peak flowering was perhaps too late to gather early and middle inflorescences in paclobutrazol treated plants. In *Lotus corniculatus* L., Supanjani (1991) reported that early inflorescences retained only 39%-52% live umbels at 30 days after anthesis, and that the application of paclobutrazol further reduced umbel survival in early inflorescences. In addition, in terms of seed moisture content levels, inflorescences from paclobutrazol treated

Plate 4.7. White clover inflorescences showing different stages of maturity

plants ripened earlier than those from control plants (Chapter 5A), and subsequently might have shed earlier. These possibilities perhaps also occurred in the triapenthenol November application. This shedding aspect needs further investigation.

While there were no changes in either the number of florets per inflorescence or the number of seeds per floret, all paclobutrazol treatments and triapenthenol applied in October reduced thousand seed weight (Table 4.6). This reduction might be because inflorescences harvested were not fully mature. However, this seemed unlikely since the percentage of unripe inflorescences harvested in these treatments did not differ from the control (Table 4.6), and there were no differences in either reproductive node composition (Table 4.4) or in the date of peak flowering (Figure 4.3). Furthermore, the thousand seed weight data recorded were still within the range of thousand seed weight recorded for cv. Grasslands Pitau by Clifford (1986a). Clifford (1986a) has also reported that floral expression imposes additional competition for nutrients, at the expense of fertilization and subsequent provisioning levels. As total dry matter (Figure 4.1) and seeds per floret (Table 4.6) were not different, but the number of inflorescences was increased at peak flowering (Table 4.4 and Figure 4.3), this suggests that a more likely cause of the seed weight reduction was competition for assimilate available for seed provisioning among and within inflorescences.

As a consequence of the plant growth regulator effects on yield components already discussed, both potential and actual seed yield were unaffected following plant growth regulator application (Table 4.7). However, irrespective of treatment, average actual seed yield was about 317 kg/ha. This was a reasonable yield for a second year crop, considering the average seed yield of cv. Grasslands Pitau is only 300 kg/ha (Appendix 3.2), and climatic conditions in the 1989/1990 growing season (Appendix 3.3), were not entirely favourable for flowering and seed development (dull and cool weather). These results are less conclusive than those reported by Rijckaert (1991), where paclobutrazol and triapenthenol gave the highest increases

in white clover seed yield (by 271% and 145% respectively), when they were applied under less favourable climatic conditions in Belgium. However, in this case, the control seed yield was only 124 kg/ha. Nevertheless, although seed yields were not improved, all plant growth regulators used improved seed quality as indicated by results from germination testing, in which they increased normal seedlings by reducing abnormal seedlings (Appendix 4.2). Marshall and Hides (1991b) also reported that paclobutrazol increased the germination of seeds of white clover cvs. Menna and Olwen. These results, as in the 1988/1989 trial, further indicate that plant growth regulators do not reduce seed quality.

Despite inducing some changes in growth and development, plant growth regulators failed to consistently increase potential and actual seed yields in a second year crop. The reason why paclobutrazol and triapenthenol, despite increasing inflorescences at peak flowering did not significantly increase seed yields, requires further investigation.

CHAPTER 5

EFFECT OF PACLOBUTRAZOL ON THE VEGETATIVE AND REPRODUCTIVE GROWTH AND DEVELOPMENT OF INDIVIDUAL PLANTS OF WHITE CLOVER CV. GRASSLANDS PITAU PROPAGATED BY SEED AND BY CLONAL PROPAGATION

This chapter deals with two years of experiments on the effects of paclobutrazol on the vegetative and reproductive growth and development of individual white clover plants. The introduction, materials and methods, and results for each year's experiment are presented separately, while the discussion for the two experiments is combined in one section.

5A. THE 1990/1991 TRIAL - INDIVIDUAL PLANTS FROM SEED

5A.1. INTRODUCTION

Studies on the effect of plant growth regulators on the reproductive growth and development of cv. Grasslands Pitau white clover under sward conditions over two consecutive growing seasons (1988/1989 and 1989/1990) showed that paclobutrazol had the potential to increase seed yield by improving the inflorescence production capacity of the plant. In a first year crop, paclobutrazol (1.0 kg a.i/ha) applied either during reproductive initiation or at the appearance of the first reproductive bud increased potential harvestable seed yield by 71 % and 57 % respectively (Chapter 3). Although not statistically significant, actual seed yield was also increased 25-26 % by both treatments. These increases were solely attributed to the significant increase in the number of inflorescences produced, as the other seed yield components (florets/inflorescence, seeds/floret and seed weight) were not affected. This finding was in agreement with the recent results reported by Marshall and Hides (1989, 1991b) and Hampton (1991). On the other hand, paclobutrazol did not

increase either potential harvestable seed yield or actual seed yield in a second year crop (Chapter 4). These contradicting results were perhaps not surprising as Marshall and Hides (1986) also reported no significant effects of paclobutrazol in white clover in an earlier trial. However, paclobutrazol still significantly increased the number of inflorescences (ripe and unripe) in the second year crop.

As the number of inflorescences produced per unit area is considered the most significant component for increasing seed yield in white clover (Clifford, 1987), the results above lead to the conclusion that paclobutrazol is still worth further investigation; in particular the mechanism of paclobutrazol for increasing inflorescence numbers, to see if the contradictory results can be explained. This should allow a general conclusion for the use of paclobutrazol in white clover to be drawn.

This experiment was therefore conducted with the following objectives:

1. To investigate the effect of paclobutrazol on vegetative and reproductive growth of individual plants propagated from seed, which represent a random but small population of cv. Grasslands Pitau white clover.

2. To investigate the possible ways by which inflorescence numbers may be increased by paclobutrazol.

5A.2. MATERIALS AND METHODS

5A.2.1. Experimental site, management and treatments

The experiment was conducted on the nursery block of the Pasture and Crop Research Unit, Massey University, Palmerston North during the 1990/1991 growing season. The site selected had not been previously used for any growth regulator experiments. The soil type was a Tokomaru silt loam. A full description of the soil is presented in Appendix 3.1B. Soil fertility analysis of the site is presented in Appendix 5A.1.

White clover plants cv. Grasslands Pitau were grown from breeders seed (Grasslands No. C5890) supplied by AgResearch Grasslands, Palmerston North. Four seeds were sown per Jiffy 7 peat pellet on 23 June 1990 and placed inside an heated glasshouse (20°/15°C). Seed emergence occurred a week later. Thinning was conducted to obtain one healthy plant/peat pellet and the plants were defoliated to 5 cm height on 4 September 1990. All plants were then brought outside for hardening for one week before transplanting into the field on 11 September 1990.

No field cultivation was applied. The field had been cleared from weeds on 7 September 1990 using the herbicide Roundup (glyphosate) at a rate of 3.6 kg a.i/ha or 10 l Roundup/ha. Roundup is a non-selective herbicide for the control of most annual and perennial grass and broadleaf weeds (Anon., 1990). It is active only through green plant tissue but translocates from the foliage to all parts of the plant, including the roots. It is inactivated on soil contact and therefore has no residual activity. 57 plants were transplanted directly into the ground using a square planting of 80 cm and nine plants were transplanted into pots sunken into the ground. Each of the pots had a volume of 20 l with drainage holes and contained top soil obtained from an area adjacent to the field used in this trial. Nitrogen was applied on 28 September 1990 at a rate of 46 kg N/ha or 100 kg urea/ha. Irrigation was conducted during the first month after transplanting with a volume of 2 l water/plant/day for

the first 15 days and 30 minutes of sprinkler irrigation/day using a garden sprinkler which delivered water at a rate of 10 l/m^2/hour for the last 15 days. During the growing period, weeds were controlled by hand weeding. Mesurol (methiocarb) was applied at a rate of 200 g a.i/ha or 10 kg Mesurol pellets/ha on 15 September 1990 to control slugs and snails. Mavrik Aquaflow (fluvalinate) insecticide was applied on 16 November 1990 at a rate of 36 g a.i/ha or 150 ml Mavrik Aquaflow/ha to control caterpillars. Mavrik Aquaflow is not toxic to honey bees.

Three paclobutrazol treatments were applied: 1 kg a.i/ha applied either during reproductive initiation (6 November 1990) or when the reproductive buds became visible (23 November 1990), and nil paclobutrazol as a control. Reproductive development was determined by dissecting one growing point/plant under a microscope at 40X magnification every week starting in early October 1990. A definition and indication of reproductive initiation is presented in section 3.2.1. Reproductive initiation (6 November 1990) was when more than 75 % of the plants were initiating reproductive buds at their terminal buds. At 23 November 1990 more than 50 % of the plant population had reproductive buds emerging on their stolons. For further reference, the 6 and 23 November applications have been designated as paclobutrazol 1 and paclobutrazol 2 respectively. Paclobutrazol was applied with water at a volume equivalent to 500 l/ha using a knapsack sprayer with single fan nozzle covered by an upper shield and held 25-30 cm above the plant canopy. The plant and surrounding soil within an area of 80 X 80 cm were covered by paclobutrazol during the application. 22 plants received the paclobutrazol 1 treatment, 19 plants received the paclobutrazol 2 treatment and 25 plants were untreated.

This trial utilized a completely randomized design using an individual plant as a replicate and for some parameters in plant growth analysis, an individual leaf and an individual inflorescence were used as a replicate. 24 plants (8 plants/treatment) were allocated for stolon growth, seed yield components and seed yield analysis, 15 plants (5 plants/treatment) were allocated for inflorescence growth and development

analysis and 18 plants (3 plants/treatment at every growth stage) were allocated for destructive plant growth analysis. In addition, 9 plants grown in pots (3 plants/treatment) were used for root:shoot ratio analysis.

5A.2.2. Plant measurements and statistical analysis

Plant growth analysis was conducted at four stages of growth:

- The first growth analysis was done during reproductive initiation (5 November 1990). Three untreated plants were harvested and used for the analysis before treatment paclobutrazol 1 was applied.
- The second growth analysis was conducted when reproductive buds became visible (22 November 1990). Plant samples were harvested before treatment paclobutrazol 2 was applied. Three untreated and three paclobutrazol 1 treated plants were used for this analysis.
- The third growth analysis was conducted during peak flowering (31 December 1990). Nine plants (3 from each treatment) were used for this analysis.
- The fourth growth analysis was conducted when most inflorescences were ripe (i.e. at harvest). 24 plants (8 from each treatment) were used for the analysis. Because the flowering period of every individual plant was not similar, plant samples were not harvested at the same time. However, most of the plants were harvested in late February 1992.

At each stage, leaf and stolon dry matter was measured following drying in an oven at a temperature of 80°C for two days. Reproductive dry matter was also measured at peak flowering and harvest. At peak flowering, reproductive bud and inflorescence dry matter were recorded, while at harvest, seed yield at 0 % SMC and peduncle and inflorescence chaff dry matter were recorded. Measurements were also taken at each stage for petiole length and leaf score (Williams *et al.*, 1964) from 60 randomly selected mature unfolded leaves/treatment, although at harvest 160 leaves/treatment were measured. Peduncle length was measured from 60

inflorescences/treatment selected at random at peak flowering and 160 inflorescences/treatment at harvest. In addition, root:shoot ratio was analyzed at harvest using the 9 plants grown in pots. Roots were separated from shoots and carefully collected by washing the soil from them. Root and shoot dry weights were determined.

Stolon growth analysis was conducted using 24 plants on which one main stolon developed in August 1990 was tagged. Tagging began in September 1990 and continued at monthly intervals up to harvest. Tagging was carried out by placing coloured wire at the position immediately behind the node bearing the youngest unfolded leaf. Different coloured wire was used for each tagging. The following measurements were taken from tagged stolons:

- number of nodes emerging from the terminal bud
- number of inflorescences formed along main stolons
- number of secondary, tertiary, quaternary and quinary branches and number of inflorescences formed on those branches
- total inflorescences produced from a whole main stolon (a main stolon and its branches)
- final stolon length

At final harvest, the composition of total stolons produced/plant was determined using criteria defined by Thomas (1987a), and presented in section 2.1 of the literature review. Because harvest was not done at the same time for every individual plant, the composition of different stolon categories is presented both in real values and percentages to give a clear picture of the comparison between treatments.

The effects of paclobutrazol on leaf chlorophyll content per unit area were non-destructively measured using a portable chlorophyll photometer (Chlorophyllometer) developed by the then Plant Physiology Division, DSIR, now HortResearch, CRI (Hardacre *et al.*, 1984). The Chlorophyllometer has two range settings, i.e. low

range, which is accurate for reading chlorophyll concentrations between 9 and 36 $\mu g/cm^2$, and high range, which is accurate for reading chlorophyll concentrations between 26 and 89 $\mu g/cm^2$. The Chlorophyllometer was set at the high range reading as white clover leaves contained high chlorophyll content, i.e. > 50 $\mu g/cm^2$ (detected during calibration). 24 plants were used for the examination. Measurements were taken from 10 mature unfolded leaves/plant by clamping the leaf sample between the spring-loaded jaws of the Chlorophyllometer handpiece and recording the display reading. The measurements were done at weekly intervals in the afternoon (4-6 pm) during the intensive flowering period from 13 December 1990 to 25 January 1991. The thickness of the leaf samples were also measured at the same time using a micrometer. Because calibration curves were available only for maize and kiwi fruit, the meter was calibrated by placing 5 mm diameter white clover leaf disks between the jaws of the meter handpiece and recording the display reading. The leaf disk area was calculated and chlorophyll concentration was determined using N,N-Dimethylformamide (DMF) extraction (Moran, 1982) to obtain chlorophyll content per unit area. 80 leaf disks from a wide range of leaf growth stages or ages were used to form the calibration curve.

The effects of paclobutrazol on individual inflorescence growth were also determined. Measurements were conducted at two inflorescence growth stages:

- from bud appearance to anthesis
- from anthesis to ripening stage

The first measurement was done by tagging all reproductive buds from 5 plants/treatment, and then retagging them at anthesis (Plate 5A.1). The average time needed (days) from first tagging to second tagging in each plant was calculated and used as a unit for comparison. The ripening stage of white clover seed development lasts 26 ± 5 days and is indicated by the decline of seed moisture content down to around 10 % SMC (Hyde, 1950). Based on this, the effects of paclobutrazol at the second stage were determined by measuring the moisture content of seeds from one

inflorescence at three different times: 22, 24 and 26 days after anthesis or the second tagging. 25 inflorescences/treatment at each time were used for this determination. Inflorescences were harvested in the afternoon (4-6 pm). Seeds were immediately extracted from every individual inflorescence and their moisture contents were measured using an oven method at a temperature of 130°C for one hour (ISTA, 1985).

Inflorescence production was determined by harvesting every single mature inflorescence from the 24 plants used for stolon growth analysis. Actual seed yield was obtained from these inflorescences using the method described in section 3.2.2, and is expressed at 10 % SMC. Other seed yield components (florets/inflorescence, seeds/floret and thousand seed weight) were measured at harvest. Florets/ inflorescence were determined from 20 inflorescences/plant. Seeds/floret were examined from 100 florets/plant using an X-ray method as described in section 3.2.2. Thousand seed weight (TSW) was calculated by weighing 4 X 100 seeds/plant and is expressed at 10 % SMC.

Data collected in this experiment were analyzed according to a completely randomized design analysis by the use of analysis of variance and Fisher's LSD test at P=0.05 to detect any differences between treatments.

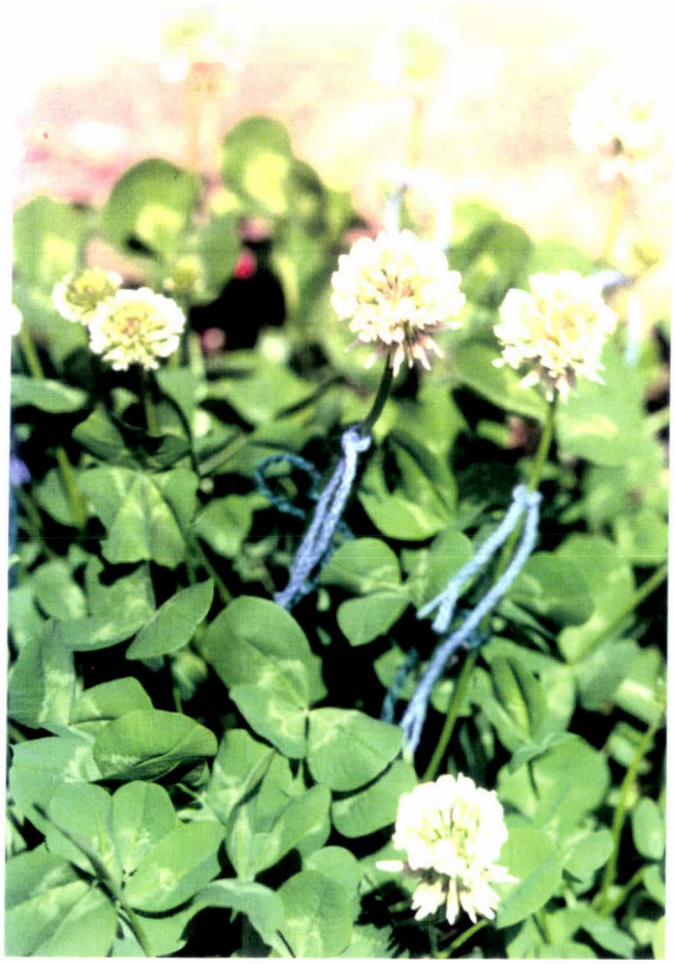


Plate 5A.1. Tagged inflorescences at anthesis.

5A.3. RESULTS

5A.3.1. Meteorological conditions

During transplanting and establishment (September), temperatures were cooler than average with deviations from minimum and maximum temperatures of - 0.9°C and - 0.3°C respectively. Throughout the rest of the trial period (October-March), temperatures were warmer than average, with the exception of the maximum temperature in December (Appendix 5A.2). The 1990/1991 growing season was a dull season as the number of sunshine hours was lower than average, except in December and March (Appendix 5A.2). During early growth (September and October), rainfall was below average, but higher in November (reproductive initiation). In December (flowering) rainfall again was below average, while the rest of the trial period, with the exception of March, was wet (Appendix 5A.2).

5A.3.2. Effect of paclobutrazol on plant growth and development

5A.3.2.1. Morphological characteristics

The growth of the leaf, petiole and peduncle was significantly retarded following paclobutrazol application both during reproductive initiation and at reproductive visible bud (Table 5A.1). However, there was a visual indication, particularly in treatment paclobutrazol 1, that paclobutrazol lost its retardation effects within two months after application. The leaf size (shown as leaf score in Table 5A.1) and petiole and peduncle lengths of paclobutrazol 1 treated plants did not differ from the control by peak flowering (approximately two months after application). However, the retardation effects occurred again at harvest (approximately four months after application). At two months after the paclobutrazol 2 treatment, leaf size, petiole and peduncle lengths were not measured, but the treatment reduced the growth of these parameters at harvest (approximately 3.5 months after application). Regardless of the treatment effects,

the growth patterns of the leaf and petiole of individual plants were incremental. Leaves and petioles increased their average size and length respectively up to harvest.

Table 5A.1. Effect of paclobutrazol on leaf score and petiole and peduncle lengths

	Leaf score				Petiole length (mm)				Peduncle length (mm)	
	Stage				Stage				Stage[1]	
	1	2	3	4	1	2	3	4	3	4
Control	17.0	18.8	20.2	21.4	79	92	148	240	206	220
Paclobutrazol 1		17.5	20.5	19.6		86	154	192	226	199
Paclobutrazol 2			19.6	19.9			115	191	176	177
LSD (P=0.05)		0.4	0.4	0.3		5	11	13	17	7
CV (%)		6.4	6.0	7.1		15	22	28	23	16

Note: 1. Stage 1 = reproductive initiation, stage 2 = reproductive visible bud, stage 3 = peak flowering and stage 4 = harvest.

5A.3.2.2. Chlorophyll content

Following the applications, the leaves of paclobutrazol treated plants were darker than the control (e.g. Plate 5A.2). The measurements of leaf chlorophyll content indicated that paclobutrazol significantly increased leaf chlorophyll content per unit area during the first three weeks of intensive flowering, which was between one to two months after application (Table 5A.2). The effects lasted until mid January. Similarly, during the first three weeks of intensive flowering, the leaves of paclobutrazol treated plants were thicker than the control, while in the fourth week there was no significant differences (Table 5A.2). However, there was no correlation between leaf thickness and leaf chlorophyll content.

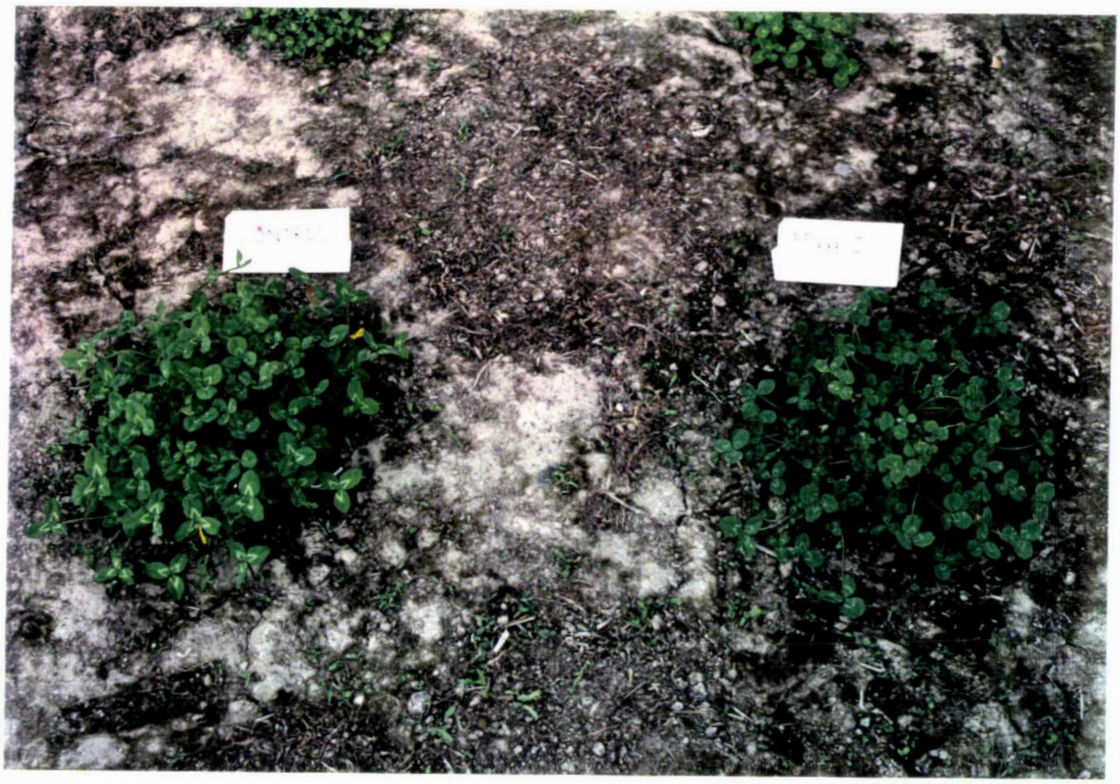


Plate 5A.2. Leaf colour change following paclobutrazol application (Photographed three weeks after application).

Table 5A.2. Effect of paclobutrazol on leaf chlorophyll content and leaf thickness

	13 Dec 1990		27 Dec 1990		19 Jan 1991		25 Jan 1991	
	CC ($\mu g/cm^2$)	LT (mm)	CC ($\mu g/cm^2$)	LT (mm)	CC ($\mu g/cm^2$)	LT (mm)	CC ($\mu g/cm^2$)	LT[1] (mm)
Control	63.10	0.183	62.99	0.186	68.06	0.165	73.85	0.178
Paclobutrazol 1	67.90	0.226	67.38	0.216	71.65	0.180	76.01	0.181
Paclobutrazol 2	66.37	0.204	67.42	0.210	71.70	0.174	75.43	0.179
LSD (P=0.05)	1.95	0.018	3.17	0.016	2.63	0.012	NS	NS
CV (%)	2.9	8.5	4.6	7.5	3.6	6.6	3.0	6.1

Note: 1. CC = chlorophyll content and LT = leaf thickness.

5A.3.2.3. Dry matter accumulation and distribution

The accumulation of leaf and stolon dry matter at reproductive visible bud was not significantly affected by paclobutrazol (Table 5A.3). Similarly, paclobutrazol did not affect the accumulation of leaf, stolon reproductive and dead dry matter at peak flowering (Table 5A.3). However, paclobutrazol significantly reduced leaf and dead dry matter at harvest, while stolon and reproductive dry matter were not affected (Table 5A.3).

When measured at harvest, the root:shoot ratio of plants was not significantly affected following paclobutrazol applications. The ratios of root and shoot were 11 : 89, 10 : 90 and 13 : 87 for control, paclobutrazol 1 and paclobutrazol 2 respectively.

Table 5A.3. Effect of paclobutrazol on leaf, stolon, reproductive and dead dry matter (g/plant)

	Initiation		Bud visible		Peak flowering				Harvest			
	Leaf	Stolon	Leaf	Stolon	Leaf	Stolon	Repro	Dead	Leaf	Stolon	Repro	Dead
Control	1.89	0.70	5.04	2.54	67.25	57.62	15.15	9.79	302.73	279.26	64.38	141.30
Paclobutrazol 1			3.75	1.89	60.30	58.55	38.25	13.51	179.27	195.14	35.60	55.73
Paclobutrazol 2					69.76	60.25	23.62	10.20	159.21	164.34	49.01	73.36
LSD (P=0.05)			NS	NS	NS	NS	NS	NS	114.25	NS	NS	66.00
CV (%)			37	39	38	20	60	72	51	58	97	70

5A.3.2.4. Growth and development of main stolons

There was no significant effect on the structure of developed main stolons following paclobutrazol applications (Plate 5A.3). Paclobutrazol also did not significantly affect the number of branches (secondary, tertiary, quaternary and quinary branches) and axillary buds formed on main stolons (Table 5A.4). However, the size of untreated plants was greater than treated plants as the final stolon length was significantly reduced by paclobutrazol (Table 5A.5). The number of nodes emerging from terminal buds and the development of inflorescences along main stolons were not significantly affected by paclobutrazol (Table 5A.5).

Table 5A.4. Effect of paclobutrazol on main stolon branch development

	Secondary branches	Tertiary branches	Quaternary branches	Quinary branches	Axillary buds
Control	22	110	49	4	11
Paclobutrazol 1	25	102	54	2	12
Paclobutrazol 2	19	91	47	2	9
LSD (P=0.05)	NS	NS	NS	NS	NS
CV (%)	34	62	121	165	49

Secondary and tertiary branches of main stolons developed inflorescences, but not the quaternary and quinary branches. However, paclobutrazol did not significantly affect the total number of inflorescences produced by total secondary and tertiary branches, with the result that there was no difference in the total inflorescences produced on a whole developed main stolon (Table 5A.5).

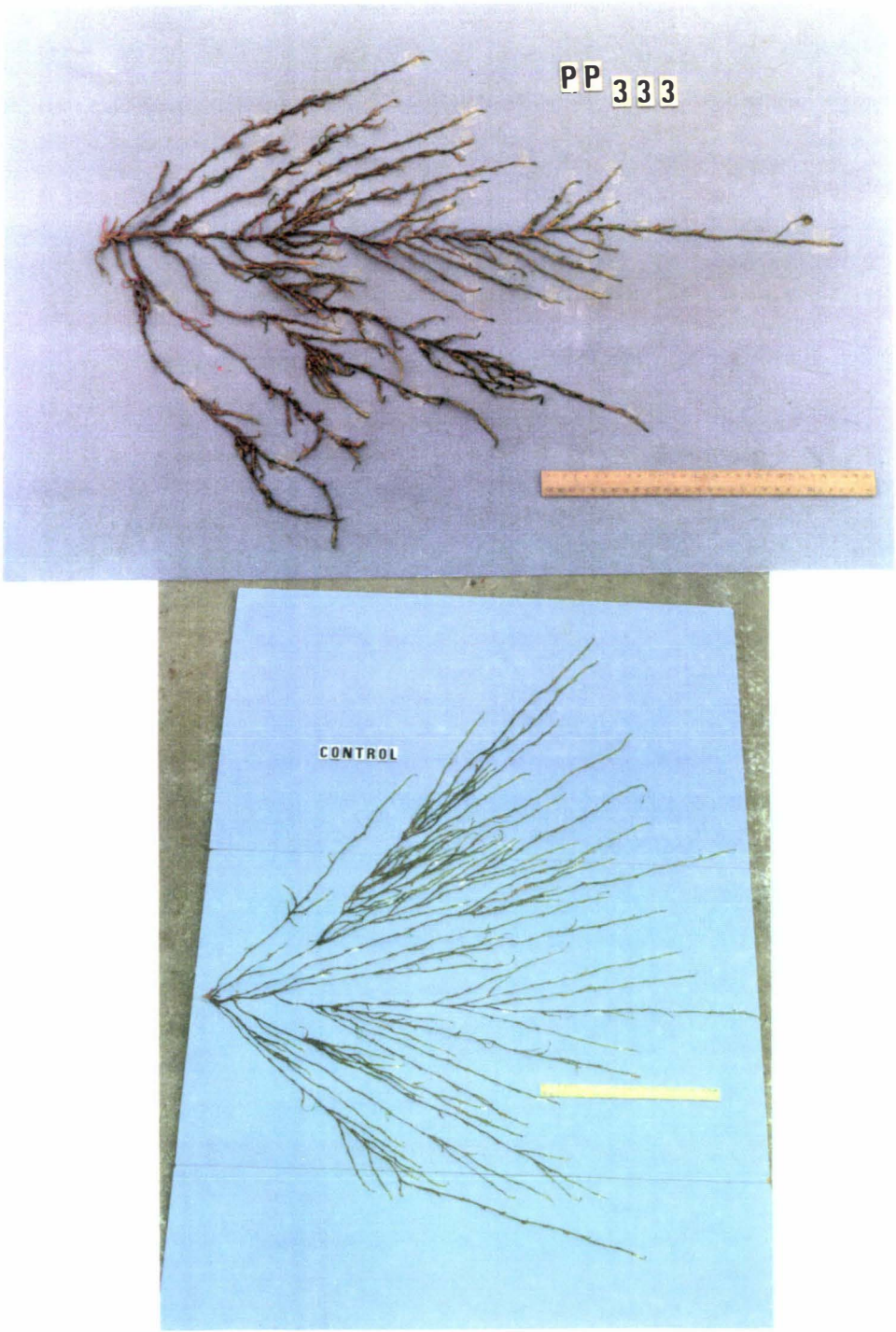


Plate 5A.3. Structure of developed main stolons for both control and paclobutrazol treated main stolons (Photographed at harvest).

Table 5A.5. Effect of paclobutrazol on stolon length, nodes and inflorescences formed on main stolons, and inflorescences developed on total secondary branches, total tertiary branches and a whole developed main stolon.

	main stolon length (cm)	number of nodes	inflorescence numbers			
			along MS	from total SB	from total TB	from a whole MS[1]
Control	86	41	1.9	10.9	1.7	14.5
Paclobutrazol 1	62	46	4.5	13.4	3.0	20.9
Paclobutrazol 2	62	38	3.3	10.4	3.1	16.8
LSD (P=0.05)	20	7	NS	NS	NS	NS
CV (%)	27	15	76	113	178	107

Note: 1. MS = main stolon, SB = secondary branch and TB = tertiary branch.

5A.3.2.5. Total number and proportion of whole plant stolons at harvest

Paclobutrazol applied at reproductive initiation significantly reduced total main stolon numbers, but it did not affect total lateral stolon and lateral branch numbers (Table 5A.6). However, when the numbers were converted into percentages of the total per plant, paclobutrazol applied at reproductive initiation also significantly reduced the proportion of main stolons but significantly increased the proportion of lateral branches. There was no significant effect on the proportion of lateral stolons. Paclobutrazol applied at reproductive visible bud did not differ from the control both in the number and proportion of different stolon categories of a whole plant.

Table 5A.6. Effect of paclobutrazol on the total number and proportion of lateral branches, lateral stolons and main stolons at harvest.

	Lateral branches		Lateral stolons		Main stolons	
	no/plant	%	no/plant	%	no/plant	%
Control	799	69.1	277	23.6	88	7.3
Paclobutrazol 1	689	75.7	193	19.7	47	4.6
Paclobutrazol 2	664	72.9	187	20.1	59	6.9
LSD (P=0.05)	NS	4.7	NS	NS	39	2.2
CV (%)	41	6.3	51	18	57	33

5A.3.3. Inflorescence growth

The growth of reproductive buds from emergence to anthesis was not affected by paclobutrazol. The average time from emergence to anthesis was 13.7, 13.6 and 13.9 days for control, paclobutrazol 1 and paclobutrazol 2 respectively. However, inflorescences from paclobutrazol treated plants ripened earlier than the control as indicated by significant differences in the moisture content of total seeds per inflorescence (Figure 5A.1).

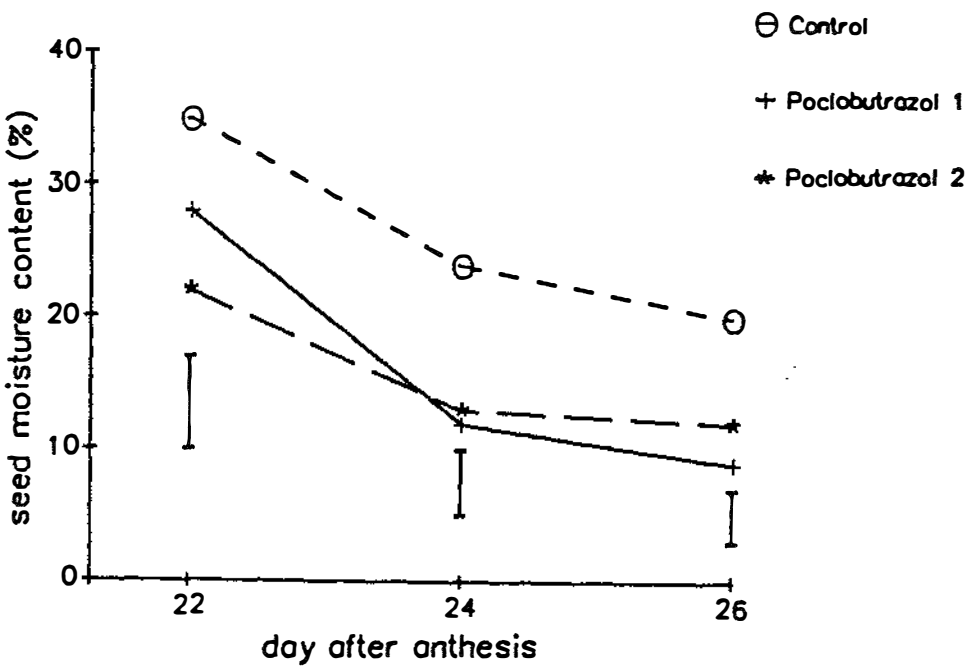


Figure 5A.1. Effect of paclobutrazol on seed moisture content during seed development.

5A.3.4. Seed yield components and seed yield

Seed yield components are presented in Table 5A.7. There was no significant difference in total inflorescence numbers produced per plant following paclobutrazol applications. However, the capacity of the plants to produce inflorescences varied extremely, ranging from 0 to more than 300 inflorescences/plant for both treated and control plants. Florets/inflorescence, seeds/floret and thousand seed weight were also not affected by paclobutrazol. As a result, seed yield from paclobutrazol treated plants did not differ from the control (Table 5A.7).

Table 5A.7. Effect of paclobutrazol on yield components and seed yield

	Inflorescences/ plant	Florets/ inflorescence	Seeds/ floret	TSW (g)	Yield (g)
Control	179	77	4.6	0.709	34.33
Paclobutrazol 1	137	73	4.4	0.725	17.06
Paclobutrazol 2	151	74	5.0	0.740	26.24
LSD (P=0.05)	NS	NS	NS	NS	NS
CV (%)	107	12	18	15	94

5B. INDIVIDUAL PLANTS FROM CLONAL PROPAGATION 1991/1992

5B.1. INTRODUCTION

The 1990/1991 trial using plants grown from seed failed to test the effect of paclobutrazol on seed yield per plant. Variation among plants was very high, i.e. CV > 90 % (Table 5A.7). Plants in that trial ranged for inflorescences from 0 to more than 300 inflorescences/plant for both treated and control plants. Since white clover is an outcrossing species (Williams, 1987a), this extreme variation is presumably due mainly to genotypic variation. Phenotypic variation might also contribute, but no measurements were conducted to distinguish this from genotypic variation. However, since all plants in that trial experienced the same environment (weather, soil fertility, soil moisture and weed and pest control), it is likely that phenotypic variation did not contribute much to the extreme variation of inflorescence production capacity among genotypes. Therefore, this trial was conducted with the same objectives of the 1990/1991 trial but with methods designed to eliminate genotypic variations, i.e. by using clonal plant material.

5B.2. MATERIALS AND METHODS

5B.2.1. Experimental site, management and treatments

The 1991/1992 experiment was conducted on the nursery block of the Pasture and Crop Research Unit, Massey University, Palmerston North. The site selected had not been previously used for any growth regulator experiments. The soil type was a Tokomaru silt loam, and the soil fertility analysis of the site is presented in Appendix 5B.1.

Three genotypes of cv. Grasslands Pitau white clover were used. Two genotypes with a clearly distinctive characteristic, high and low inflorescence production, were supplied by Dr J. Caradus of AgResearch Grasslands, Palmerston North. Another

genotype with an unknown capacity for inflorescence production was collected from a plant established in the previous year on the Frewin's block (Chapter 6). For ease of reference, the unknown genotype has been termed genotype I, the high inflorescence producer genotype as genotype II and the low inflorescence producer genotype as genotype III.

Clones (ramets) were collected on 3 July 1991 by cutting more than 30 stolon tips (growing points) with 2-3 nodes from each genotype. As the unknown genotype was growing under sward conditions, its clones were carefully collected by digging up an area of 1 m^2 surrounding the plant and carefully separating the unknown genotype from the rest by tracing stolons back to the root. All clones were grown for rooting in pots containing sand, peat and soil with a ratio of 5:3:2 respectively inside an heated glasshouse (20°/15°C) for one month. On 1 August 1991, all plants were brought outside for hardening for 4 weeks and defoliated to 5 cm height.

Transplanting into the field was carried out on 29 August 1991 using a square planting of 1 m. 24 healthy plants from each genotype were used for this experiment. No cultivation was applied. The field had been cleared of weeds using the herbicide Buster (glufosinate ammonium) at a rate of 0.75 kg a.i/ha or 3.75 l Buster/ha on 20 August 1991. Buster is a non-selective herbicide which is active against a wide range of grass and broadleaf weeds and clovers (Anon., 1990). It is primarily contact in action, but also has a slight systemic effect. There is no residual life in the soil and no uptake by roots of established plants. No fertilizer was applied. Controlling weeds during the growing period was done by hand weeding. Irrigation was applied only during the first month after transplanting with a volume of 2 l water/plant/day for the first 15 days and 30 minutes sprinkler irrigation at a rate of 10 l/m^2/hour for the last 15 days. Mesurol (methiocarb) was applied at the rate of 200 g a.i/ha or 10 kg Mesurol pellets/ha on 25 September 1991 and 6 November 1991 to control slugs and snails. Mavrik Aquaflow (fluvalinate) insecticide was applied at the rate of 36 g a.i/ha or 150 ml Mavrik Aquaflow/ha on 8 November 1991 to control caterpillars.

Three paclobutrazol treatments were applied: 1.0 kg a.i/ha applied either on 3 October 1991 or 14 November 1991 and nil paclobutrazol as a control. Application in October was intended as an application during reproductive initiation and the November application at reproductive visible bud. However, because of the nature of the genotypes, the application on 3 October coincided with reproductive visible bud in genotype I and genotype II, and reproductive initiation in genotype III. Application on 14 November coincided with the anthesis of early inflorescences in genotype I and genotype II, and reproductive visible bud in genotype III. For ease of reference, the time of paclobutrazol applications has been termed as October and November application. Paclobutrazol was applied with water at a volume equivalent to 500 l/ha using a knapsack sprayer with one fan nozzle held 25-30 cm above the plant and covering a 1 m^2 area around each plant.

The experiment was arranged as a split plot design trial with genotype as a main plot and paclobutrazol treatment as a sub plot. Each main plot was replicated eight times (3 plants per main plot and 1 plant per sub plot).

5B.2.2. Plant measurements and statistical analysis

Stolon growth analysis was conducted by tagging stolons at two weeks intervals starting before transplanting (1 August 1991) and continuing until 2 January 1991. Final counting was conducted at final harvest on 2 February 1992. Tagging was done by placing coloured wire at the position immediately behind the node bearing the youngest unfolded leaf. Each new tagging was carried out using different coloured wire. Growth analysis was conducted for the original main stolons, two early secondary branches growing from the original main stolons, two early tertiary branches growing from the secondary branches analysed and two early quaternary branches growing from the tertiary branches analysed from every plant (subplot). Main stolon tagging was started on 1 August 1991, secondary branch tagging was started on 25 September 1991, tertiary branch tagging was started on 16 November 1991 and quaternary branch tagging was started on 1 December 1991.

The following measurements were taken from tagged stolons:

- Number of nodes emerging from the terminal bud of each stolon
- Number of inflorescences produced along stolons
- Number of total branches formed on stolons and the proportion of vegetative and fertile branches, i.e. branches bearing inflorescence(s)
- final stolon length at harvest (2 February 1992)

In addition, to distinguish between nodes initiated before and after paclobutrazol applications, one day prior to application, the number of unexpanded leaves and leaf primordia present in the terminal apex was determined by microscopic examination of 10 dissected stolon apices from each genotype (except that only six stolon apices were dissected for genotype III in October) at a magnification of 40 X (Figure 5B.1). It was found from the examination that the number of leaf primordia enclosed in the terminal apex was six in genotype I and seven in genotypes II and III. Thus, nodes emerging prior to the paclobutrazol applications and nodes present in the terminal apex were initiated before the applications. The sequence of node initiation in relation to the time of paclobutrazol application is presented in Figure 5B.2. The comparison of paclobutrazol effects on node growth and development, including inflorescences produced along stolons, was conducted only among nodes initiated following paclobutrazol applications.

At final harvest, the composition of total stolons produced was determined using criteria defined by Thomas (1987a), as presented in section 2.1 of the literature review.

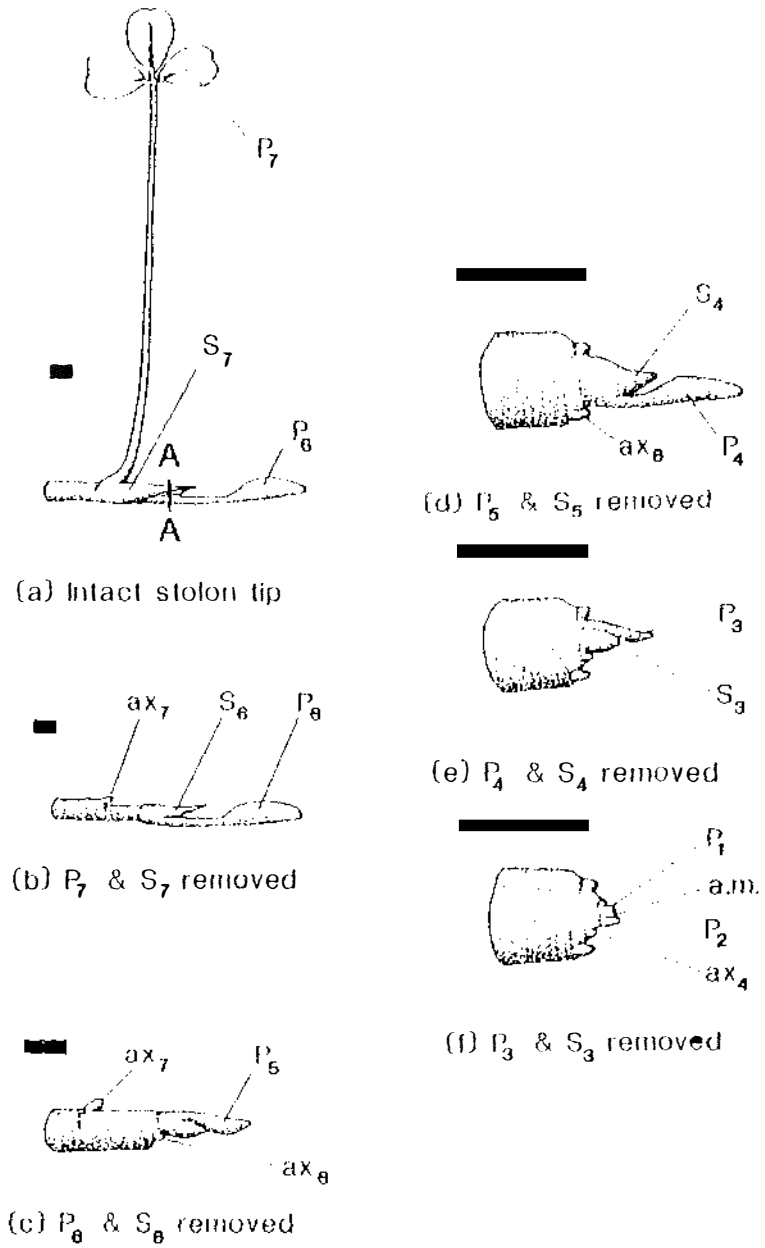
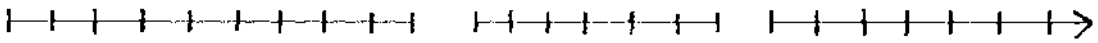


Figure 5B.1. Structure of the terminal apex of a stolon as revealed by successive stages of dissection. Leaf primordia and leaves present in the apical bud of the stolon tip in (a) are labelled P_1 - P_7 in sequence from youngest to oldest, their axillary buds are labelled ax_1 - ax_7 respectively, their stipules S_1 - S_7, and the stem apical meristem a.m. The scale is indicated by horizontal bars, each of which represents 4 mm (Redrawn from Thomas, 1987b).



nodes emerging prior to paclobutrazol application

node primordia enclosed in the terminal apex

nodes initiated following paclobutrazol application

Figure 5B.2. The sequence of node initiation in relation to the time of paclobutrazol applications.

The inflorescence production patterns of the plants were observed by harvesting ripe inflorescences (section 4.2.2) every week starting on 8 December 1991 and continuing to final harvest on 2 February 1992. Seed yield was obtained from the harvested inflorescences using the same method described in section 3.2.2 and is expressed at 10 % SMC. Other seed yield components (florets/ inflorescence, seeds/floret and thousand seed weight) were measured from three different inflorescence categories (early, middle and late inflorescences). Ovule numbers/carpel and initial floret numbers/inflorescence from the three inflorecence categories were also recorded. Measurements for early inflorescences were conducted on 20 November 1991 for genotype I and genotype II, and on 20 December 1991 for genotype III. Measurements for middle inflorescences were conducted on 20 December 1991 for genotype I and genotype II, and on 9 January 1992 for genotype III. Measurements for late inflorescences were conducted only for genotype I and genotype II on 9 January 1992. At each stage, three white inflorescences/subplot (plant) were harvested for initial floret numbers/inflorescence measurements and only one inflorescence/subplot was used to determine ovule numbers/carpel. At the same time, five white inflorescences/subplot were tagged. These tagged inflorescences were used for floret numbers/inflorescence and seeds/floret measurements at the ripening stage. As the seed develoment process of white clover seeds takes 26 ± 5 days, depending on weather conditions (Hyde,

1950), the tagged inflorescences from early and middle stages were harvested 30 days later, but only 25 days later for late inflorescences. Weather conditions during the growth of early and middle inflorescences (November and December 1991) were cool, dull (very low sunshine hours) and wet (only in November), while during the growth of late inflorescences (January 1992) the weather conditions were warmer and had more sunshine hours than in November and December (Appendix 5A.2). Thousand seed weight (TSW) was measured by weighing 4 x 100 seeds/subplot from seedlots obtained from inflorescences harvested at the same date as the tagged inflorescences were harvested. Seed weight is expressed at 10 % SMC.

The measurements of ovule numbers/carpel and seeds/floret were conducted for florets from three different position on the inflorescences, i.e. top, middle and bottom position. Ovule numbers/carpel were examined under a microscope at 40X magnification. 10 florets from each position per subplot were examined for each inflorescence category. Thus, for all three inflorescence categories, a total of 5760 florets were used. Seeds/floret were examined using an X-ray method as described in section 3.2.2. For every inflorescence category, 20 florets/position obtained from two inflorescences/subplot were used for examination.

The effects of paclobutrazol on other plant growth and development parameters were also measured. Plant height was measured weekly during the intensive flowering period (5 December 1991-10 January 1992) by placing a ruler in the mid-point of every single plant canopy. Peduncle length was measured from 10 middle inflorescences/subplot on 20 December 1991. Time needed for inflorescences to elevate above the canopy was measured by tagging reproductive buds at the time they emerged from their surrounding stipular sheaths and counting the number of days needed by them to elevate just above the canopy. Three middle inflorescences/ subplot from genotype I and genotype II were used for this observation. No measurements were taken for genotype III because it had not produced enough inflorescences when the observation was conducted. Determining the effect of paclobutrazol on the erectness of the peduncle (in order to keep the inflorescence

above the canopy during the ripening stage) was conducted by tagging five inflorescences/subplot. Middle inflorescences were used for genotype I and genotype II, and early inflorescence for genotype III. The angle between the peduncle and the ground surface (Plate 5B.1) was measured using a protractor. The measurements were taken from five inflorescences/subplot selected randomly and conducted on 3 January 1992.

The changes in plant structure and morphology following paclobutrazol application was also indirectly determined by measuring light penetration under the canopy using a LI-COR Integrating Quantum/Radiometer/Photometer model LI-188B. Measurements were carried out only for four plants/treatment/genotype. Light intensities above (direct light from the sun) and below the canopy were measured five times (1 second/measurement) at every observation. Observations were made in the morning (8 am), midday (1 pm) and afternoon (6 pm) for both full sun and overcast conditions. The time set was delayed one hour to match the position of the sun as there was a one hour day light saving. Data recorded (in Quantum millivolt units) were calculated to obtain the percentage of light penetration using the formula:

$$\% \text{ light penetration} = \frac{X \text{ (light intensity below the canopy)}}{Y \text{ (light intensity above the canopy)}} \times 100\ \%$$

Data collected in this experiment were mostly analysed according to a split plot design analysis by the use of analysis of variance and Fisher's LSD test at P=0.05 to detect any differences between main plots, subplots or main plotXsubplot interactions. For ovule numbers/carpel and seeds/floret, another factor, i.e. floret position on the inflorescences (top, middle and bottom), was added into the experimental design. As the floret position factor is fixed, data for these two parameters were analysed according to a split block split plot design with genotype

as a main plot, floret position as a subplot or subblock and paclobutrazol treatment as a subsubplot (Dr I. Gordon of Plant Science Department, Massey University, pers. comm.). Considering the distinct genotype characteristic, the comparison among early, middle and late inflorescences for some parameters was done for each genotype. Data for the growth and development of nodes and inflorescences along stolons from paclobutrazol October and November applications were separately compared to the control (nil paclobutrazol) as the treatment effects took place at different times.

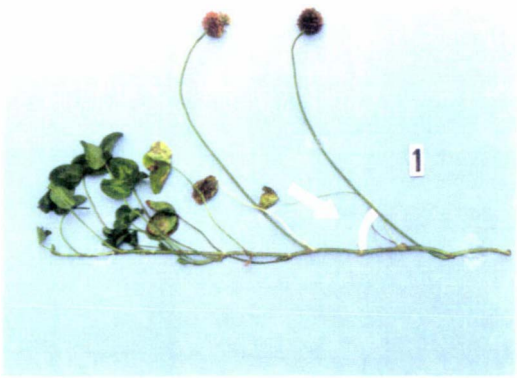


Plate 5B.1. The angle between the peduncle and the ground surface.

5B.3. RESULTS

5B.3.1. Meteorological conditions

In the 1991/1992 growing season, temperatures were warmer during the early part of the season (August and September), but temperatures were much cooler than average for the rest of the season, with the exception of January (Appendix 5A.2). Similarly, the number of sunshine hours was lower than average throughout the season, except in October (Appendix 5A.2). Apart from November, rainfall throughout the trial period was generally lower than average (Appendix 5A.2).

5B.3.2. Effect of paclobutrazol on plant growth and development

5B.3.2.1. Plant morphology

There were significant differences between genotypes for plant height (Plate 5B.2, 5B.3 and 5B.4, and Figure 5B.3). Genotype III had the tallest plants (P=0.05), and genotype I plants were significantly taller than genotype II plants. Paclobutrazol applied in October significantly increased plant height compared to the control when measurements were taken within two to three months after application (Figure 5B.4). On the other hand, paclobutrazol applied in November significantly reduced plant height within four to five weeks after application (12-19 December), but retardation effects began to disappear in the following weeks (Figure 5B.4). There were significant genotypeXpaclobutrazol interactions on plant height on 27 December 1991. The November application significantly increased plant height of genotype III (26.0 cm) compared to the control (23.4 cm) but not of genotype I and genotype II, while the October application significantly increased plant height in all genotypes. There were no significant genotypeXpaclobutrazol interactions for plant height at the other measurement times, except for 5 December 1991, when the November application significantly reduced the plant height of genotype I (10.0 cm) compared to the control (13.3 cm) but not of genotype II and genotype III, while the

Plate 5B.2. Effect of paclobutrazol on the height of genotype I.
Top= Control, Middle= Oct application and Bottom= Nov application
(Photographed on 9 December 1991).

Plate 5B.3. Effect of paclobutrazol on the height of genotype II.
Top= Control, Middle= Oct application and Bottom= Nov application
(Photographed on 9 December 1991).

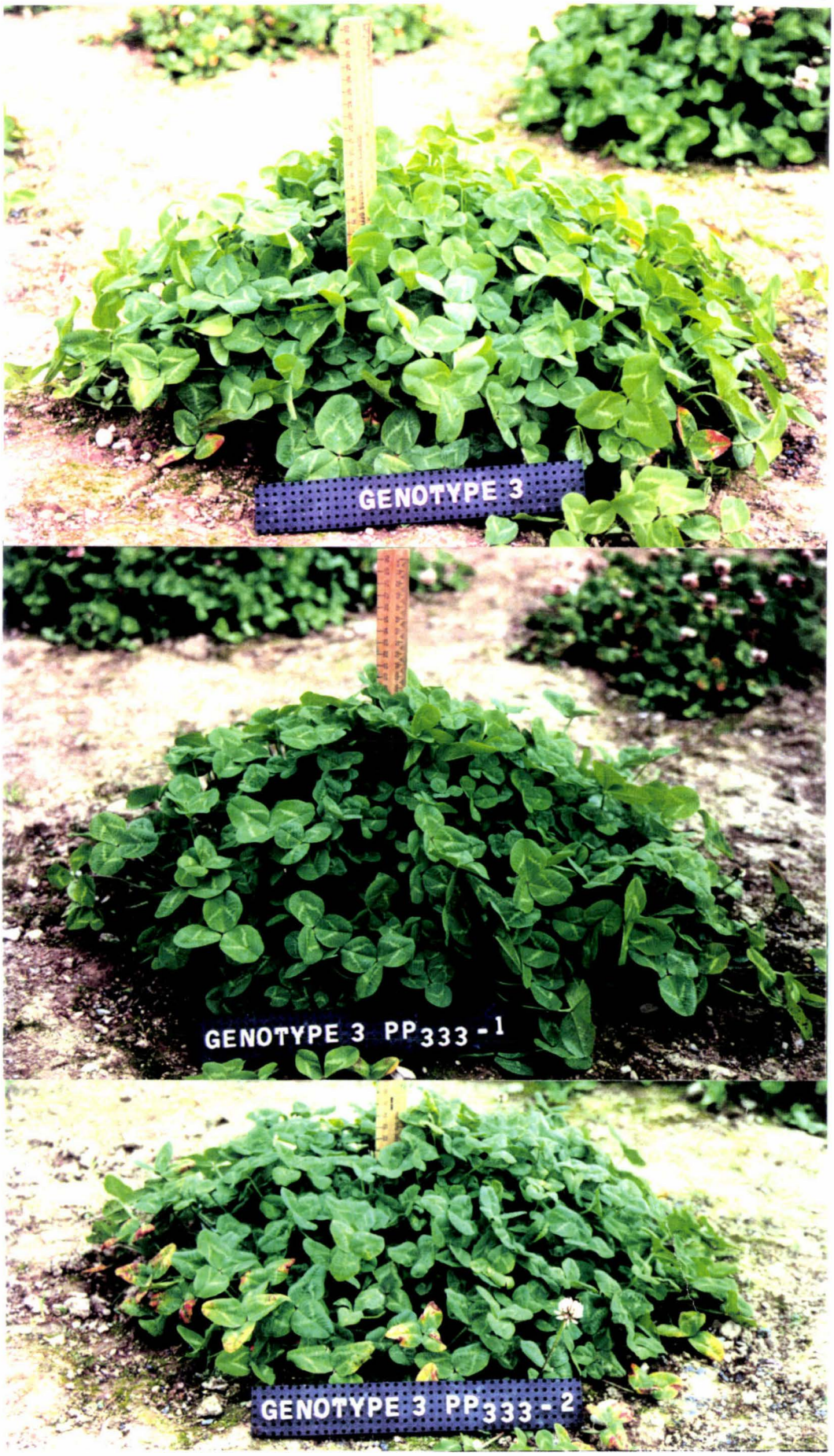


Plate 5B.4. Effect of paclobutrazol on the height of genotype III.
Top= Control, Middle= Oct application and Bottom= Nov application
(Photographed on 9 December 1991).

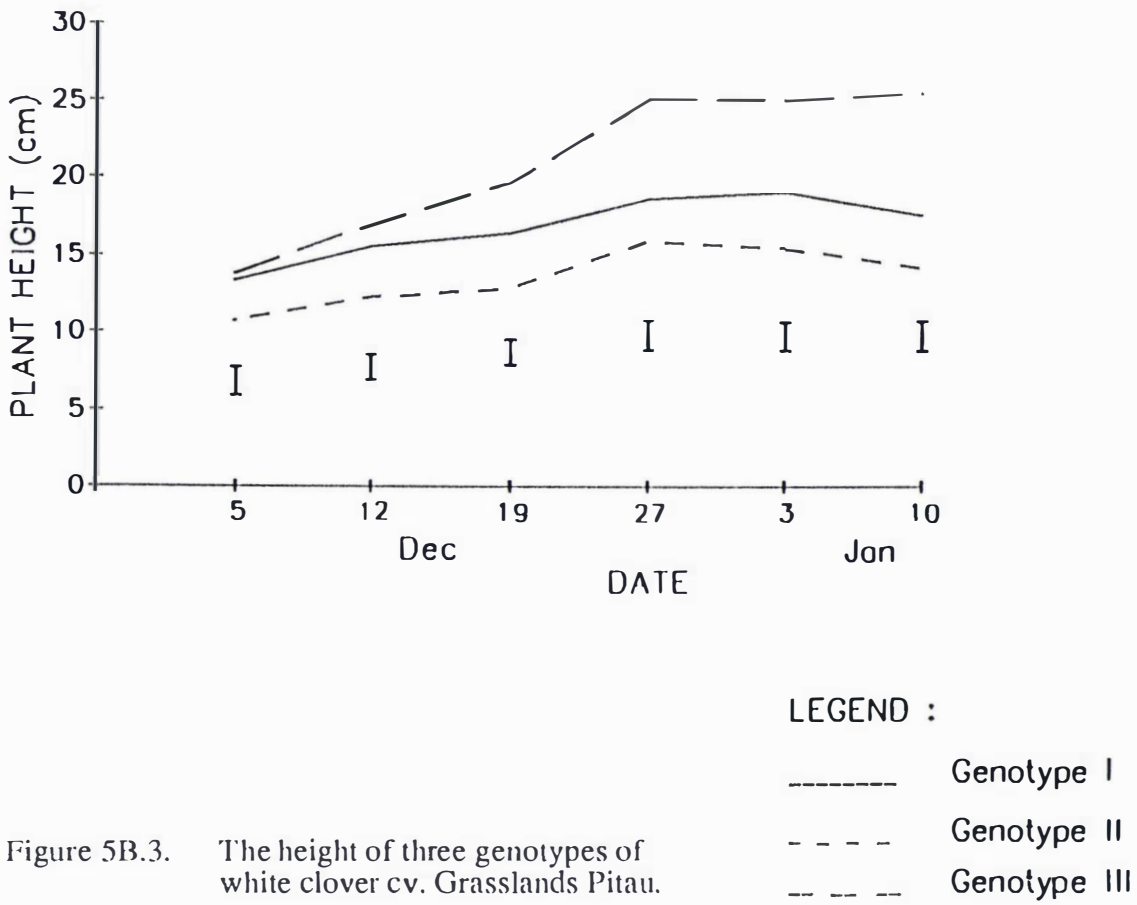


Figure 5B.3. The height of three genotypes of white clover cv. Grasslands Pitau.

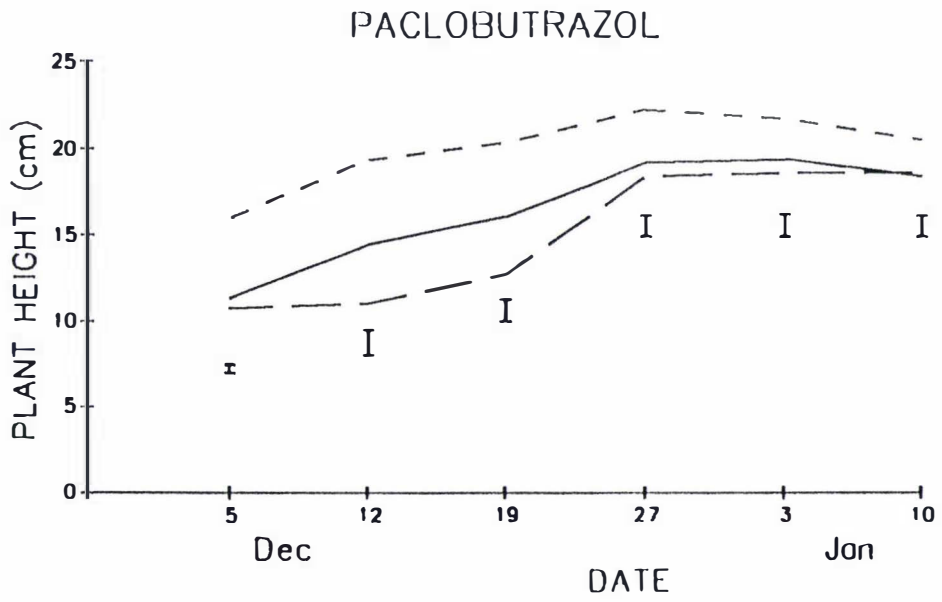


Figure 5B.4. Effect of paclobutrazol on the height of white clover cv. Grasslands Pitau.

October application significantly increased plant height in all genotypes. Despite the significant differences between genotypes and significant effects of paclobutrazol on plant height, the pattern of plant height growth was not different among genotypes and it was not affected by paclobutrazol (Figures 5B.3 and 5B.4). Plant height gradually increased from 5 December 1991, reached its peak between 27 December 1991 and 3 January 1992, and leveled off until the measurements stopped on 10 January 1992.

Stolon lengths for the main stolon, secondary branches and tertiary branches in genotype III were significantly shorter than those of the other two genotypes, while stolon lengths in genotype I did not differ from the stolon lengths in genotype II (Table 5B.1). Genotype I and genotype II also did not differ in their quaternary and quinary branch lengths (Appendix 5B.2). There was no comparison with genotype III as its quaternary branches were not measured and it did not produce quinary branches. Paclobutrazol applied in November significantly reduced main stolon, secondary branch and tertiary branch lengths compared to the control (Table 5B.1). On the other hand, the October application did not affect main stolon length, but it significantly reduced the lengths of secondary and tertiary branches compared to the control (Table 5B.1). However, there were significant genotypeXpaclobutrazol interactions in tertiary branch length. The October application significantly reduced tertiary branch length in genotype III but not in genotype I and genotype II, while the November application significantly reduced tertiary branch length in all genotypes (Table 5B.1). There were no significant interactions between genotypes and paclobutrazol applications for main stolon and secondary branch lengths. In addition, both the October and November paclobutrazol applications did not affect quaternary and quinary branch lengths in genotype I and genotype II, and there were no significant interactions between genotypes and paclobutrazol applications for these parameters (Appendix 5B.2).

Table 5B.1. Effect of paclobutrazol on stolon length for main stolons, secondary branches and tertiary branches in three different genotypes.

A. Main stolon length (cm)

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	74.2	75.2	63.0	70.8
Genotype II	68.9	68.1	65.8	67.6
Genotype III	63.4	54.1	52.1	56.5
Treatment means	68.8	65.8	60.3	

LSD (P=0.05) : genotype means = 6.5, treatment means= 5.2, genotypeXtreatment= NS.
CV : main plots = 9.3 %, sub plots = 13.7 %

B. Secondary branch length (cm)

Genotype I	71.6	67.0	57.8	65.5
Genotype II	65.7	59.8	61.1	62.2
Genotype III	56.6	49.5	45.7	50.6
Treatment means	64.6	58.8	54.9	

LSD (P=0.05) : genotype means = 4.9, treatment means = 3.8, genotypeXtreatment = NS.
CV : main plots = 7.7 %, sub plots = 11.0 %

C. Tertiary branch length (cm)

Genotype I	70.8	67.3	50.3	62.8
Genotype II	62.7	61.7	50.7	58.4
Genotype III	41.0	30.9	30.5	34.1
Treatment means	58.2	53.3	43.8	

LSD (P=0.05) : genotype means = 5.2, treatment means = 4.6, between treatments for the same genotype = 8.0, between treatments for different genotypes = 8.3.
CV : main plots = 9.3 %, sub plots = 15.3%

Based on the percentage of light penetration below the canopy, the plant canopy of genotype II was more open than the plant canopies of genotype I and genotype III. Genotype II had significantly more light being recorded below its canopy compared to the other two genotypes, while genotype I did not differ from genotype III (Appendices 5B.3 and 5B.4). Paclobutrazol altered canopy structure in all three genotypes by making them more closed and compact, as indicated by the significant reduction of light penetrating under the canopies compared to the control (Appendices 5B.3 and 5B.4). However, there were significant genotypeXpaclobutrazol interactions in % light penetration when the measurements were taken at 8 am under overcast conditions, 1 pm both in full sun and under overcast conditions, and at 6 pm in full sun, while interactions for the other time measurements were not significant. At midday in full sun, the October application significantly reduced light penetration compared to the control in genotype II but not in genotype I and genotype III, while it significantly reduced light penetration in genotype I and genotype II but not in genotype III when measured at 1 pm in overcast conditions and 6 pm in full sun (Appendices 5B.3 and 5B.4). On the other hand, the November application significantly reduced light penetration in all genotypes at all times in both full sun and overcast conditions (Appendices 5B.3 and 5B.4). In fact, the November application resulted in the % light penetration in the more open plant (genotype II) being similar with the % light penetration in genotype I and genotype III when the measurements were taken at 1 pm in full sun and at 8 am in overcast conditions (Appendices 5B.3 and 5B.4).

5B.3.2.2. Peduncle growth

Peduncle length of genotype II was significantly shorter than that of the other two genotypes, while the peduncles of genotype I and genotype III did not differ significantly in length (Table 5B.2). When measured at peak flowering, paclobutrazol applied in October had significantly increased peduncle length compared to the control, while the November application did not (Table 5B.2). However, there were significant genotypeXpaclobutrazol interactions. While the

Table 5B.2. Effect of paclobutrazol on peduncle length (mm) for three different genotypes.

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	279	319	244	281
Genotype II	232	268	207	236
Genotype III	260	302	279	280
Treatment means	257	296	243	

LSD (P=0.05) : genotype means = 15.5, treatment means = 14.4,
between treatments for the same genotype = 24.9,
between treatments for different genotypes = 25.5.
CV : main plots = 5.4 %, subplots = 9.3 %

Table 5B.3. Effect of paclobutrazol on the angle (°) between the peduncle and the ground surface for three different genotypes.

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	41.8	37.0	25.5	34.8
Genotype II	43.8	41.3	27.3	37.5
Genotype III	50.3	46.8	43.0	46.7
Treatment means	45.3	41.7	31.9	

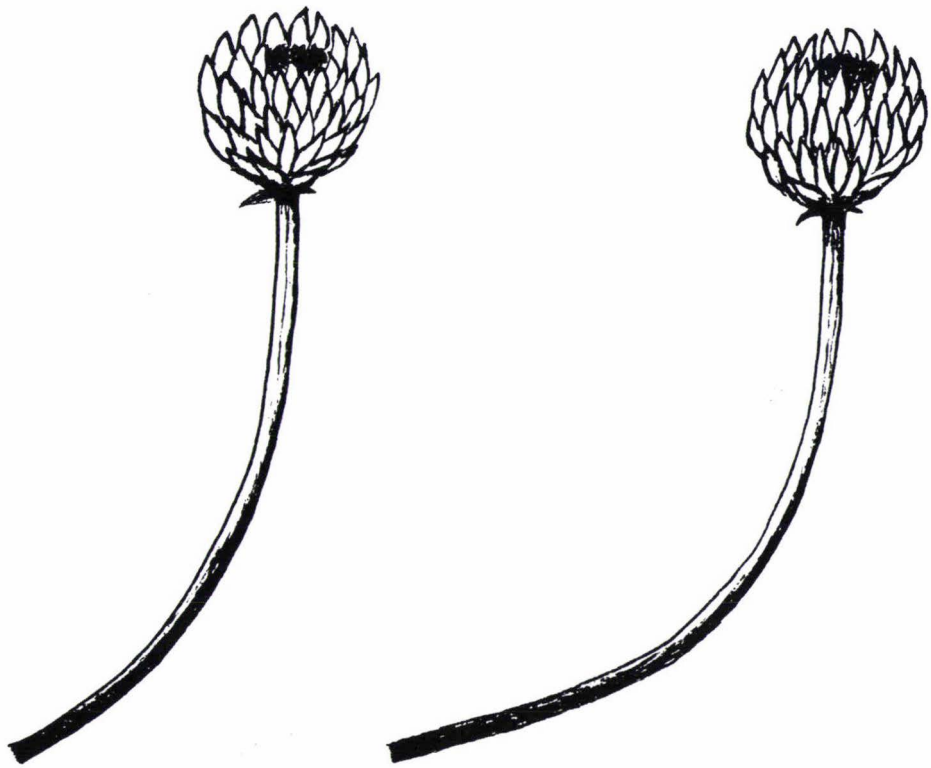
LSD (P=0.05) : genotype means = 1.7, treatment means = 2.0,
between treatments for the same genotype = 3.4,
between treatments for different genotypes = 3.3.
CV : main plots = 4.1 %, subplots = 8.5 %

October application significantly increased peduncle length in all genotypes, the November application significantly reduced peduncle length compared to the control in genotype I and genotype II but not in genotype III (Table 5B.2).

Peduncle growth in genotype III was the most erect among the three genotypes, and peduncle growth in genotype II was more erect than that in genotype I. This was indicated by a significantly larger angle between the peduncle of genotype III and the ground surface, while the angle in genotype II was significantly larger than the angle in genotype I (Table 5B.3). Paclobutrazol applications resulted in peduncle growth being oriented more towards the ground than the control (Figure 5B.5). However, there were significant genotypeXpaclobutrazol interactions. The October application significantly reduced the angle between the peduncle and the ground surface in genotype I and genotype III but not in genotype II, while the November application significantly reduced the angle compared to the control in all genotypes (Table 5B.3).

The peduncle of genotype III was more erect and compact than the peduncles of genotype I and genotype II as indicated by the significantly lower percentage of its inflorescences falling under the plant canopy at the ripening stage than the percentages in the other genotypes, while the peduncles of genotype I and genotype II were not significantly different in their erectness (Table 5B.4). Paclobutrazol significantly reduced the percentage of inflorescences falling under the plant canopy (Table 5B.4). However, there were significant genotypeXpaclobutrazol interactions. Paclobutrazol applied in both October and November significantly strengthened the erectness of peduncles in genotype I and genotype II, but it did not have an effect in genotype III (Table 5B.4).

Peduncle growth in genotype II was faster than peduncle growth in genotype I as indicated by significantly fewer days being required for the inflorescences of genotype II to elevate above the plant canopy than the days for the inflorescences of genotype I (Table 5B.5). There was no measurement for genotype III as there were



Control Paclobutrazol

Figure 5B.5. The position of peduncle growth following paclobutrazol application (original).

Table 5B.4. Effect of paclobutrazol on the percentage of inflorescences falling below the plant canopy at the ripening stage for three different genotypes.

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	95.0	70.0	15.0	60.0
Genotype II	95.0	75.0	25.0	65.0
Genotype III	10.0	0.0	0.0	3.3
Treatment means	66.7	48.3	13.3	

LSD (P=0.05) : genotype means = 12.5, treatment means = 10.6, between treatments for the same genotype = 18.4, between treatments for different genotypes = 19.6.
CV : main plots = 27 %, subplots = 43 %

Table 5B.5. Effect of paclobutrazol on the time (days) needed for inflorescences to elevate above the plant canopy for two different genotypes.

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	10.6	10.8	9.8	10.4
Genotype II	10.2	9.8	9.6	9.9
Treatment means	10.4	10.3	9.7	

LSD (P=0.05) : genotype means = 0.4, treatment means = 0.4, genotypeXtreatment = NS.
CV : main plots = 3.5 %, subplots = 5.2 %

insufficient inflorescences produced (see section 5B.2.2). Paclobutrazol applied in November significantly reduced the time needed by inflorescences to elevate above the plant canopy compared to the control, while the October application did not differ (Table 5B.5). There were no significant genotypeXpaclobutrazol interactions.

5B.3.2.3. Vegetative and reproductive nodes formed along stolons

There were significant differences among genotypes in the number of nodes formed along main stolons and their secondary and tertiary branches but not along their quaternary and quinary branches. When node numbers were counted starting from the seventh node (for genotype I) or eighth node (for genotype II and genotype III) which emerged from stolon tips following paclobutrazol applications, genotype III produced the significantly highest number of total nodes (vegetative and reproductive nodes) along main stolons and secondary branches, while genotype I did not differ from genotype II, except that the number of total nodes formed along its secondary branches were significantly lower than the numbers in genotype II when they were counted following the November paclobutrazol application (Tables 5B.6 and 5B.7). Genotype III did not differ from genotype II in the total number of nodes formed along tertiary branches, while genotype I produced the lowest numbers (Table 5B.8).

However, high total node numbers were not always reflected in high reproductive nodes formed. Genotype III produced the greatest number of vegetative node numbers along main stolons, and also produced the lowest number of reproductive nodes, except that it did not differ from genotype I when the numbers were counted following the November paclobutrazol application (Table 5B.6). On the other hand, genotype II produced the highest numbers of reproductive nodes and the lowest number of vegetative nodes along main stolons (Table 5B.6). Apart from the similarity with genotype III in the number of reproductive nodes as mentioned before, the capacity of genotype I to produce vegetative and reproductive nodes along main stolons lay between genotype II and genotype III (Table 5B.6).

Table 5B.6. Effect of paclobutrazol on the number of nodes formed along main stolons in three different genotypes.

A. Vegetative nodes (genotypeXtreatment = NS)

Genotypes	A[1)] Paclobutrazol		Genotype means	B Paclobutrazol		Genotype means
	Control	October	(for A)	Control	November	(for B)
Genotype I	17.5	17.5	17.5	12.3	11.0	11.7
Genotype II	15.1	16.1	15.6	8.9	10.7	9.8
Genotype III	23.6	24.9	24.3	15.8	15.0	15.4
Treatment means	18.7	19.5		12.3	12.2	

LSD (P=0.05) for A : genotype means = 1.4, treatment means = NS,
CV : main plots = 6.8 %, sub plots = 12.9 %
LSD (P=0.05) for B : genotype means = 1.8, treatment means = NS
CV : main plots = 14 %, sub plots = 17 %

B. Reproductive nodes (genotypeXtreatment = NS)

Genotype I	2.8	2.6	2.7	0.1	0.4	0.3
Genotype II	5.6	5.3	5.5	3.0	3.4	3.2
Genotype III	1.6	1.4	1.5	0.7	0.4	0.6
Treatment means	3.3	3.1		1.3	1.4	

LSD (P=0.05) for A : genotype means = 0.7, treatment means = NS,
CV : main plots = 22 %, sub plots = 21 %
LSD (P=0.05) for B : genotype means = 0.9, treatment means = NS
CV : main plots = 60 %, sub plots = 84 %

C. Total nodes (genotypeXtreatment = NS)

Genotype I	20.3	20.1	20.2	12.4	11.4	11.9
Genotype II	20.7	21.4	21.1	11.9	14.1	13.0
Genotype III	25.2	26.3	25.8	16.5	15.4	16.0
Treatment means	22.0	22.6		13.6	13.6	

LSD (P=0.05) for A : genotype means = 1.4, treatment means = NS,
CV : main plots = 6.0 %, sub plots = 11.0 %
LSD (P=0.05) for B : genotype means = 2.2, treatment means = NS
CV : main plots = 15 %, sub plots = 19 %
Note: 1. A and B are analysed separately (details in section 5B.2.2).

Table 5B.7. Effect of paclobutrazol on the number of nodes formed along secondary branches in three different genotypes.

A. Vegetative nodes (genotypeXtreatment = NS)

Genotypes	A[1)] Paclobutrazol		Genotype means (for A)	B Paclobutrazol		Genotype means (for B)
	Control	October		Control	November	
Genotype I	15.1	16.9	16.0	10.3	10.7	10.5
Genotype II	15.3	15.4	15.4	10.0	10.0	10.0
Genotype III	21.1	23.4	22.3	15.1	15.5	15.3
Treatment means	17.2	18.6		11.8	12.1	

LSD (P=0.05) for A : genotype means = 1.8, treatment means = 1.4,
CV : main plots = 9.5 %, sub plots = 13.0 %
LSD (P=0.05) for B : genotype means = 1.1, treatment means = NS
CV : main plots = 8.9 %, sub plots = 14.9 %

B. Reproductive nodes (genotypeXtreatment = NS)

Genotype I	3.2	3.0	3.1	0.4	0.3	0.4
Genotype II	4.9	5.8	5.4	2.8	3.4	3.1
Genotype III	1.3	1.0	1.2	0.2	0.1	0.2
Treatment means	3.1	3.3		1.1	1.3	

LSD (P=0.05) for A : genotype means = 0.6, treatment means = NS,
CV : main plots = 18 %, sub plots = 27 %
LSD (P=0.05) for B : genotype means = 0.6, treatment means = NS
CV : main plots = 48 %, sub plots = 60 %

C. Total nodes (genotypeXtreatment = NS)

Genotype I	18.3	19.9	19.1	10.7	11.0	10.9
Genotype II	20.2	21.2	20.7	12.8	13.4	13.1
Genotype III	22.4	24.4	23.4	15.3	15.6	15.5
Treatment means	20.3	21.8		12.9	13.3	

LSD (P=0.05) for A : genotype means = 1.8, treatment means = NS,
CV : main plots = 7.8 %, sub plots = 12.6 %
LSD (P=0.05) for B : genotype means = 1.5, treatment means = NS
CV : main plots = 10.4 %, sub plots = 14.6 %
Note: 1. A and B are analysed separately (details in section 5B.2.2).

Table 5B.8. Effect of paclobutrazol on the number of nodes formed along tertiary branches in three different genotypes.

A. Vegetative nodes (genotypeXtreatment = NS)

Genotypes	A[1)] Paclobutrazol		Genotype means	B Paclobutrazol		Genotype means
	Control	October	(for A)	Control	November	(for B)
Genotype I	15.7	16.1	15.9	11.3	10.9	11.1
Genotype II	17.1	16.6	16.9	12.1	10.8	11.5
Genotype III	21.3	21.4	21.4	13.7	13.3	13.5
Treatment means	18.0	18.0		12.4	11.7	

LSD (P=0.05) for A : genotype means = 1.8, treatment means = NS,
CV : main plots = 9.1 %, sub plots = 12.5 %
LSD (P=0.05) for B : genotype means = 1.9, treatment means = NS
CV : main plots = 15 %, sub plots = 16 %

B. Reproductive nodes (genotypeXtreatment = NS)

Genotype I	2.6	2.7	2.7	0.6	0.6	0.6
Genotype II	4.8	5.1	5.0	3.0	2.9	3.0
Genotype III	0.6	0.8	0.7	0.2	0.4	0.3
Treatment means	2.7	2.9		1.3	1.3	

LSD (P=0.05) for A : genotype means = 0.6, treatment means = NS,
CV : main plots = 20 %, sub plots = 19 %
LSD (P=0.05) for B : genotype means = 0.4, treatment means = NS
CV : main plots = 31 %, sub plots = 54 %

C. Total nodes (genotypeXtreatment = NS)

Genotype I	18.3	18.8	18.6	11.9	11.5	11.7
Genotype II	21.9	21.7	21.8	15.1	13.7	14.4
Genotype III	21.9	22.2	22.1	13.9	13.7	13.8
Treatment means	20.7	20.9		13.6	13.0	

LSD (P=0.05) for A : genotype means = 1.7, treatment means = NS,
CV : main plots = 7.8 %, sub plots = 11.5 %
LSD (P=0.05) for B : genotype means = 2.0, treatment means = NS
CV : main plots = 14 %, sub plots = 17 %

Note: 1. A and B are analysed separately (details in section 5B.2.2).

The results of the comparison between genotypes for vegetative and reproductive nodes formed along secondary and tertiary branches were similar to the results of the comparison between genotypes for vegetative and reproductive nodes formed along main stolons, except that genotype II did not differ from genotype I in the number of vegetative nodes formed along the secondary and tertiary branches (Table 5B.7 and 5B.8). There were no significant differences between genotype I and genotype II in node numbers for both vegetative and reproductive nodes formed along quaternary and quinary branches (Appendices 5B.5 and 5B.6). No measurements were taken for the quaternary branches of genotype III, and this genotype did not produce quinary branches.

Apart from the October paclobutrazol application which significantly increased the number of vegetative nodes along secondary branches compared to the control, paclobutrazol did not affect the initiation and development of vegetative, reproductive and total nodes along main stolons and their four different category branches, and there were no significant genotypeXpaclobutrazol interactions in all these parameters (Tables 5B.6, 5B.7 and 5B.8, and Appendices 5B.5 and 5B.6).

5B.3.2.4. Main stolon branching and the proportion of vegetative and fertile branches

The number of vegetative branches, the number of fertile branches and the number of total branches for secondary, tertiary, quaternary and quinary branches formed on the original main stolons are presented in Tables 5B.9, 5B.10 and 5B.11, and Appendix 5B.7. There were no significant genotypeXpaclobutrazol interactions for these parameters.

Table 5B.9. Effect of paclobutrazol on the number of secondary branches/plant (original main stolon) for three different genotypes.

A. Vegetative branches

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	7.1	10.4	9.1	8.9
Genotype II	1.9	2.3	3.4	2.5
Genotype III	21.0	23.9	22.3	22.4
Treatment means	10.0	12.2	11.6	

LSD (P=0.05) : genotype means = 2.0, treatment means = 2.1, genotypeXtreatment = NS.
CV : main plots = 17 %, subplots = 32 %

B. Fertile branches

Genotype I	11.3	13.1	11.4	**11.9**
Genotype II	14.9	17.3	18.4	**16.8**
Genotype III	13.4	15.5	15.3	**14.7**
Treatment means	13.2	15.3	15.0	

LSD (P=0.05) : genotype means = 1.8, treatment means = 1.7, genotypeXtreatment = NS.
CV : main plots = 12 %, subplots = 21 %

C. Total branches

Genotype I	18.4	23.5	20.5	20.8
Genotype II	16.8	19.6	21.8	19.4
Genotype III	34.4	39.4	37.6	37.1
Treatment means	23.2	27.5	26.6	

LSD (P=0.05) : genotype means = 1.9, treatment means = 1.8, genotypeXtreatment = NS.
CV : main plots = 6.8 %, subplots = 12.0 %

Table 5B.10. Effect of paclobutrazol on the number of tertiary branches/ secondary branch for three different genotypes.

A. Vegetative branches

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	6.9	9.6	7.9	8.1
Genotype II	3.4	3.0	3.7	3.4
Genotype III	19.6	23.1	22.1	21.6
Treatment means	10.0	11.9	11.2	

LSD (P=0.05) : genotype means = 1.6, treatment means = 1.5, genotypeXtreatment = NS.
CV : main plots = 14 %, subplots = 24 %

B. Fertile branches

Genotype I	7.9	9.8	9.0	8.9
Genotype II	9.6	13.1	13.4	12.0
Genotype III	3.0	3.5	3.8	3.4
Treatment means	6.8	8.8	8.7	

LSD (P=0.05) : genotype means = 1.4, treatment means = 1.2, genotypeXtreatment = NS.
CV : main plots = 16 %, subplots = 26 %

C. Total branches

Genotype I	14.8	19.4	16.9	17.0
Genotype II	13.0	16.1	17.1	15.4
Genotype III	22.6	26.6	25.9	25.0
Treatment means	16.8	20.7	19.9	

LSD (P=0.05) : genotype means = 1.3, treatment means = 1.3, genotypeXtreatment = NS.
CV : main plots = 6.3 %, subplots = 12.0 %

Table 5B.11. Effect of paclobutrazol on the number of quaternary branches/ tertiary branch for three different genotypes.

A. Vegetative branches

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	8.0	8.7	8.4	8.4
Genotype II	4.2	5.3	4.0	4.5
Genotype III	15.2	15.4	16.8	15.8
Treatment means	9.1	9.8	9.7	

LSD (P=0.05) : genotype means = 2.0, treatment means = NS, genotypeXtreatment = NS.
CV : main plots = 20 %, subplots = 23 %

B. Fertile branches

Genotype I	4.8	5.5	5.5	5.3
Genotype II	6.9	9.1	7.9	8.0
Genotype III	0.0	0.0	0.0	0.0
Treatment means	3.9	4.9	4.5	

LSD (P=0.05) : genotype means = 1.2, treatment means = 0.7, genotypeXtreatment = NS.
CV : main plots = 24 %, subplots = 29 %

C. Total branches

Genotype I	12.8	14.2	13.9	13.6
Genotype II	11.1	14.4	11.9	12.5
Genotype III	15.2	15.4	16.8	15.8
Treatment means	13.0	14.7	14.2	

LSD (P=0.05) : genotype means = 2.2, treatment means = 1.4, genotypeXtreatment = NS.
CV : main plots = 15 %, subplots = 17 %

Genotype I and genotype II produced secondary, tertiary, quaternary and quinary branches from their main stolons, while genotype III also produced all those branches but the quinary ones. Genotype III produced significantly higher total branch numbers for secondary and tertiary branches among the three genotypes, while its total quaternary branch numbers were significantly higher than the numbers in genotype II but not in genotype I. Genotype I did not differ from genotype II in total branch numbers for tertiary, quaternary and quinary branches, but its total secondary branch numbers were significantly higher. Genotype III also produced significantly higher vegetative branch numbers for secondary, tertiary and quaternary branches, and genotype I produced significantly higher vegetative branch numbers for those three stolon branch categories than genotype II. However, genotype I did not differ from genotype II in the number of vegetative quinary branches. On the other hand, genotype II produced the highest fertile branch numbers for secondary, tertiary and quaternary branches, while it did not differ from genotype I in the number of fertile quinary branches. Genotype I produced significantly smaller fertile secondary branch numbers than genotype III, but it produced significantly greater fertile branch numbers for tertiary and quaternary branches. In fact, genotype III did not produced fertile quaternary branches.

As a consequence of the data above, genotype II had the highest fertile branch proportions for secondary, tertiary and quaternary branches, and the proportions in genotype I were higher than the proportions in genotype III. The proportion figures (%) were:

		Genotypes		
		I	II	III
fertile secondary branches	:	57	87	40
fertile tertiary branches	:	52	78	14
fertile quaternary branches	:	39	64	0

Paclobutrazol applied in both October and November significantly increased total branch numbers compared to the control for secondary and tertiary branches, but only the October application significantly increased total quaternary branch numbers. Total quinary branch numbers were not affected by paclobutrazol. Similarly, both the October and November applications significantly increased fertile branch numbers compared to the control for secondary and tertiary branches, while only the October application significantly increased fertile quaternary branch numbers. Paclobutrazol did not affect fertile quinary branch numbers. Vegetative branch numbers for secondary and tertiary branches were increased significantly by paclobutrazol applied in October compared to the control, but they were not affected by the November application. Paclobutrazol did not affect the number of vegetative branches in quaternary and quinary branches. The proportion (%) of fertile branches following paclobutrazol applications were:

		Paclobutrazol treatments		
		Control	Oct	Nov
fertile secondary branches	:	57	56	56
fertile tertiary branches	:	41	43	44
fertile quaternary branches	:	30	33	32

Thus, paclobutrazol had no effect on the proportion of fertile branches.

5B.3.2.5. Total stolon number and stolon composition at harvest

Genotype I produced a significantly greater number of total stolons (vegetative and fertile stolons), while genotype II did not differ from genotype III (Table 5B.12A). On the other hand, genotype II produced a significantly greater number of fertile stolons, and the fertile stolon numbers in genotype I were significantly higher than the numbers in genotype III (Table 5B.12B). Paclobutrazol did not affect stolon numbers for either fertile or total stolons (Table 5B.12). There were no significant genotypeXpaclobutrazol interactions for these parameters.

When stolons were separated into main stolons, lateral stolons and lateral branches, genotype I also produced the highest stolon numbers for all three stolon categories, and genotype II produced significantly higher stolon numbers for main and lateral stolons than genotype III (Table 5B.13). Genotype II did not differ from genotype III in the number of lateral branches (Table 5B.13C). Paclobutrazol did not affect the number of lateral branches and there were no significant genotypeXpaclobutrazol interactions (Table 5B.13C). On the other hand, paclobutrazol affected main and lateral stolon numbers. However, there were significant genotypeXpaclobutrazol interactions. Paclobutrazol applied in both October and November significantly reduced stolon numbers compared to the control for main and lateral stolons in genotype I, but not in genotype II and genotype III (Tables 5B.13A and 5B.13B).

Genotype I produced significantly higher fertile stolon numbers for main and lateral stolons, but it did not produce fertile lateral branches (Table 5B.14). Genotype III produced significantly lower fertile stolon numbers for main and lateral stolons, but it also did not produce fertile lateral branches (Table 5B.14). Fertile stolon numbers for main and lateral stolons produced by genotype II lay in between those produced by genotype I and genotype III, and genotype II was the only genotype that produced fertile lateral branches (Table 5B.14). Paclobutrazol applied in October significantly increased fertile stolon numbers compared to the

Table 5B.12. Effect of paclobutrazol on stolon number/plant for fertile and total stolons at harvest for three different genotypes.

A. Fertile stolons

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	144	145	139	143
Genotype II	150	193	195	179
Genotype III	24	27	25	25
Treatment means	106	122	120	

LSD (P=0.05) : genotype means = 20, treatment means = NS, genotypeXtreatment = NS.
CV : main plots = 16 %, subplots = 26 %

B. Total stolons

Genotype I	591	535	476	534
Genotype II	326	402	342	357
Genotype III	305	357	360	341
Treatment means	407	431	393	

LSD (P=0.05) : genotype means = 64, treatment means = NS, genotypeXtreatment = NS.
CV : main plots = 14 %, subplots = 22 %

Table 5B.13. Effect of paclobutrazol on stolon number/plant for main stolons, lateral stolons and lateral branches at harvest for three different genotypes.

A. Main stolons

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	100	79	74	84
Genotype II	57	60	51	56
Genotype III	28	30	29	29
Treatment means	62	56	51	

LSD (P=0.05) : genotype means = 7.0, treatment means = 6.1,
between treatments for the same genotype = 10.6
between treatments for different genotypes = 11.2
CV : main plots = 12 %, subplots = 19 %

B. Lateral stolons

Genotype I	160	138	110	136
Genotype II	81	89	70	80
Genotype III	60	59	62	60
Treatment means	100	95	81	

LSD (P=0.05) : genotype means = 12.5, treatment means = 11.3,
between treatments for the same genotype = 19.5
between treatments for different genotypes =20.3
CV : main plots = 13 %, subplots = 21 %

C. Lateral branches

Genotype I	330	319	292	314
Genotype II	187	253	222	221
Genotype III	217	268	269	251
Treatment means	245	280	261	

LSD (P=0.05) : genotype means = 53, treatment means = NS,
genotypeXtreatment = NS.
CV : main plots = 19 %, subplots = 26 %

Table 5B.14. Effect of paclobutrazol on fertile stolon number/plant for main stolons, lateral stolons and lateral branches at harvest for three different genotypes.

A. Fertile main stolons

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	85	69	70	75
Genotype II	57	60	51	56
Genotype III	21	24	26	24
Treatment means	54	51	49	

LSD (P=0.05) : genotype means = 7.5, treatment means = NS,
between treatments for the same genotype = 11.5
between treatments for different genotypes = 12.0
CV : main plots = 14 %, subplots = 22 %

B. Fertile lateral stolons

Genotype I	60	75	69	68
Genotype II	53	67	59	60
Genotype III	3	3	3	3
Treatment means	39	48	44	

LSD (P=0.05) : genotype means = 6.5, treatment means = 8.2,
genotypeXtreatment = NS.
CV : main plots = 14 %, subplots = 32 %

C. Fertile lateral branches

Genotype I	0	0	0	0
Genotype II	43	66	85	65
Genotype III	0	0	0	0
Treatment means	14	22	28	

LSD (P=0.05) : genotype means = 10.4, treatment means = 8.0,
between treatments for the same genotype = 13.9
between treatments for different genotypes = 15.4

control for lateral stolons and lateral branches, while the November application only significantly increased fertile lateral branch numbers (Tables 5B.14B and 5B.14C). There were no significant genotypeXpaclobutrazol interactions for these parameters. Although paclobutrazol affected fertile main stolon numbers, there were significant genotypeXpaclobutrazol interactions. The October and November application significantly reduced the number of fertile main stolons compared to the control in genotype I, but not in genotype II and genotype III (Table 5B.14A).

5B.3.2.6. Vegetative dry weight at harvest

Genotype I produced significantly higher vegetative dry weight at harvest, while genotype II did not differ from genotype III (Table 5B.15). Paclobutrazol affected vegetative dry weight at harvest, but there were significant genotypeXpaclobutrazol interactions. When compared to the control, paclobutrazol applied in November significantly reduced vegetative dry weight in genotype I but not in genotype II and genotype III, while the October application did not have significant effects in all genotypes (Table 5B.15).

Table 5B.15. Effect of paclobutrazol on vegetative dry weight (g/plant) at harvest in three different genotypes.

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	506	432	335	424
Genotype II	225	246	196	222
Genotype III	253	270	251	258
Treatment means	328	316	261	

LSD (P=0.05) : genotype means = 55, treatment means = 47, between treatments for the same genotype = 81, between treatments for different genotypes = 86.
CV : main plots = 17 %, subplots = 27 %

5B.3.3. Effect of paclobutrazol on seed yield components and seed yield

5B.3.3.1. Inflorescence production and its pattern

The duration of inflorescence production differed among genotypes (Figure 5B.6). Genotype I and genotype II were early reproductive initiating plants with a long flowering duration. However, although they started to flower at the same time, genotype I finished its reproductive initiation earlier than genotype II as shown by the fact that genotype I reached peak inflorescence production one week earlier than genotype II. Genotype III was a late reproductive initiating plant and it had a short flowering span. Paclobutrazol did not alter the duration of inflorescence production in any genotype (Figure 5B.6).

As determined earlier, there were significant differences in the capacity of genotypes to produce inflorescences (Plate 5B.5). Genotype II (high inflorescence producer) produced the highest total inflorescence numbers/plant (mean = 400 inflorescences/plant), while genotype III (low inflorescence producer) produced the lowest total inflorescence numbers/plant (mean = 30 inflorescences/plant). Genotype I, with an unknown capacity to produce inflorescence(s), differed significantly from both genotype II and genotype III (mean = 320 inflorescences/plant). Paclobutrazol did not affect total inflorescence numbers/plant. However, there were significant paclobutrazol effects on inflorescence numbers at particular harvest times during the long span of inflorescence production, though there were also significant genotypeXpaclobutrazol interactions. The results for each genotype are presented in Figure 5B.6. Only inflorescence production in genotype II was affected by paclobutrazol. Inflorescences harvested within the time frame 22 December 1991 to 19 January 1992, (and termed middle inflorescences), were significantly increased by the October application compared to the control, while the November application significantly increased inflorescence numbers only on 12 January 1992 and 2 February 1992 (late inflorescences). In addition, the October application significantly reduced unripe inflorescences compared to the control at final harvest, while the November application did not differ from the control.

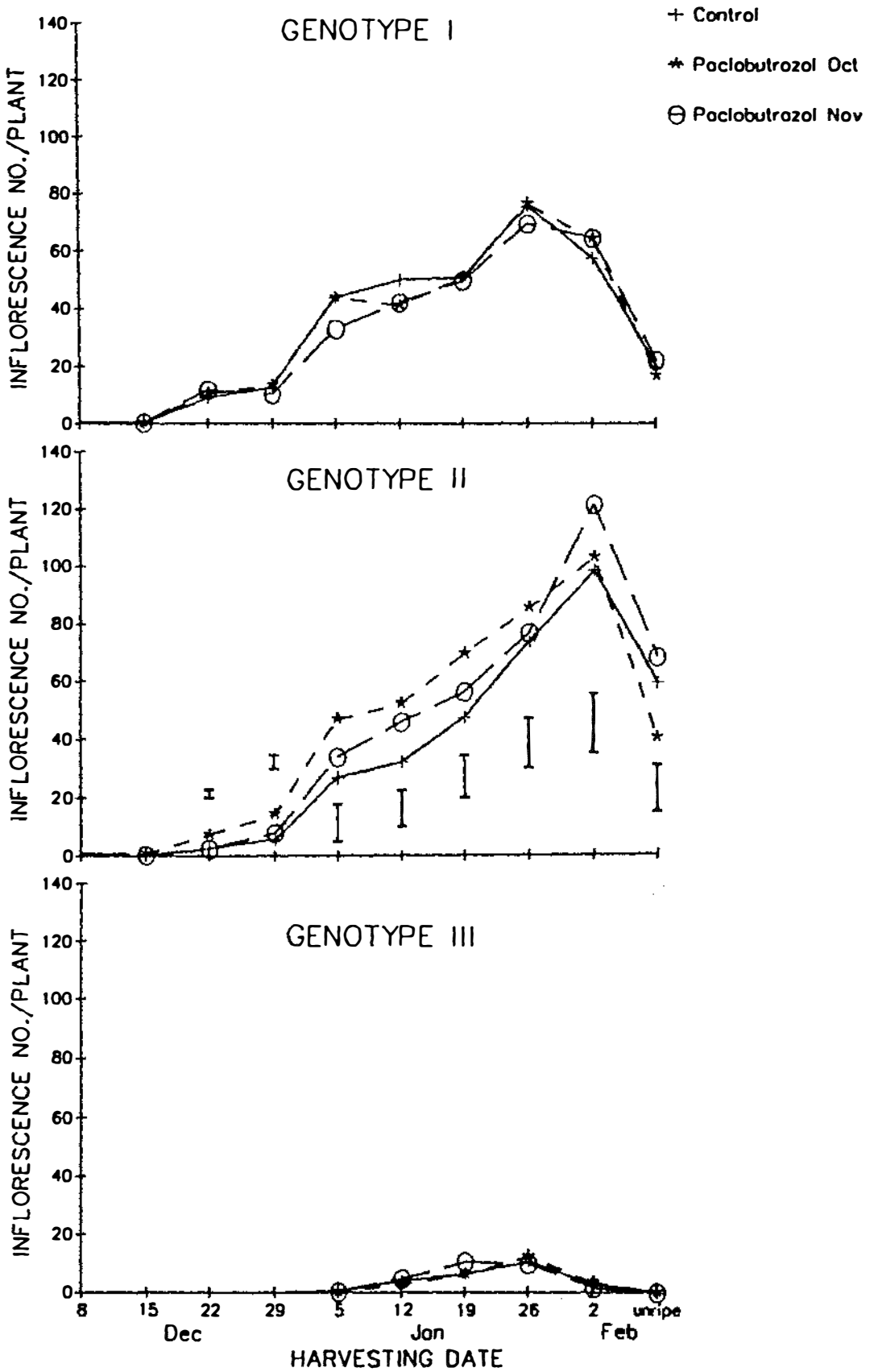


Figure 5B.6. Effect of paclobutrazol on inflorescence numbers/plant in three cv. Grasslands Pitau white clover genotypes. I = LSD (P=0.05) between treatments for the same genotype (applies all figures).

Plate 5B.5. Inflorescence production capacity for three genotypes.
Top = Genotype I, Middle = Genotype II and Bottom = Genotype III
(Photographed on 17 January 1992).

5B.3.3.2. Initial and final floret numbers per inflorescence

The number of initial florets/inflorescence for early, middle and late inflorescences differed significantly among genotypes, but was not affected by paclobutrazol (Table 5B.16). Among the three genotypes, genotype I had significantly higher numbers of florets/inflorescence for all three inflorescence categories, and the floret numbers/inflorescence for early and middle inflorescences in genotype II were significantly higher than the numbers in genotype III. No measurements were taken for late inflorescences in genotype III. There were no significant genotypeXpaclobutrazol interactions in initial floret numbers/ inflorescence for all three inflorescence categories.

The final number of florets/inflorescence retained (i.e. at ripening) for early, middle and late inflorescences also differed significantly among genotypes, with the significant high number to low number ranking being genotype I, genotype II and genotype III respectively (Table 5B.17). Paclobutrazol did not affect floret numbers/inflorescence in early and middle inflorescences, but it affected the numbers in late inflorescences (Table 5B.17). However, there were significant genotypeXpaclobutrazol interactions in floret numbers/inflorescence for all three inflorescence categories. In early inflorescences, paclobutrazol applied in October significantly increased floret numbers/inflorescence in genotype I and genotype II when compared to the control (Table 5B.17A). On the other hand, the November application significantly reduced floret numbers/inflorescence in genotype I, but it significantly increased the numbers in genotype II (Table 5B.17A). Floret numbers/inflorescence from the early inflorescences of genotype III were not affected by both the October and November applications (Table 5B.17A). Although there were significant genotypeXpaclobutrazol interactions for middle inflorescences, both the October and November applications did not affect floret numbers/inflorescence when treatments were compared for the same genotypes (Table 5B.17B). In late inflorescences, the October application significantly increased floret numbers/inflorescence compared to the control in genotype I but not in genotype II, while the November application did not change floret numbers (Table 5B.17C).

Table 5B.16. Effect of paclobutrazol on the initial number of florets/inflorescence in different genotypes.

A. Early inflorescences

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	108.3	107.1	98.9	104.8
Genotype II	84.5	86.1	89.3	86.6
Genotype III	67.9	63.0	69.5	66.8
Treatment means	86.9	85.4	85.9	

LSD (P=0.05) : genotype means = 10.1, treatment means = NS, genotypeXtreatment = NS.
CV : main plots = 11 %, subplots = 14 %

B. Middle inflorescences

Genotype I	122.4	122.5	123.1	122.7
Genotype II	93.1	89.3	91.1	91.2
Genotype III	63.0	59.5	61.4	61.3
Treatment means	92.8	90.4	91.9	

LSD (P=0.05) : genotype means = 6.3, treatment means = NS, genotypeXtreatment = NS.
CV : main plots = 6.4 %, subplots = 9.7 %

C. Late inflorescences

Genotype I	109.6	133.1	111.1	117.9
Genotype II	103.1	101.3	95.9	100.1
Treatment means	106.4	117.2	103.5	

LSD (P=0.05) : genotype means = 15.6, treatment means = 11.6, genotypeXtreatment = NS.
CV : main plots = 12 %, subplots = 15 %

Table 5B.17. Effect of paclobutrazol on the final number of florets/inflorescence in different genotypes.

A. Early inflorescences

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	98.5	104.8	92.6	98.6
Genotype II	79.8	85.8	85.9	83.8
Genotype III	67.6	62.9	66.6	65.7
Treatment means	82.0	84.5	81.7	

LSD (P=0.05) : genotype means = 3.2, treatment means = NS,
between treatments for the same genotype = 5.6,
between treatments for different genotypes = 6.1.
CV : main plots = 4.6 %, subplots = 6.7 %

B. Middle inflorescences

Genotype I	112.8	119.8	109.8	114.1
Genotype II	88.8	87.5	87.0	87.8
Genotype III	63.4	61.1	59.9	61.5
Treatment means	88.3	89.5	85.2	

LSD (P=0.05) : genotype means = 4.1, treatment means = NS,
between treatments for the same genotype = 7.2,
between treatments for different genotypes = 7.2.
CV : main plots = 4.4 %, subplots = 8.1 %

C. Late inflorescences

Genotype I	94.6	109.9	93.3	99.3
Genotype II	93.0	90.6	88.5	90.7
Treatment means	93.8	100.3	90.9	

LSD (P=0.05) : genotype means = 8.2, treatment means = 6.2,
between treatments for the same genotype = 8.7,
between treatments for different genotypes = 10.9.
CV : main plots = 7.3 %, subplots = 9.0 %

Irrespective of treatment effects, in genotype I, middle inflorescences had significantly higher initial floret numbers/inflorescence than early inflorescences but not late inflorescences, while late inflorescences also did not differ from early inflorescences (Table 5B.18). The middle inflorescences of genotype I also retained significantly more florets/inflorescence at ripening, while early inflorescences did not differ from late inflorescences (Table 5B.18). On the other hand, in genotype II, late inflorescences had a significantly greater number of initial florets/inflorescence, while early inflorescences did not differ from middle inflorescences (Table 5B.18). The late inflorescences of genotype II had significantly higher floret numbers/ inflorescence retained at ripening than early inflorescences but not middle inflorescences, while middle inflorescences also did not differ from early inflorescences (Table 5B.18). There were no significant differences between early and middle inflorescences in genotype III in both initial floret numbers/ inflorescence and final floret numbers/inflorescence (Table 5B.18). Regardless of genotype characteristics, floret abortion in late inflorescences was high, i.e. 10 % or more, while floret abortion in early and middle inflorescences was less than 10 % (Table 5B.18).

5B.3.3.3. Ovule numbers/carpel and seed numbers/floret

There were significant differences between genotypes in ovule numbers/carpel, and the numbers also differed depending upon the position of the florets (top, middle or bottom) on the inflorescences. However, there were significant genotypeXfloret position interactions. Comparing ovule numbers/carpel between genotypes at the same floret position on the early inflorescences, genotype I had significantly higher ovule numbers/carpel at the top, middle and bottom position, while genotype II did not differ from genotype III, except that the numbers from its florets at the bottom position were significantly higher (Table 5B.19). Genotype I also had higher ovule numbers/carpel from florets at the top, middle and bottom position on the middle and late inflorescences, and genotype II had significantly higher ovule numbers/carpel at all position on the middle inflorescences than

Table 5B.18. Initial floret numbers/inflorescence, floret numbers/inflorescence at ripening and floret abortion in different inflorescence categories for three different genotypes.

	Inflorescence categories	Initial florets/ inflorescence	Final florets/ inflorescence	Abortion (%)
Genotype I	Early	105	99	5.7
	Middle	123	114	7.3
	Late	118	99	16.1
LSD (P=0.05)		16	14	
CV (%)		7.0	6.8	
Genotype II	Early	87	84	3.5
	Middle	91	88	3.3
	Late	100	90	10.0
LSD (P=0.05)		5	5	
CV (%)		2.9	2.6	
Genotype III	Early	67	66	1.5
	Middle	61	61	0.0
LSD (P=0.05)		NS	NS	
CV (%)		4.3	3.4	

Table 5B.19. Effect of paclobutrazol on ovule numbers/carpel from early inflorescences for three different genotypes.

Genotypes	Floret position	Paclobutrazol treatments			Genotype means
		Control	October	November	
Genotype I	Top	6.1	6.5	6.4	
	Middle	7.0	7.5	7.3	
	Bottom	7.8	8.1	7.9	7.18
Genotype II	Top	6.0	6.0	5.8	
	Middle	6.2	6.2	6.0	
	Bottom	6.4	6.4	6.3	6.14
Genotype III	Top	5.8	5.9	5.8	
	Middle	6.1	6.1	6.0	
	Bottom	6.2	6.2	6.1	6.02

GenotypeXfloret position means

Floret position	Genotype I	Genotype II	Genotype III	Position means
Top	6.33	5.93	5.83	6.03
Middle	7.27	6.13	6.07	6.49
Bottom	7.93	6.37	6.17	6.82

GenotypeXpaclobutrazol means

Paclobutrazol treatments	Genotype I	Genotype II	Genotype III	Treatment means
Control	6.97	6.20	6.03	6.40
October	7.37	6.20	6.07	6.55
November	7.20	6.03	5.97	6.40

LSD (P=0.05):
genotype means = 0.11, position means = 0.05, treatment means= 0.12,
between genotypes for the same or different position = 0.15,
between position for the same genotypes = 0.11,
between treatments for the same genotype = 0.29,
between treatments for different genotypes = 0.20.
floret positionXpaclobutrazol = NS, genotypeXpositionXpaclobutrazol = NS.

genotype III (Tables 5B.20 and 5B.21). There were no measurements for late inflorescences in genotype III. Although ovule numbers/carpel from florets at the bottom and middle position in genotype III were not statistically different for both early and middle inflorescences, the earlier florets developed within the inflorescences, i.e. from bottom to top position, the higher the ovule numbers/carpel developed (Tables 5B.19, 5B.20 and 5B.21).

Paclobutrazol affected ovule numbers/carpel in all inflorescence categories. There were significant genotypeXpaclobutrazol interactions in ovule numbers/carpel for early and middle inflorescences. In early inflorescences, paclobutrazol applied in October significantly increased ovule numbers/carpel compared to the control in genotype I but not in genotype II and genotype III, while the November application did not have an effect (Table 5B.19). On the other hand, in middle inflorescences, the November application significantly reduced ovule numbers/carpel compared to the control in genotype I but not in genotype II and genotype III, while the October application did not have an effect (Table 5B.20). However, in late inflorescences, the November application significantly increased ovule numbers/carpel compared to the control in all genotypes, while the October application did not differ (Table 5B.21).

There were no significant floret positionXpaclobutrazol or genotypeXfloret positionXpaclobutrazol interactions in ovule numbers/carpel for all three inflorescence categories.

As with ovule numbers/carpel, seed numbers/floret retained at ripening differed among genotypes, and the numbers also differed depending upon the position of the florets on the inflorescences. However, there were significant genotypeXposition interactions as well. Comparing seed numbers/floret among genotypes at the same floret position on the early inflorescences, genotype II had significantly higher numbers at the top, middle and bottom position, and genotype I had significantly higher seed numbers/floret than genotype III, except that the numbers from its

Table 5B.20. Effect of paclobutrazol on ovule numbers/carpel from middle inflorescences for three different genotypes.

Genotypes	Floret position	Paclobutrazol treatments			Genotype means
		Control	October	November	
Genotype I	Top	7.6	7.5	6.9	
	Middle	7.9	7.8	7.4	
	Bottom	8.1	8.2	7.7	7.67
Genotype II	Top	6.1	6.1	6.1	
	Middle	6.4	6.4	6.6	
	Bottom	6.8	6.7	6.8	6.44
Genotype III	Top	5.7	5.8	5.7	
	Middle	5.9	6.0	6.0	
	Bottom	6.0	6.1	6.1	5.92

GenotypeXfloret position means

Floret position	Genotype I	Genotype II	Genotype III	Position means
Top	7.33	6.10	5.73	6.39
Middle	7.70	6.47	5.97	6.71
Bottom	8.00	6.77	6.07	6.95

GenotypeXpaclobutrazol means

Paclobutrazol treatments	Genotype I	Genotype II	Genotype III	Treatment means
Control	7.87	6.43	5.87	6.72
October	7.83	6.40	5.97	6.73
November	7.33	6.50	5.93	6.59

LSD (P=0.05):
genotype means = 0.17, position means = 0.06, treatment means= 0.10,
between genotypes for the same or different position = 0.19,
between position for the same genotypes = 0.12,
between treatments for the same genotype = 0.24,
between treatments for different genotypes = 0.22.
floret positionXpaclobutrazol = NS, genotypeXpositionXpaclobutrazol = NS.

Table 5B.21. Effect of paclobutrazol on ovule numbers/carpel from late inflorescences for two different genotypes.

Genotypes	Floret position	Paclobutrazol treatments			Genotype means
		Control	October	November	
Genotype I	Top	6.2	6.3	6.5	
	Middle	7.4	7.8	7.7	
	Bottom	8.0	8.0	8.0	7.32
Genotype II	Top	6.0	6.0	6.1	
	Middle	6.4	6.4	6.6	
	Bottom	6.9	6.9	7.1	6.49
Treatment means		6.82	6.90	7.00	

	GenotypeXfloret position means		
Floret position	Genotype I	Genotype II	Position means
Top	6.33	6.03	6.18
Middle	7.63	6.47	7.05
Bottom	8.00	6.97	7.49

LSD (P=0.05):
genotype means = 0.14, position means = 0.07, treatment means= 0.10,
between genotypes for the same or different position = 0.17,
between position for the same genotypes = 0.11,
GenotypeXtreatment = NS, floret positionXpaclobutrazol = NS,
genotypeXpositionXpaclobutrazol = NS.

florets at the top position did not differ (Table 5B.22). Genotype II also had significantly higher seed numbers/floret at the bottom and middle position on the middle inflorescences (Table 5B.23). On the other hand, although its seed numbers/floret at the top position were still significantly higher than the numbers in genotype I, the numbers were significantly lower than the numbers in genotype III, i.e. genotype III had the highest numbers (Table 5B.23). Nevertheless, genotype I did not differ from genotype III in seed numbers/floret at the middle position, and in fact, its numbers at the bottom position were significantly higher than numbers in genotype III (Table 5B.23). In late inflorescences, genotype II did not differ from genotype I in seed numbers/floret at the bottom and middle position, but the numbers from its florets at the top position were significantly higher (Table 5B.24). No measurements were taken for late inflorescences in genotype III.

Similar to ovule numbers/carpel, florets that were developed early within the inflorescences had higher seed numbers/floret retained at the ripening stage than the numbers in florets developed later (Plate 5B.6). However, not all differences were statistically significant. In genotype I, seed numbers/floret from florets at the bottom and middle position on the early inflorescences were not significantly different, while in genotype II, the numbers from florets at the bottom and middle position were not significantly different in both early and late inflorescences (Table 5B.22 and 5B.24). On the other hand, the non significant difference which occurred in genotype III was between seed numbers/floret from florets at the bottom and middle position on the middle inflorescences (Table 5B.23).

Paclobutrazol applied in both October and November significantly increased seed numbers/floret compared to the control in early inflorescences, while seed numbers/floret in late inflorescences were not affected (Table 5B.22 and 5B.24). There were no significant genotypeXpaclobutrazol interactions in seed numbers/floret for early and late inflorescences. Although paclobutrazol affected seed numbers/floret in middle inflorescences, there were significant genotypeXpaclobutrazol interactions. Paclobutrazol applied in October significantly increased

Table 5B.22. Effect of paclobutrazol on seed numbers/floret from early inflorescences for three different genotypes.

Genotypes	Floret position	Paclobutrazol treatments			Genotype means
		Control	October	November	
Genotype I	Top	2.4	3.8	3.4	
	Middle	4.5	4.9	4.9	
	Bottom	4.6	5.3	5.0	4.31
Genotype II	Top	4.0	4.7	4.3	
	Middle	5.2	5.4	5.3	
	Bottom	5.3	5.7	5.4	5.03
Genotype III	Top	3.0	3.5	3.1	
	Middle	4.3	4.1	4.2	
	Bottom	4.4	4.5	4.5	3.96
Treatment means		4.19	4.66	4.46	

GenotypeXfloret position means

Floret position	Genotype I	Genotype II	Genotype III	Position means
Top	3.20	4.33	3.20	3.58
Middle	4.77	5.30	4.20	4.76
Bottom	4.97	5.47	4.47	4.97

LSD (P=0.05):
genotype means = 0.43, position means = 0.15, treatment means= 0.23,
between genotypes for the same or different position = 0.47,
between position for the same genotypes = 0.25,
GenotypeXtreatment = NS, floret positionXpaclobutrazol = NS,
genotypeXpositionXpaclobutrazol = NS.

Table 5B.23. Effect of paclobutrazol on seed numbers/floret from middle inflorescences for three different genotypes.

Genotypes	Floret position	Paclobutrazol treatments			Genotype means
		Control	October	November	
Genotype I	Top	1.7	2.5	2.4	
	Middle	4.3	5.0	4.3	
	Bottom	5.2	5.3	4.8	3.94
Genotype II	Top	3.1	3.7	3.3	
	Middle	4.8	5.5	5.4	
	Bottom	5.6	6.0	5.7	4.79
Genotype III	Top	3.7	4.1	4.3	
	Middle	4.3	4.8	4.8	
	Bottom	4.6	4.8	5.0	4.49

GenotypeXfloret position means

Floret position	Genotype I	Genotype II	Genotype III	Position means
Top	2.20	3.37	4.03	3.20
Middle	4.53	5.23	4.63	4.80
Bottom	5.10	5.77	4.80	5.22

GenotypeXpaclobutrazol means

Paclobutrazol treatments	Genotype I	Genotype II	Genotype III	Treatment means
Control	3.73	4.50	4.20	4.14
October	4.27	5.07	4.57	4.64
November	3.83	4.80	4.70	4.44

LSD (P=0.05):
genotype means = 0.21, position means = 0.12, treatment means= 0.15, between genotypes for the same or different position = 0.29,
between position for the same genotypes = 0.23,
between treatments for the same genotype = 0.38,
between treatments at different genotypes = 0.30.
floret positionXpaclobutrazol = NS, genotypeXpositionXpaclobutrazol = NS.

Table 5B.24. Effect of paclobutrazol on seed numbers/floret from late inflorescences for two different genotypes.

Genotypes	Floret position	Paclobutrazol treatments			Genotype means
		Control	October	November	
Genotype I	Top	2.4	2.6	2.7	
	Middle	4.7	4.8	4.7	
	Bottom	5.4	5.3	5.2	4.20
Genotype II	Top	3.8	3.8	3.2	
	Middle	5.0	5.1	5.0	
	Bottom	5.1	5.2	5.0	4.58
Treatment means		4.40	4.47	4.30	

GenotypeXfloret position means

Floret position	Genotype I	Genotype II	Position means
Top	2.57	3.60	3.09
Middle	4.73	5.03	4.88
Bottom	5.30	5.10	5.20

LSD (P=0.05):
genotype means = 0.32, position means = 0.29, treatment means= NS,
between genotypes for the same or different position = 0.49,
between position for the same genotypes = 0.40,
GenotypeXtreatment = NS, floret positionXpaclobutrazol = NS,
genotypeXpositionXpaclobutrazol = NS.

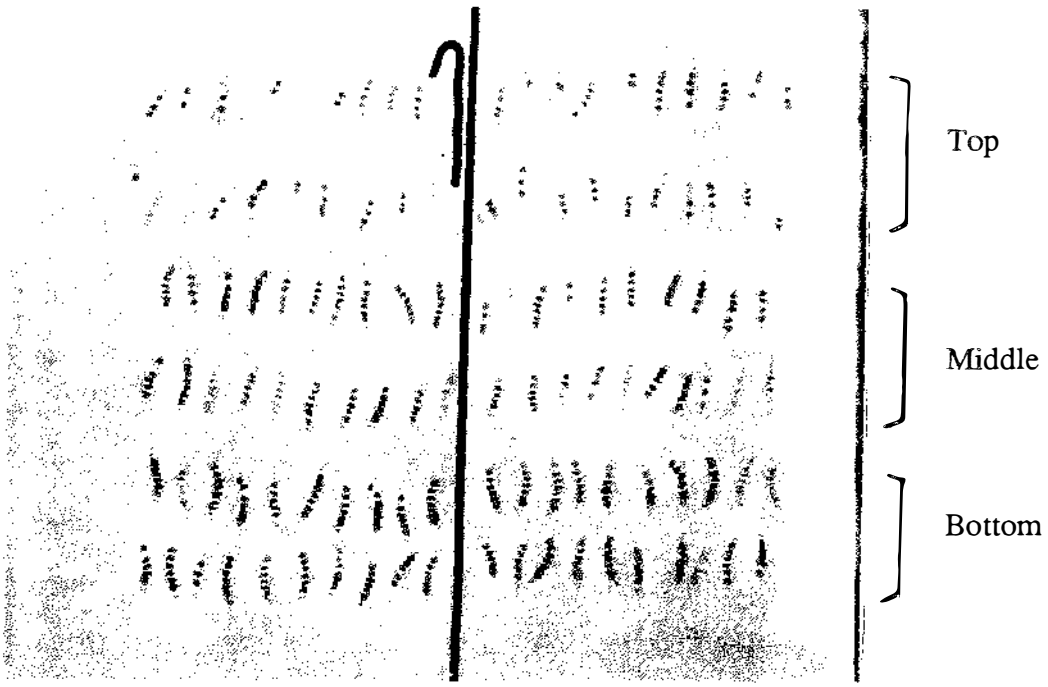


Plate 5B.6. Seeds/floret from florets at the top, middle and bottom position on the inflorescences. X-rayed from two randomly selected genotype II inflorescences (separated by the middle line).

seed numbers/floret compared to the control in genotype I and genotype II but not in genotype III, while the November application significantly increased the numbers in genotype III but not in genotype I and genotype II (Table 5B.23).

There were no significant floret positionXpaclobutrazol or genotypeXfloret positionXpaclobutrazol interactions in seed numbers/floret for all three inflorescences categories.

Irrespective of floret position and paclobutrazol effects, ovule numbers/carpel and seed numbers/floret from early, middle and late inflorescences in genotype I were not significantly different, hence ovule/seed abortion was also not different among the three inflorescence categories (Table 5B.25). On the other hand, in genotype II, late inflorescences had significantly higher ovule numbers/carpel than early inflorescences but not middle inflorescences, while middle inflorescences also did not differ from early inflorescences (Table 5B.25). However, there were no significant differences among inflorescence categories in seed numbers/floret (Table 5B.25). This indicated that late inflorescences in genotype II aborted significantly more ovules/seeds than early inflorescences but not middle inflorescences, while middle inflorescences also did not differ from early inflorescences (Table 5B.25). In genotype III, there was no significant difference between early and middle inflorescences in ovule numbers/carpel, but middle inflorescences had higher seed numbers/floret than early inflorescences (Table 5B.25). Thus, early inflorescences significantly aborted more ovules/seeds than middle inflorescences (Table 5B.25). Regardless of some differences mentioned above, the average ovule/seed abortion in genotype I (44 %) was far higher than in the other two genotypes, which had an average ovule/seed abortion of less than 30 %, i.e. 25 % and 29 % for genotype II and genotype III respectively.

Table 5B.25. Mean ovule number/carpel, seed number/floret and % seed abortion in different inflorescence categories for three different genotypes.

	Inflorescence categories	Ovule number/ carpel	Seed number/ floret	Abortion (%)
Genotype I	Early	7.18	4.31	41
	Middle	7.67	3.94	49
	Late	7.32	4.20	44
LSD (P=0.05)		NS	NS	NS
CV (%)		3.1	8.6	10
Genotype II	Early	6.14	5.03	18
	Middle	6.44	4.79	26
	Late	6.49	4.58	30
LSD (P=0.05)		0.29	NS	11
CV (%)		1.5	5.3	17
Genotype III	Early	6.02	3.96	34
	Middle	5.92	4.49	24
LSD (P=0.05)		NS	0.51	7
CV (%)		2.6	12	24

5B.3.3.4. Thousand seed weight

Thousand seed weight (TSW) for early, middle and late inflorescences differed significantly among genotypes (Table 5B.26). Genotype I developed a significantly higher TSW for all three inflorescence categories. TSW from the early inflorescences of genotype III was significantly higher than TSW from the early inflorescences of genotype II, but TSW from the middle inflorescences of genotype II was significantly higher than TSW from the middle inflorescences of genotype III. There was no measurement for late inflorescences in genotype III. Paclobutrazol significantly increased TSW compared to the control in early and middle inflorescences, but it did not affect late inflorescences (Table 5B.26). However, there were significant genotypeXpaclobutrazol interactions in TSW for late inflorescences, while there were not for early and middle inflorescences. When compared to the control, paclobutrazol applied in October also significantly increased TSW from the late inflorescences of genotype I but not from the late inflorescences of genotype II, while the November application did not differ (Table 5B.26).

Regardless of genotype and treatment differences, TSW from early and middle inflorescences in genotype I did not differ, but it was significantly higher than TSW from late inflorescences (Table 5B.27). Similarly, there were no differences between TSW from early and middle inflorescences in genotype II (Table 5B.27). However, only TSW from middle inflorescences was significantly higher than TSW from late inflorescences, while TSW from early inflorescences did not differ (Table 5B.27). There were no significant differences between TSW from early and middle inflorescences in genotype III (Table 5B.27).

Table 5B.26. Effect of paclobutrazol on thousand seed weight (g) in different genotypes.

A. Early inflorescences

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	0.726	0.758	0.764	0.749
Genotype II	0.574	0.614	0.636	0.608
Genotype III	0.628	0.640	0.637	0.635
Treatment means	0.643	0.671	0.679	

LSD (P=0.05) : genotype means = 0.023, treatment means = 0.027, genotypeXtreatment = NS.
CV : main plots = 3.2 %, subplots = 7.1 %

B. Middle inflorescences

Genotype I	0.743	0.778	0.764	0.762
Genotype II	0.639	0.642	0.662	0.648
Genotype III	0.608	0.625	0.630	0.621
Treatment means	0.663	0.682	0.685	

LSD (P=0.05) : genotype means = 0.017, treatment means = 0.015, genotypeXtreatment = NS.
CV : main plots = 2.3 %, subplots = 3.7 %

C. Late inflorescences

Genotype I	0.693	0.724	0.701	0.706
Genotype II	0.596	0.578	0.574	0.583
Treatment means	0.645	0.651	0.638	

LSD (P=0.05) : genotype means = 0.022, treatment means = NS,
between treatments for the same genotype = 0.026
between treatments for different genotypes = 0.030
CV : main plots = 2.8 %, subplots = 3.9 %

Table 5B.27. Thousand seed weight in different inflorescence categories for three different genotypes.

Genotypes	Inflorescence categories	Thousand seed weight (g)
Genotype I	Early	0.749
	Middle	0.762
	Late	0.706
LSD (P=0.05)		0.036
CV (%)		2.5
Genotype II	Early	0.608
	Middle	0.648
	Late	0.583
LSD (P=0.05)		0.041
CV (%)		3.4
Genotype III	Early	0.635
	Middle	0.621
LSD (P=0.05)		NS
CV (%)		1.5

5B.3.3.5. Seed yield/plant

Total seed yield/plant differed significantly among genotypes. Genotype I produced a significantly higher total seed yield/plant (mean = 74.04 g/plant), and genotype II produced a significantly higher total seed yield/plant than genotype III (mean = 56.45 g/plant and 3.76 g/plant for genotype II and genotype III respectively). Paclobutrazol did not affect total seed yield/plant. However, there were significant paclobutrazol effects on seed yield at particular harvest times. Paclobutrazol applied in October significantly increased seed yield/plant obtained from inflorescences harvested on 22 and 29 December 1991 compared to the control, with the mean seed yield/plant for the October paclobutrazol and control plants being 2.20 g/plant and 1.35 g/plant respectively on 22 December 1991, and 2.62 g/plant and 1.84 g/plant respectively on 29 December 1991. On the other hand, the November application did not have any effects on yield. There were no significant genotypeXpaclobutrazol interactions in seed yield/plant harvested at these two harvest dates. At the 5 and 12 January harvests, there were genotypeXpaclobutrazol interactions. When compared to the control, paclobutrazol applied in October significantly increased seed yield/plant in genotype II but not in genotype I and genotype III at both the 5 and 12 January harvests (Figure 5B.7). In contrast, the November application significantly reduced seed yield in genotype I but not in genotype II and genotype III at the 5 January harvest, while it did not differ from the control at the 12 January harvest (Figure 5B.7). There were no significant paclobutrazol effects on seed yield/plant at the other harvest times (Figure 5B.7).

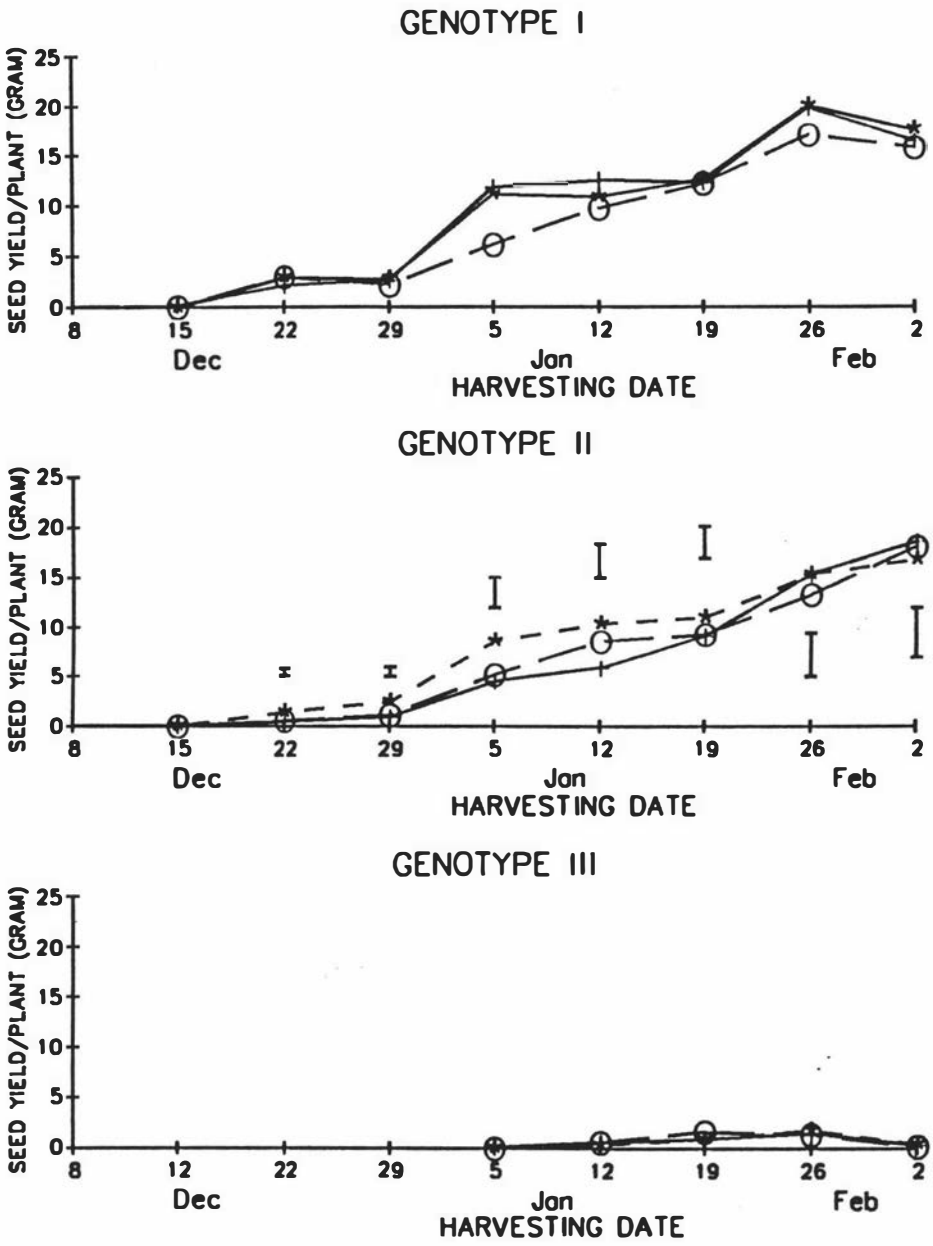


Figure 5B.7. Effect of paclobutrazol on seed yield/plant in three cv. Grasslands Pitau white clover genotypes.
I = LSD (P=0.05) between treatments for the same genotype.

5. DISCUSSION

In experiment 5A, although no quantitative measurements were made, clear visible differences in plant morphological characteristics, particularly plant height and stolon length were observed among genotypes within the population of white clover cv. Grasslands Pitau. These differences were confirmed in experiment 5B, where plant height and stolon length differed significantly among genotypes (Figure 5B.3 and Table 5B.1). Since within experiments the plants experienced the same environmental conditions (weather, soil fertility, soil moisture and weed and pest control), the morphological differences were presumably due to genetic factors. White clover is an outcrossing species and hence is genetically variable (Williams, 1987a). White clover populations in permanent grassland are characterized by remarkable genetic diversity within very small areas. A study by Burdon (1980) showed that in a random sample of 50 plants taken from one 60-year-old pasture in North Wales, nearly every plant was found to differ significantly from every other plant in at least one out of 22 characters recorded.

Despite their morphological differences, growth of all genotypes in experiment 5A was initially retarded by paclobutrazol, as indicated by reduced leaf size and petiole length (Table 5A.1), but the effects did not last long. Similarly in experiment 5B, the retardation effects following the November application were evident for only four to five weeks after application, and no retardation effects following the October application were detected when measurements were taken two months after application (Figure 5B.4). Paclobutrazol also induced only short term growth retardation in China aster (*Callistephus chinensis* L.) (Phetpradap L., 1992) and dahlia (Phetpradap S., 1992). However, Hampton and Hebblethwaite (1984a) reported that paclobutrazol continued to reduce stem length in ryegrass up to harvest (14 weeks after application). The reason for these differences is not known. Nevertheless, one thing is similar in white clover, dahlia and China aster; they are indeterminate plants which have the characteristic of active vegetative growth which continues during the reproductive growth stage. On the other hand, ryegrass is a

species which has a determinate growth pattern, i.e. vegetative growth ceases at the onset of the reproductive growth stage. It is possible that indeterminate plants may have a capacity to relatively rapidly overcome the interference of paclobutrazol in blocking gibberellin biosynthesis. However, whether this actually happened is not known. In addition, although paclobutrazol is a plant growth retardant, it is interesting to note that in experiment 5B plant growth increased (as shown by the increase in plant height) in three different genotypes within two to three months after paclobutrazol application in October, or during recovery from paclobutrazol retarding effects (Figure 5B.4), while the November application increased plant height only in one genotype (section 5B.3.2.1). Ogata and Saito (1987) also reported that vigorous regrowth occurred (as indicated by higher growth rate) when cherry and apple trees recovered from paclobutrazol treatments. The reason for this again is not known. A study of changes in hormonal balance within the plant following paclobutrazol application might help to explain the short duration of paclobutrazol retardation effects and also the effects during recovery from paclobutrazol in white clover.

Although leaf size and petiole length were reduced, plant dry weight was not affected by paclobutrazol (Table 5A.3). This suggests that whole plant photosynthetic performance was not affected by paclobutrazol. There are three possibilities to explain this, as follows:

1. The first possibility is related to chlorophyll content. As in the previous sward trials, the leaves of paclobutrazol treated plants were darker than those of the control following application. Measurements indicated that paclobutrazol significantly increased leaf chlorophyll content per unit leaf area (Table 5A.2). Similar increases in chlorophyll content following paclobutrazol application have also been reported in other species, e.g. sugar beet (*Beta vulgaris* L.) (Jaggard *et al.*, 1982), pecan (*Carya illinoensis*) (Wood, 1984) and soybean (*Glycine max* (L.) Merrill) (Sankhla *et al.*, 1985). However, it is not clear whether increased chlorophyll content is due to enhanced chlorophyll biosynthesis or is simply a 'concentrating effect' due to reduced cell size. In

sugar beet, it is reported that the number of chloroplasts per cell and number of cells per unit leaf area were not affected following paclobutrazol application, while chlorophyll content per unit leaf area was doubled (Dalziel and Lawrence, 1984). This suggests that each chloroplast must presumably contain more chlorophyll (Dalziel and Lawrence, 1984; Lawrence and Mayne, 1991). It is also possible that paclobutrazol reduces chlorophyll catabolism, at least in senescing tissues. For example, excised leaves from triazole treated Kentucky bluegrass (*Poa pratensis* L.) retained their chlorophyll longer than leaves from untreated plants when placed in the dark (Kane and Smiley, 1983). Davis *et al.*, (1988) suggested that studies on the activities of enzymes involved with chlorophyll synthesis, e.g. ALA synthetase, and breakdown, e.g. chlorophyllase, would be useful in further assessing the influence of paclobutrazol on chlorophyll content. Chlorophyll, which has the function of absorbing light energy, is essential for the photosynthetic process. However, whether increased chlorophyll content in response to treatment with paclobutrazol has a favourable influence on the rate of photosynthesis cannot be answered adequately as yet. Results from paclobutrazol trials in sugar beet (Jaggard *et al.*, 1982) and soybean (Sankhla *et al.*, 1985) do not support the suggestion of such an influence. However, if increased chlorophyll content is linked to a prolonged functioning of the photosynthetic apparatus, an overall higher photosynthetic performance is to be expected simply as a result of delayed senescence (Jung, 1979; Kane and Smiley, 1983; Sankhla *et al.*, 1985). In this regard, several investigators, i.e. Jaggard *et al.*, (1982) in sugar beet, Hampton and Hebblethwaite (1985a) in perennial ryegrass (*Lolium perenne* L.) and Marini (1987) in peach (*Prunus persica*), have noted that leaves on paclobutrazol treated plants were retained longer than comparable leaves on controls. However, this 'stay-green' phenotype might be also due to fungicidal properties of paclobutrazol (Hampton, 1983; Sugavanam, 1984). The fungicidal activity of paclobutrazol might control leaf microflora which play an active part in the senescence process (Dickinson, 1973).

2. The second possibility is related to carbon dioxide exchange. There are many literature references to correlations between photosynthesis rate per unit leaf area and leaf thickness (Barden, 1977; Nobel, 1977; Patterson *et al.*, 1978; Jaggard *et al.*, 1982; Yun and Taylor, 1986; Anderson *et al.*, 1988). Thick leaves have, per unit leaf area, larger surface areas of mesophyll cells exposed to the intercellular air spaces and therefore provide improved opportunities for CO_2 exchange to the sites of photosynthesis which might result in an increase of net photosynthetic rate per unit leaf area. In the present trial (experiment 5A), leaf thickness was increased following paclobutrazol application (Table 5A.2). It could be expected that this would result in increased net photosynthetic rate per unit leaf area, and hence might offset reduced photoassimilate production due to reduced leaf area.
3. The third possibility is related to increased leaf numbers. Although Jaggard *et al.*, (1982) reported that the rate of net photosynthesis per unit leaf area in sugar beet was increased following paclobutrazol application, other authors have reported that paclobutrazol generally has little direct effect on net photosynthetic rates on a leaf area basis, e.g. in sunflower (*Helianthus annuus* L.) (Wample and Culver, 1983), nectarine (*Prunus nectarina*) (Dejong and Doyle, 1984), pecan (*Carya illinoensis*) (Wood, 1984; Andersen and Aldrich, 1987) and apple (*Malus pumila*) (Wieland and Wample, 1985). The rate of net photosynthesis per unit leaf area was not measured in the present experiments. If paclobutrazol also did not increase the rate of net photosynthesis per unit leaf area in white clover, even though leaf size was reduced, the fact that dry weight did not change was presumably because total leaf area for the whole plant did not change, since paclobutrazol increased the number of secondary and tertiary branches (Tables 5B.9 and 5B.10) and vegetative nodes formed along secondary branches (Table 5B.7), which in turn increased the number of leaves. In addition, although petioles were shortened following paclobutrazol application (Figure 4B.4), they also appeared to be thickened. However, unfortunately no quantitave measurements of petiole thickness were made.

Although these three possibilities need to be verified, results from the present experiments showed that the application of paclobutrazol generally did not have a detrimental effect on the plant's assimilate production capacity, and so growth and development did not differ from that of the control.

In experiment 5A, paclobutrazol reduced dry weight and also petiole length at harvest (Tables 5A.1 and 5A.3). The reason for this was perhaps the result of high rainfall in February 1991 (Appendix 5A.2). High soil moisture caused by high rainfall might have reactivated paclobutrazol residues in the soil. Hampton and Hebblethwaite (1985a) reported that paclobutrazol residues did not show an effect on *Lolium perenne* L. regrowth at three weeks after harvest, but five months after application the residues showed their effects in reducing plant growth. This suggests that paclobutrazol perhaps needs time to penetrate into the root zone, provided that it easily reaches the soil surface after it is applied to foliage and can be reactivated when soil moisture is high. The reactivation of paclobutrazol coupled with the decline of white clover seasonal growth which occurs in February (Brougham, 1959, Chapter 4) might have caused reduced plant growth and resulted in decreased plant dry weight at harvest. On the other hand, during the period of experiment 5B, rainfall was lower than average (Appendix 5A.2). Therefore, the reduced vegetative dry weight in genotype I following November paclobutrazol application might be simply because genotype I was more sensitive in response to a retardation effect of paclobutrazol than the other two genotypes. Genotype I has the largest leaf size. Proportionally, the leaf size of the three genotypes might have been similarly reduced, but in terms of actual leaf area, the reduction of leaf size in genotype I was greater than in the other two genotypes. Although no measurements were made, this possible high reduction in total leaf area might have severely reduced the capacity of the plant for photosynthesis. Thus, although there is a possibility that the rate of net photosynthesis per unit leaf area is increased by paclobutrazol (Jaggard *et al.*, 1982), any increase in the rate of net photosynthesis per unit leaf area was perhaps insufficient to compensate for the reduced leaf area in genotype I. This finding again

indicates that the secondary effects of paclobutrazol, such as its effect on photosynthesis in white clover, need to be further investigated.

The growth and development of inflorescences from their emergence to ripeness can be divided into three phases:

1. The first phase is when inflorescence heads are immature and grow below or within the canopy.
2. The second phase is from when inflorescence heads rise above the canopy to anthesis.
3. The third phase is during seed set, development and seed maturation.

Paclobutrazol altered the white clover plant canopy by making it more closed and compact, and therefore there was less light penetrating into the canopy (Appendices 5B.3 and 5B.4). This lower light penetration is considered unfavourable for inflorescence development from the time of emergence up to elevation above the canopy (Thomas, 1961b, 1987c; Dunn *et al.*, 1962; Pasumarty *et al.*, 1991). However, although the time taken from inflorescence emergence to anthesis was not affected by paclobutrazol (section 5A.3.3), in experiment 5B the November paclobutrazol application reduced the time needed by inflorescences to rise just above the plant canopy (Table 5B.5), irrespective of differences in speeds of inflorescence growth among genotypes, presumably because petiole length was reduced. This decrease in the duration of phase I might to some extent offset the potentially unfavourable effects of shade. Alternatively, the compactness of the plant canopy might provide the advantage that it prevents inflorescences from falling below the canopy. Following paclobutrazol application, peduncles initially grew more horizontally than those in the controls, but eventually grew vertically later on towards the source of light (Figure 5B.5). Although the treated peduncles could become taller than the control, as in experiment 5B, they generally maintained their erectness up to the ripening stage, as shown by a lower percentage of inflorescences falling below the plant canopy (Table 5B.4). Nevertheless, whether

this was because the peduncles were supported by the compact plant canopy or because paclobutrazol actually hardened and thickened the peduncles, or both, is not known. Observations of peduncle cell structure were not made. This matter needs further investigation. In addition, in the present trial, it was found that the ripening stage of the seed development process was speeded up by paclobutrazol, as indicated by a faster reduction in seed moisture content (Figure 5A.1). As mentioned above, inflorescences from treated plants maintained their erectness to keep their heads above the plant canopy up to the ripening stage. On the other hand, more than 65 % of inflorescences in control plants fell below the canopy (Table 5B.4). Thus, the faster reduction of seed moisture content might be simply because inflorescences from paclobutrazol treated plants were more exposed to sunshine and dry air than those from untreated plants, which experienced lower light intensity and moister conditions (high humidity) below the plant canopy.

Root:shoot ratio at harvest was not affected by paclobutrazol application (Section 5A.3.2.3). This result is contrary to results reported with other species, e.g. sugar beet (Jaggard *et al.*, 1982) and *Lolium perenne* L. (Hampton and Hebblethwaite, 1985a). Davis *et al.*, (1988) also suggested that paclobutrazol treated plants had increased root:shoot ratio due to relatively inhibited shoot growth. Generally, total white clover shoot dry weight, including vegetative organs (leaves and stolons) and reproductive organs (inflorescences and inflorescence buds), was not affected by paclobutrazol during the active growth period, i.e. one to two months after application (Table 5A.3 and Chapter 4). Therefore, the result from the present trial may support the suggestion that paclobutrazol did not reduce the plant's assimilate production, but only altered the growth pattern of the shoot, in which a reduction of dry weight in one part of the shoot was compensated by an increase of dry weight in another part of shoot (Chapters 3 and 4). However, root:shoot ratios were not determined during the period of one to two months after application. Thus, a further investigation of root:shoot ratio conducted during that period would be useful to verify whether paclobutrazol affects root growth at that time in white clover.

No evidence that paclobutrazol directly influenced inflorescence initiation was obtained in the present trial (Tables 5B.6, 5B.7 and 5B.8 and Appendix 5B.5). This is contradictory to findings under sward conditions (Marshall and Hides, 1991a,b; Chapter 4), but in agreement with the results from glasshouse experiments (Marshall and Hides, 1987, 1989). The differences might have arisen from the methods used. Under sward conditions, stolons were indiscriminately selected for observation, while in individual plant trials, both in the field and glasshouse, stolon categories were carefully determined. In the present trial it was found that all stolon categories, i.e. main stolons and their secondary, tertiary, quaternary and quinary branches, were capable of developing inflorescences. However, their capabilities are dependent on genotypic characteristics. Quaternary and quinary branches in some genotypes did not develop inflorescences. In fact, a few genotypes in experiment 5A did not produce inflorescences at all. In addition, in the present trial, node initiation was closely monitored before and after paclobutrazol application, while in the sward trial (Chapter 4) 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 needs to be done to verify this.

The capacity of white clover plants to develop stolon branches is dependent on their genetic characteristics. Some genotypes (such as genotypes I and II in the present trial) can produce up to five different branches from their main stolons. On the other hand, some genotypes such as genotype III do not produce quinary branches. Similarly, when stolons were classified according to Thomas' (1987a) definition at harvest, there were significant differences in total stolon numbers among genotypes (Table 5B.12). However, regardless of genotypic differences in the ability to develop stolon branches, paclobutrazol significantly increased the production of secondary and tertiary, and to a lesser extent quaternary branches in all three genotypes (Tables 5B.9, 5b.10 and 5B.11). This is in agreement with results reported for *Lotus uliginosus* Schkuhr. (Clifford and Hare, 1987; Hampton *et al.*, 1989; Tabora, 1991) and *Lotus corniculatus* L. (Hampton *et al.*, 1989). These

investigators suggested that branches were increased as a result of the suppression of apical dominance by paclobutrazol. From a seed production point of view, an increase of stolon branches can be considered as an advantage for increasing seed yield, i.e. more stolons mean more sites for inflorescence production. However, this is not necessarily the case for all genotypes. From the present trial, only genotype II produced more inflorescences during the mid flowering period, while genotypes I and III did not. This variation perhaps was due chiefly to different requirements for cold temperatures between genotypes, which in turn affect plant responses to day length for inflorescence initiation (Thomas, 1987c). Genotypic variations in inflorescence production capacity were also found in cv. Grasslands Huia by Maldonado (1985). Nevertheless, the results point out that environmental factors, such as low temperature and day length, and their relation with unidentified growth substances within the plant which induce inflorescence initiation (Thomas, 1987c), are also, along with genetic factors, important in determining seed yield in white clover. Cultural practice manipulation, e.g. to increase stolon numbers, will only have a limited effect on inflorescence numbers if stolons are developed when ambient temperatures exceed the critical low temperatures needed for inflorescence initiation. Nevertheless, the results in the present trial suggest that an increase of inflorescences following paclobutrazol application under sward conditions is most likely to be the result of an increase in stolon numbers. Reports by Marshall and Hides (1991a,b) and results in Chapters 3 and 4 indirectly support this possibility. In addition, variation due to genetic differences in response to the floral stimulus might be also a major cause of non-significant results for seed yield components and seed yield in experiment 5A, although phenotypic variation might also contribute. Therefore, the following discussion of the effect of paclobutrazol on other seed yield components and seed yield is mainly based on the results from experiment 5B.

The number of florets initiated per inflorescence in cv. Grasslands Pitau for three inflorescence categories, i.e. early, middle and late inflorescences, differed among genotypes, and was not affected by paclobutrazol (Table 5B.16). However, paclobutrazol significantly affected the number of florets per inflorescence retained

at the ripening stage, although the effects differed with genotype, inflorescence category and application time (Table 5B.17). The October application significantly increased the number of florets per inflorescence at harvest for early inflorescences in genotypes I and II and for late inflorescences in genotype I, but had no effect on genotype III. No effects on floret numbers occurred for middle inflorescences in any of the three genotypes. On the other hand, the November application only increased the number of florets per inflorescence at harvest for early inflorescences in genotype II. In fact, it significantly reduced the number of florets per inflorescence at harvest in early inflorescences in genotype I. Marshall and Hides (1991b) also found that paclobutrazol applied at the stage when reproductive buds were first visible reduced the number of florets per inflorescence. They suggested that this reduction occurred because paclobutrazol application reduced leaf size. Management that reduces leaf size similarly reduces the number of florets per inflorescence (Clifford, 1987). In the 1991/1992 trial, the effect of paclobutrazol on leaf size was not measured. However, the November paclobutrazol application significantly reduced plant height and stolon length, particularly in genotype I, during early flowering. Thus, it could be expected that leaf size in genotype I might also have been reduced by the November application. Nevertheless, the results indicate that paclobutrazol has the potential to reduce floret abortion, although this was less pronounced in the present trial, particularly in middle inflorescences. In *Lotus uliginosus* Schkuhr, Tabora (1991) reported that paclobutrazol reduced floret abortion in the middle inflorescences but not in the early and late inflorescences. Hampton and Hebblethwaite (1985b) also reported that paclobutrazol reduced floret abortion in *Lolium perenne* L. as a result of better assimilate supplies to the basal, intermediate and terminal sections of the ear. The less pronounced effect in the present trial might be because the floret abortion which occurred in both treated and untreated plants was relatively low. Had floret abortion been high due to adverse climatic conditions, paclobutrazol perhaps could have shown a greater response in terms of reducing floret abortion. However, this matter needs to be verified. In addition, under sward conditions (for commercial seed production), the harvest mostly comes from middle (mid season) inflorescences. The results from the present

trial might explain why the number of florets per inflorescence in sward trials (Chapters 3 and 4) was not affected by paclobutrazol when measured at harvest. Regardless of treatment and genotype differences, late inflorescences aborted more florets than early and middle inflorescences (Table 5B.18). Under natural conditions, floret numbers per inflorescence decrease from spring to summer (Clifford, 1979; Thomas, 1981d). Therefore, the increase of abortion in late inflorescences was perhaps simply a manifestation of seasonal effects.

Number of ovules per carpel differed among genotypes (Tables 5B.19, 5B.20 and 5B.21). However, the three different genotypes in experiment 5B were similar in that the basal (lower) florets on an inflorescence possessed more ovules than the apical (upper) florets. In Ladino clover, the lower florets on an inflorescence also tend to contain approximately one more ovule per carpel than the upper florets (Dessureaux, 1951). Pasumarty (1990) also reported a similar tendency in inflorescences taken from the same plants used in the 1989/1990 sward trial (Chapter 4). From a study with *Lupinus luteus*, which has a similar ovule formation pattern, Van Steveninck (1957) suggested that this pattern might occur, in part, because the upper florets have vascular supplies which are not as well developed as those to the lower florets, and this might affect availability of assimilates for ovule formation. Whether this also occurs in white clover is not known. This matter needs to be investigated further.

Generally, paclobutrazol had little effect on ovule formation (Tables 5B.19, 5B.20 and 5B.21). However, it still showed some effects which were conflicting. The October application increased ovules per carpel only in the early inflorescences of genotype I. On the other hand, the November application reduced ovules per carpel in the middle inflorescences of genotype I, but it increased ovules per carpel in the late inflorescences of genotypes I and II. The results suggest that genotype I was more sensitive to paclobutrazol application than the other two genotypes, and that the effect of paclobutrazol on ovule formation might be related to its effect on assimilate availability. During the period when early inflorescences were initiating

and developing ovules, the October application started to lose its retarding effect, and plants began to increase growth. This increased growth might have resulted in more assimilate being available for ovule formation in early inflorescences. However, if this is true, why did the increase in ovule formation only occur in one genotype, although all three genotypes showed increased growth ? Furthermore, the October application increased plant growth during a period when middle inflorescences were initiating and developing ovules, but it had no effect on ovule formation in these middle inflorescences. This might be because increased growth suggests increased demand to support this growth, and consequently assimilates for ovule formation are still limited. Another possibility is that paclobutrazol improved the translocation of assimilates to reproductive sites, which in turn increased ovule formation. Hampton (1983) reported that paclobutrazol increased the partitioning of assimilates to the ear of *Lolium perenne* L. at the expense of vegetative tillers. However, this again does not explain why genotypes respond differently. Nevertheless, this possibility could perhaps be used to explain increased ovule formation in the late inflorescences of genotypes I and II following the November application. On the other hand, the November application retarded plant growth at the time the middle inflorescences were initiating and developing ovules (12-19 December 1991). At the same time, paclobutrazol might also have disturbed the translocation of assimilate to inflorescences and florets, and hence reduced ovule formation in the middle inflorescences of genotype I. Whether this actually did occur is not known. This aspect needs to be further investigated.

Probably of most importance is the effect of paclobutrazol in reducing seed abortion. Paclobutrazol significantly increased the number of seeds retained per floret at the ripening stage (Tables 5B.22, 5B.23 and 5B.24). Both the October and November applications increased seed numbers per floret in early inflorescences for all three genotypes in experiment 5B. The October application also increased seed numbers per floret in the middle inflorescences for genotypes I and II, but not genotype III. On the other hand, the November application increased seed numbers per floret in the middle inflorescences only for genotype III. The number of seeds

per floret in late inflorescences was not affected by paclobutrazol. The results clearly indicate that, in terms of persistency, October application consistently affected plant growth and development within two to three months of application. Non significant differences in the number of seeds per floret for the middle inflorescences of genotype III arose simply because the inflorescences in that genotype were developed more than three months after the October application, by which time the chemical was perhaps no longer effective and hence did not reduce seed abortion. On the other hand, the November application had shorter-lived effects than the October application in genotypes I and II but not in genotype III. The reason for this is not clear. However, the results demonstrate that genotypes respond differently to paclobutrazol application. Irrespective of the differences discussed above, paclobutrazol has promising potential for reducing seed abortion in white clover. Tabora (1991) also found that paclobutrazol reduced seed abortion in the middle inflorescences of *Lotus uliginosus* Schkuhr. There are two possible explanations as to how paclobutrazol might reduce seed abortion. Firstly, it may increase assimilate availability to support seed development processes, in particular, ovule provisioning through increased assimilate production as a result of increased net photosynthetic rate (Jaggard *et al.*, 1982) and improved assimilate translocation to reproductive sites (Hampton, 1983; Hampton and Hebblethwaite, 1985b). Insufficient assimilate during ovule provisioning may result in higher seed abortion (Atwood, 1940; Clifford, 1986a; Pasumarty *et al.*, 1992a,b). Secondly, paclobutrazol application tended to result in inflorescences being elevated above the plant canopy as petiole length was decreased, and hence these inflorescences received a greater light intensity, which is favourable for the seed development process. Inflorescences that are shaded tend to develop fewer seeds. This is because shading increases ovule sterility (Pasumarty and Thomas, 1990; Pasumarty *et al.*, 1992a,b), and may also reduce *in situ* photosynthesis (Pasumarty, 1987), and hence result in more competition for assimilates among florets within an inflorescence and among seeds within a floret. Also a combination of the above two possibilities is clearly likely. Further study on photosynthesis and assimilate translocation following paclobutrazol application may help to evaluate these possibilities.

Thousand seed weight differed among genotypes (Table 5B.26). Irrespective of these differences though, paclobutrazol significantly increased thousand seed weight for all the three genotypes in experiment 5B. In addition, it also tended to increase thousand seed weight in experiment 5A. A possible reason for this might be, as discussed earlier, the improvement of assimilate availability for ovule provisioning following paclobutrazol application. However, the results differed from those obtained in sward trials. Paclobutrazol either did not affect or tended to reduce thousand seed weight under sward conditions (Chapters 3 and 4). This might be because competition for assimilate in individual plants was lower than competition in sward conditions. The growth of individual plants is not restricted by available space, and there is no shading by neighbouring plants. Therefore, the plants can more fully utilize light and nutrients available in the soil for photosynthesis. On the other hand, in plants under sward conditions, growth is restricted by available space and by the shading caused by neighbouring plants, so that the plants cannot fully utilize light and nutrients, as competition among plants within a restricted area is high. Pasumarty (1990) also suggested that in terms of inflorescence development, spaced plants with an open canopy were better than a denser sward with a closed canopy because:

1. Fertility of ovules is reduced when shading occurs at the time inflorescence heads are still immature and grow below the plant canopy.
2. Post fertilization abortion of fertile ovules is increased when shading occurs during seed set and seed maturation due to insufficient production of photo-assimilates.

This insufficient photoassimilate production also reduces the weight of surviving fertilized ovules during ovule provisioning. There are two ways by which shading reduces photoassimilates for ovule provisioning. A shortage of assimilates might result firstly from a change in partitioning of assimilates in favour of peduncles at the expense of flower heads due to shading (Pasumarty *et al.*, 1991), and secondly and more directly, from a reduction in *in situ* photosynthesis (Pasumarty, 1987).

Nevertheless, whether paclobutrazol actually affected photosynthesis in a dense sward is not known. This aspect was not investigated in the previous sward trials, and needs to be further studied.

Seed yield from the middle inflorescences of genotype II was significantly increased by paclobutrazol, while the other two genotypes were not affected (Figure 5B.7). In all three genotypes, however, there were some effects of paclobutrazol in reducing seed abortion (Section 5B.3.3.3), and increasing seed weight (Table 5B.26), but the plant growth regulator had no significant effect on seed yield in genotypes I and III (percentage total seed yield differences between paclobutrazol treatment and control for genotype I were - 0.2 % and - 15 % , and for genotype III were - 0.2 % and 14 % for October and November applications respectively). Only in genotype II did paclobutrazol significantly increase inflorescences (Figure 5B.6) and the significant increase of seed yield in genotype II most likely resulted from the significant increase in middle inflorescences following paclobutrazol application. This result agrees with those from sward trials, where paclobutrazol increased white clover seed yield mainly by increasing inflorescence production (Hampton, 1991; Marshall and Hides, 1989, 1991b; Chapter 3). It is interesting that although it was increased by paclobutrazol, the seed yield of genotype II was never higher than the seed yield of genotype I. This points to the importance of genetic factors in determining white clover seed yield. To improve the seed production capacity of white clover, factors such as the capability of plants to produce high numbers of inflorescences, florets per inflorescence, seeds per floret and high seed weight, should be incorporated into white clover plant breeding programmes (Van Bockstaele and Rijckaert, 1988). Initial selection for improving white clover seed production based on inflorescence production capabilities has recently been carried out in Russia (Yakuts and Kurchak, 1991).

CHAPTER 6

EFFECT OF PACLOBUTRAZOL AND DIFFERENT HARVEST TIMES ON SEED YIELD COMPONENTS AND SEED YIELD IN WHITE CLOVER CV. GRASSLANDS PITAU

6.1. INTRODUCTION

The development of white clover seed from pollination to ripeness occupies about 26 days, but may be reduced or extended by approximately five days depending on weather conditions (Hyde, 1950; Hyde *et al.*, 1959). Thus, harvesting of white clover for maximum yield of quality seed should be based on this seed development time frame. However, white clover has a protracted flowering pattern during which inflorescence buds, blooming inflorescences, young pods and mature pods ready to dehisce can be present simultaneously on an individual plant (Norris, 1984; Thomas, 1987c; Hollington *et al.*,1989; Marshall *et al.*, 1989; Chapter 5). Early harvesting can result in unripe inflorescences being gathered, but a late harvest can miss the older, earlier produced, inflorescences which may have fallen below cutting height or from which seed may have been shed or sprouted. Therefore, the most consistent method of determining an optimum harvest date may be associated with the time after peak flowering. Clifford (1985b) recommended harvesting six weeks after peak flowering to maximize seed yields with low seed losses. However, Holloway (1987) found that the best seed yield was obtained from harvesting 28 days after peak flowering. Harvest delay resulted in a linear decline in seed yields.

In a first year crop, potential harvested seed yield was significantly increased by paclobutrazol, but actual seed yield was not significantly affected (Chapter 3). This indicated that preharvest seed losses in paclobutrazol treated plants were probably higher than the losses in untreated plants. Results from the individual plant trial

showed that paclobutrazol reduced the time of seed development during the ripening stage (Chapter 5A). Therefore, optimal harvesting time for paclobutrazol-treated white clover plants might differ from that for untreated plants.

This trial examined the effect of paclobutrazol and different harvest times, based on the days from peak flowering, on seed yield components and seed yield, and whether there was any interaction between paclobutrazol and harvest time in seed yield recovery.

6.2. MATERIALS AND METHODS

6.2.1. Experimental site, management and treatments

The trial was conducted on the Frewin's block of the Pasture and Crop Research Unit, Massey University, Palmerston North during the 1990/1991 growing season. The site selected had not been used for any plant growth regulator experiments previously. A full description of soil type and soil fertility is presented in Appendices 3.1A and 3.1B. The site was cultivated by ploughing on April 1990, left fallow over winter, rotary hoed on 10 September 1990 and rolled on 11 September 1990. The same plant materials as described in section 5A.2.1 were used for this trial. The experiment was conducted as a sward simulation by transplanting 252 plants into an area of 30 m^2 using an interrow spacing of 45 cm and intrarow spacing of 15 cm (42 plants per plot) on 12 September 1990. Nitrogen as urea was applied on 28 September 1990 at the rate of 46 kg N/ha. Plants were irrigated during the first month after transplanting using a garden sprinkler, which delivered water at a rate of 10 l/m^2/hour, for 30 minutes irrigation/day. To control weeds, the herbicide MCPB was applied on 8 November 1990 at a rate of 1.2 kg a.i/ha or 3 l MCPB/ha. MCPB, a selective herbicide for use in white clover to control broadleaf weeds (Anon., 1990), is absorbed mainly through leaves and readily translocates in the plant with nutrient movement. Susceptible plants convert MCPB to MCPA which interferes

with cell division and enlargement. The plants were defoliated to 5 cm from ground level using a rotary mower on 16 November 1990, and cut plant material was removed from the plots.

The trial utilized a three replicate split plot design with a paclobutrazol treatment as a main plot and harvest time as a sub plot. The size of a main plot was 2.5 x 1.25 m. Two paclobutrazol treatments were applied: paclobutrazol 1 kg a.i/ha applied on 27 November 1990 (reproductive bud visible) and nil paclobutrazol as a control. Paclobutrazol was applied with water at a volume equivalent to 500 l/ha by a knapsack sprayer with four fan nozzles held 25-30 cm above the herbage. Harvest was conducted at five different times, i.e. 25, 30, 35, 40 and 45 days after the first flowering peak (see section 6.3.2 and Figure 6.1), from a quadrat of 0.25 m^2 by cutting all plant material to ground level using an electric shearing machine.

6.2.2. Plant measurements and statistical analysis

Flowering pattern was determined by counting the number of white inflorescences within two permanent quadrats of 0.5 x 0.5 m established in each main plot at a regular interval of five days.

Seed yield components (inflorescence numbers/unit area, floret numbers/ inflorescence, seed numbers/floret and thousand seed weight), potential harvestable seed yield/unit area, actual seed yield/unit area, seed yield recovery, vegetative dry weight/unit area and harvest index were measured from harvested material obtained from a quadrat of 0.25 m^2 from each sub plot. The inflorescences produced from each sub plot were separated into two groups, i.e. ripe and unripe inflorescences (see section 4.2.2 and Plate 4.1). Floret numbers/inflorescence were counted from ten randomly selected ripe inflorescences/sub plot. Seed numbers/floret were determined from 100 florets/sub plot using an X-ray method as described in section 3.2.2. Thousand seed weight (TSW) was determined by weighing 4 x 100 seeds/sub plot and correcting to 10 % seed moisture content.

Potential harvestable seed yield/unit area, actual seed yield/unit area, vegetative dry weight/unit area and harvest index were measured as described for the 1988/1989 experiments (section 3.2.2). Only ripe inflorescences (see section 4.2.2 and Plate 4.1) were used to calculate potential harvestable seed yield. Actual seed yield was corrected to 10 % seed moisture content. Seed yield recovery was measured using the formula:

$$\text{Seed yield recovery} = \frac{\text{Actual seed yield}}{\text{Potential harvestable seed yield}} \times 100\ \%$$

Apart from data for flowering pattern, which were analyzed according to a randomized complete block design analysis, data collected in this experiment were mostly analysed according to a split plot design analysis by the use of analysis of variance and Fisher's LSD test at P=0.05 and P=0.10 (for the number of inflorescences/unit area). Variances over five harvest times for the number of infloresces and seed yield data were suspected to be heterogenous since there was no significant interaction between treatment and harvest time, while treatment differences differed considerably among five harvest times (Dr. I. Gordon, pers. comm.) Based on this assumption, a separate analysis for harvests at 25, 30 and 35 days after peak flowering and for harvests at 40 and 45 days after peak flowering was performed. However, results for treatment effects (Appendices 6.2, 6.3, 6.4 and 6.5) were similar to those from the original analysis (using five harvest times), and therefore, the results presented are based on the original anlysis. Seed yield recovery data were arcsine transformed prior to analysis. Because the significance of results from transformed data did not differ from those of the original data, the seed yield recovery results are presented using original values.

6.3. RESULTS

6.3.1. Meteorological conditions

The meteorological conditions during the 1990/1991 growing season have been described in section 5A.3.1 and data are presented in Appendix 5A.2.

6.3.2. Flowering pattern

Flowering started in early December, and two flowering peaks occurred (Figure 6.1). The first flowering peak occurred on 8 January 1991 for paclobutrazol-treated plants and on 3 January 1991 for control plants. The second flowering peaks for both the treated and untreated plants occurred on 19 January 1991. Because there were no significant differences in the number of inflorescences/m^2 produced on 3, 8, 14 and 19 January 1991 by the control plants (Appendix 6.1), harvest time for both treatments was calculated from the 8 January 1991 (the first flowering peak for paclobutrazol treated plants). Paclobutrazol significantly increased the number of inflorescences/m^2 at the first peak flowering, but there were no significant differences for any of the other dates (Figure 6.1).

6.3.3. Inflorescence numbers

Increases in total inflorescence numbers/m^2 produced following paclobutrazol application were not significant at the P=0.05 level, but were significantly increased at P=0.10 (Table 6.1). The increase was attributed to a significant increase (P=0.10) in unripe inflorescences/m^2 (Table 6.1). The ripe inflorescences/m^2 from paclobutrazol treated plants were always higher than the numbers from the control plants, but they were not statistically different (Table 6.1).

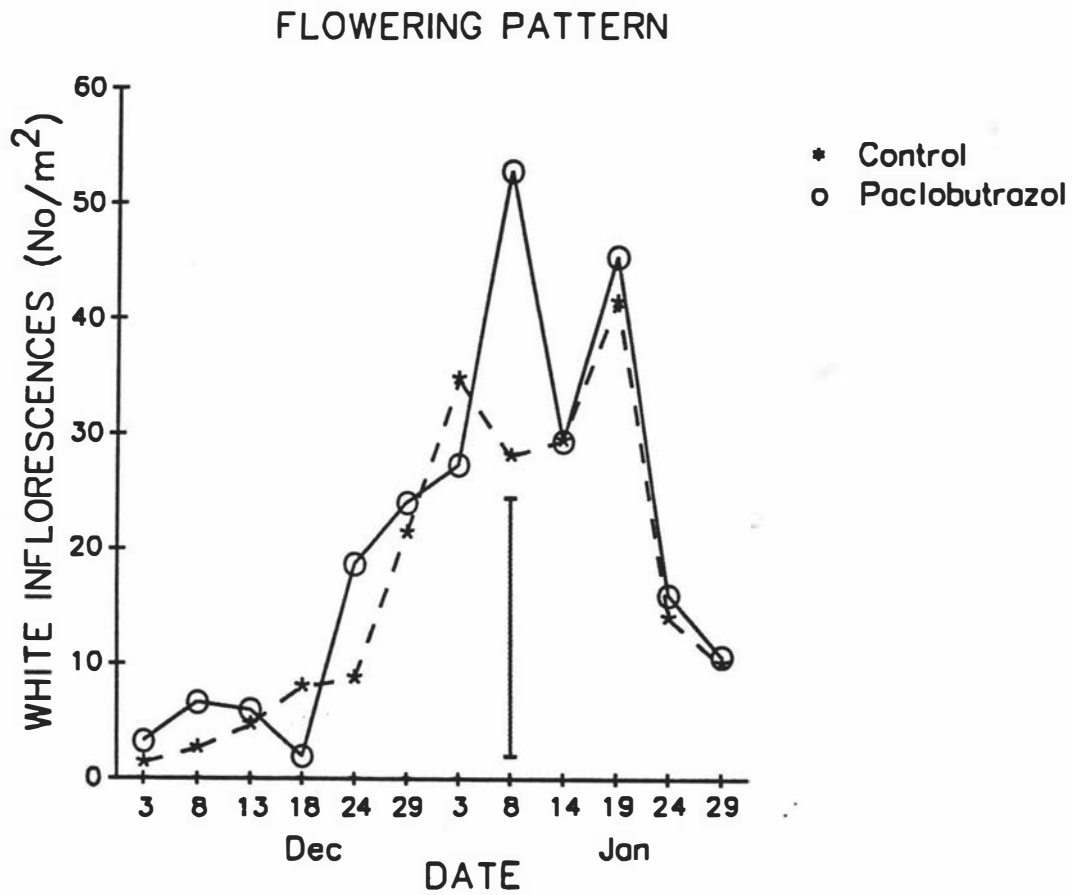


Figure 6.1. Effect of paclobutrazol on flowering pattern.
I = LSD (P=0.05) between the paclobutrazol-treated plants and the controls.

Table 6.1. Effect of paclobutrazol and different harvest times on the number of inflorescences.

A. Ripe inflorescences (no./m^2)

Treatments	Harvest times (days after peak flowering)					Treatment means
	25	30	35	40	45	
Control	181.3	153.3	188.0	142.7	70.7	147.2
Paclobutrazol	317.3	212.0	278.7	166.7	80.0	210.9
Harvest means	249.3	182.7	233.4	154.7	75.4	

LSD (P=0.05):treatment means = NS, harvest means = 63.3,treatmentXharvest= NS.
LSD (P=0.10):treatment means = NS, harvest means = 52.1,treatmentXharvest= NS.
CV: main plots = 24 %, sub plots = 29 %.

B. Unripe inflorescences (no./m^2)

Control	45.3	61.3	42.7	34.7	12.0	**39.2**
Paclobutrazol	92.0	73.3	81.3	76.0	22.7	**69.1**
Harvest means	68.7	67.3	62.0	55.4	17.4	

LSD (P=0.05): treatment means = NS, harvest means = NS, treatmentXharvest= **NS**.
LSD (P=0.10): treatment means= 20.4, harvest means= NS, treatmentXharvest= **NS**.
CV: main plots = 16 %, sub plots = 86 %.

C. Total inflorescences (no./m^2)

Control	226.6	214.6	230.7	177.4	82.7	186.4
Paclobutrazol	409.3	285.3	360.0	242.7	102.7	280.0
Harvest means	318.0	250.0	295.4	210.1	92.7	

LSD (P=0.05): treatment means=NS, harvest means= 106.6, treatmentXharvest=NS.
LSD (P=0.10): treatment means=82.8, harvest means= 87.8, treatmentXharvest=NS.
CV: main plots = 15 %, sub plots = 37 %.

The highest total inflorescences/m^2 were obtained when harvest was conducted at 25 days after the first flowering peak (Table 6.1). However, delaying harvest up to 35 days after the first flowering peak did not significantly change inflorescence numbers. On the other hand, delaying harvest to 40-45 days after the first flowering peak significantly reduced the total inflorescences/m^2 compared to harvest at 25 days after the first flowering peak. Similarly, although the highest number of ripe inflorescences/m^2 was obtained when harvest was conducted at 25 days after the first flowering peak, harvest at 35 days after the first flowering peak did not reduce the number of ripe inflorescences/m^2 compared to harvest at 25 days after the first flowering peak (Table 6.1). However, harvest at 30 days after the first flowering peak significantly reduced the numbers compared to harvest at 25 days after the first flowering peak, but it did not differ from harvest at 35 days after the first flowering peak. Delaying harvest to 40-45 days after the first flowering peak significantly reduced the number of ripe inflorescences/m^2 compared to harvest at 25 days after the first flowering peak (Table 6.1). There were no significant effects of different harvest times on the number of unripe inflorescences/m^2 (Table 6.1).

There were no significant paclobutrazolXharvest time interactions for the number of ripe, unripe and total inflorescences/m^2.

6.3.4. Floret numbers/inflorescence, seed numbers/floret and TSW

Paclobutrazol and different harvest times did not affect floret numbers/ inflorescence, seed numbers/floret or TSW, and there were no significant paclobutrazolXharvest time interactions for these parameters (Table 6.2).

Table 6.2. Effect of paclobutrazol and different harvest times on floret numbers/ inflorescence, seed numbers/floret and thousand seed weight.

A. Floret numbers/inflorescence

Treatments	Harvest times (days after peak flowering)					Treatment means
	25	30	35	40	45	
Control	68.0	67.7	63.7	61.0	66.3	65.3
Paclobutrazol	66.7	63.7	61.3	61.0	64.0	63.3
Harvest means	67.4	65.7	62.5	61.0	65.2	

LSD (P=0.05): treatment means = NS, harvest means = NS, treatmentXharvest= NS.
CV: main plots = 6.5 %, sub plots = 8.0 %.

B. Seed numbers/floret

Control	4.4	4.4	4.3	3.7	4.1	4.2
Paclobutrazol	4.3	4.6	4.5	4.1	3.6	4.2
Harvest means	4.4	4.5	4.4	3.9	3.9	

LSD (P=0.05): treatment means = NS, harvest means = NS, treatmentXharvest= NS.
CV: main plots = 11 %, sub plots = 16 %.

C. Thousand seed weight (g)

Control	0.625	0.571	0.558	0.568	0.545	0.573
Paclobutrazol	0.587	0.568	0.582	0.617	0.530	0.577
Harvest means	0.606	0.570	0.570	0.593	0.538	

LSD (P=0.05): treatment means = NS, harvest means = NS, treatmentXharvest= NS.
CV: main plots = 3.6 %, sub plots = 10.9 %.

6.3.5. Potential harvestable seed yield, actual seed yield, seed yield recovery and harvest index

Paclobutrazol increased both potential harvestable seed yield/m^2 and actual seed yield/m^2 by 56 %, but the increases were not statistically significant (Table 6.3). Harvest at 25 days after the first flowering peak gave the highest yield/m^2 for both potential harvestable yield and actual seed yield (Table 6.3). However, delaying harvest up to 35 days after the first flowering peak did not result in a significant reduction of the yields compared to harvest at 25 days after the first flowering peak. On the other hand, delaying harvest to 40-45 days after the first flowering peak significantly reduced the yields compared to harvest at 25 days after the first flowering peak. There were no significant paclobutrazolXharvest time interactions for either potential harvestable seed yield or actual seed yield. In this trial, seed yield recovery was not affected by paclobutrazol or different harvest time, and there were no significant paclobutrazolXharvest time interactions (Table 6.3). The average seed yield recovery was 70 %.

Vegetative dry weight/m^2 at harvest was not significantly affected by paclobutrazol (Table 6.4). Paclobutrazol also did not significantly affect actual seed yield/m^2 (Table 6.3). Therefore, harvest index was not significantly affected, although there was a 50 % improvement in harvest index by paclobutrazol compared to the control (Table 6.4). Different harvest times significantly affected vegetative dry weight/m^2 (Table 6.4). Although not differing significantly from harvest at 30 days after the first flowering peak, harvest at 25 days after the first flowering peak resulted in the highest vegetative dry weight/m^2 gathered. When harvest was conducted at 35-45 days after the first flowering peak, vegetative dry weight/m^2 was significantly decreased compared to harvest at 25 days after the first flowering peak. Although actual seed yield was also affected by different harvest times (Table 6.3), harvest index was strongly dependent on harvest time. Harvest at 25 to 35 days after the first flowering peak gave higher harvest index than harvest at 40 to 45 days after the first flowering peak (Table 6.4). There were no significant paclobutrazolXharvest time interactions for vegetative dry weight/m^2 or harvest index.

Table 6.3. Effect of paclobutrazol and different harvest times on potential harvestable seed yield/m^2, actual seed yield/m^2 and seed yield recovery.

A. Potential harvestable seed yield (g/m^2)

Treatments	Harvest times (days after peak flowering)					Treatment means
	25	30	35	40	45	
Control	33.6	25.8	29.8	16.9	9.6	23.1
Paclobutrazol	55.3	36.9	47.9	26.8	12.9	36.0
Harvest means	44.5	31.4	38.9	21.9	11.3	

LSD (P=0.05): treatment means= NS, harvest means= 13.2, treatmentXharvest= NS.
CV: main plots = 33 %, sub plots = 36 %.

B. Actual seed yield (g/m^2)

Control	25.5	20.8	21.1	10.0	6.2	16.7
Paclobutrazol	37.3	28.8	36.7	17.9	9.1	26.0
Harvest means	31.4	24.8	28.9	14.0	7.7	

LSD (P=0.05): treatment means= NS, harvest means= 10.0; treatmentXharvest= NS.
CV: main plots = 44 %, sub plots = 38 %.

C. Seed yield recovery (%)

Control	78	80	72	60	62	70
Paclobutrazol	66	73	72	67	72	70
Harvest means	72	77	72	64	67	

LSD (P=0.05): treatment means = NS, harvest means = NS, treatmentXharvest= NS.
CV: main plots = 9.4 %, sub plots = 15.3 %.

Table 6.4. Effect of paclobutrazol and different harvest times on vegetative dry weight/m^2 and harvest index.

A. Vegetative dry weight (g/m^2)

Treatments	Harvest times (days after peak flowering)					Treatment means
	25	30	35	40	45	
Control	409.8	419.7	343.5	355.5	362.6	378.2
Paclobutrazol	402.1	349.9	376.6	322.2	344.8	359.1
Harvest means	406.0	384.8	360.1	338.9	353.7	

LSD (P=0.05): treatment means= NS, harvest means= 42.5, treatmentXharvest= NS.
CV: main plots = 10.5 %, sub plots = 9.4 %.

B. Harvest Index (%)

Control	5.4	4.3	5.3	2.5	1.5	3.8
Paclobutrazol	7.4	6.6	7.6	4.8	2.1	5.7
Harvest means	6.4	5.5	6.5	3.7	1.8	

LSD (P=0.05): treatment means = NS, harvest means = 2.0, treatmentXharvest= NS.
CV: main plots = 35 %, sub plots = 35 %.

6.4. DISCUSSION

In 1990/1991, paclobutrazol again had no effect on the flowering duration of a first year white clover crop under simulated sward conditions (Figure 6.1). The flowering duration of the present crop was about two and a half months (early December to mid February) which was about the same duration as a first year crop in a previous experiment (Chapter 3). The number of flowering peaks was also similar, i.e. two peaks. However, there were differences in the time at which the flowering peaks occurred. In the 1990/1991 trial, the first flowering peak occurred at the end of the first week of January 1991, while it occurred at the end of the first week of December 1989 in the previous experiment. The second flowering peaks occurred at 11 and 16 days after the first flowering peak for the 1990/1991 and the 1988/1989 trials respectively. The differences might be because in the 1990/1991 trial the crop was defoliated on 16 November 1990. Therefore, there might have been a shift in flowering pattern without any reduction in the flowering duration. Clifford (1979) reported that crops closed in mid November started flowering two weeks after the final topping and peak flowering was shifted a week later compared to September and October closings. Thomas (1981b) and Maldonado (1985) also reported that late defoliation delayed flowering and inflorescence formation, and that mid November defoliation resulted in peak flowering occurring in mid January. Defoliation causes a brief cessation of growth as well as a reduction in stored assimilates until photosynthesis is reestablished at a level that meets the growth requirements of the plants (Harris, 1978). Therefore, it is to be expected that defoliation causes an initial reduction in stolon elongation and hence a delay in the date of peak flowering.

Paclobutrazol significantly increased the number of inflorescences/m^2 produced at the first flowering peak (Figure 6.1). This result was in agreement with the results from the previous trials, in both first and second year crops (Chapters 3 and 4). Paclobutrazol also increased (P=0.10) total inflorescence numbers/m^2 at harvest, which was attributed to an increase (P=0.10) in unripe inflorescences (Table 6.1).

On the other hand, although the numbers were increased by 43 % compared to the control, paclobutrazol did not significantly affect ripe inflorescence numbers. An explanation for this might be because the variation between main plots was high (CV = 24 %). This high variation was perhaps primarily because of genotypic variation (Chapter 5B). Some genotypes in the population of the 1990/1991 plant material perhaps responded positively to paclobutrazol application, as shown by the high percentage difference (43 %). However, considering the limited number of plants used in this trial (as a sward simulation), the increase might not have exceeded the neutral and negative responses by some genotypes within the population in some main plots, and therefore the result was a high variation between main plots. Whether this did occur is not known and needs to be further investigated.

There were no significant paclobutrazolXharvest time interactions in the number of inflorescences/m^2 (Table 6.1). However, the estimation of the optimum time of harvest using the time from first peak flowering technique was proved to be precise and worthwhile, as it allowed the recovery of high inflorescence numbers for both the control and paclobutrazol-treated plants. Given that weather conditions can vary from year to year, harvest could be delayed up to 35 days after peak flowering without any significant reduction in inflorescences harvested. When harvest was delayed to 40 days or more after peak flowering, the number of harvested inflorescences was reduced significantly. However, harvest at 25 days after the first peak flowering, i.e. the time of ripening stage in the seed development process (Hyde, 1950; Hyde *et al.*, 1959), resulted in the highest number of inflorescences gathered. This is particularly recommended when two flowering peaks occur. The results from this trial showed that there was a drop in inflorescence numbers at five days after the first flowering peak, before the numbers increased again to make the second peak (Figure 6.1). The pattern clearly affected the number of ripe inflorescences harvested, as shown by the significant reduction of ripe inflorescences harvested at 30 days after the first flowering peak compared to the numbers harvested at 25 days after the first flowering peak. The numbers were

increased again when harvest was conducted at 35 days after the first flowering peak or 25 days after the second flowering peak (Table 6.1). There were no differences in the number of unripe inflorescences produced over five different harvest times. This was not surprising because there are some genotypes within the cv. Grasslands Pitau white clover population which are late inflorescence-initiating plants (Chapter 5B). Furthermore, in January and February 1991, rainfall was higher than average. To a certain extent, this wet condition was also conducive for inflorescence production through the growth and development of new stolon branches which might have reproductive buds initiated at their apices (Norris, 1984; Chapter 5B).

Similar to the 1988/1989 results, paclobutrazol did not affect floret numbers/ inflorescence, seed numbers/floret or thousand seed weight in the 1990/1991 first year crop (Table 6.2). These three seed yield components were also not affected by different harvest times (Table 6.2). Differences among the five harvest times were not expected because these components basically were determined from middle inflorescences. Harvest was calculated from the 8 January 1991 (the first flowering peak), and the second peak flowering was on 19 January 1991. As the span of five consecutive harvests was only 25 days, inflorescences harvested within this time span would be expected to come from the middle inflorescence range. In addition, the results in Chapter 5 showed that there were no big differences between inflorescence categories (early, middle and late) in their floret numbers, seeds/floret and thousand seed weight. Furthermore, the data recorded in the present trial were consistent and still within the range of floret numbers/inflorescence, seed numbers/floret and thousand seed weight found for cv. Grassland Pitau previously (Clifford, 1986a; Chapters 3 and 4). In the 1990/1991 trial, both the control and paclobutrazol treated plants lost 30 % of their potential seed yields. The degree of seed losses was also not affected by different harvest times (Table 6.4). Thus, inflorescence number was again the main factor for improving seed yield. The increase in inflorescence numbers following paclobutrazol application produced an increase in both potential harvestable seed yield and actual seed yield of 56 % compared to the control. However, once again the increases were not statistically

significant. The variation among main plots was again very high (CV= 33% and 44% for potential harvestable seed yield and actual seed yield respectively). This might have contributed to the statistically non significant results. Regarding the effect of different harvest times, there was also consistency between inflorescence numbers harvested and seed yield. Harvest at 25 days after peak flowering gave the highest seed yield for both potential harvestable seed yield and actual seed yield, but in this season harvest could be delayed for up to 35 days after peak flowering without significantly reducing seed yield.

Vegetative dry weight at harvest was not affected by paclobutrazol (Table 6.4). This might be because of the wet conditions in January and February 1991 which were favourable for vegetative regrowth and development (Norris, 1984). Nevertheless, if there had been a reduction in vegetative dry weight, and hence an improved harvest index, this reduction would have had only a small advantage for improvement in seed yield recovery, as the increased seed yield in response to paclobutrazol would be so small relative to the mass of dry matter present. This statement is in contrast with the suggestion that paclobutrazol increased seed yield in white clover through a reduction in vegetative dry weight (Rijckaert, 1991). Results from the 1988/1989 trial proved that in this cultivar, a reduction of vegetative dry weight had no advantage for improvement in seed yield recovery (Chapter 3). In addition, delaying harvest can also reduce vegetative dry weight. The growth of white clover is affected by seasonal growth periodicity (Brougham, 1959; Harris and Hoglund, 1980; Harris, 1987) and, in a monoculture, growth was maximised several weeks before daily temperature maxima reached the growth maximum for white clover (24°C, Mitchell, 1956). Under the Palmerston North environment, this maximum growth occurred in December, and from this stage the growth started to level off and decline in autumn (Brougham, 1959; see also section 4.3.2.2 of Chapter 4). The results from the present experiment also demonstrated the declining growth pattern as shown by the declining pattern of vegetative dry weight over five consecutive harvests (Table 6.4). However, this reduction of vegetative dry weight did not always reflect an improvement of harvest index because delaying harvest also reduced actual seed yield.

Hare and Lucas (1984) reported that weather conditions affected seed development in *Lotus uliginosus* Schkuhr., in which the time from pollination to seed maturity (maximum seed dry weight) was 27 days under hot and dry conditions, but 35 days under cool and moist conditions. Based on these results, they suggested that after 27 days from pollination, the crop must be inspected daily and the weather monitored to decide when harvest should be conducted for this species. Seed development in white clover is also affected by weather conditions as explained by Hyde (1950) and Hyde *et al.* (1959). However, the results of the present trial showed that there was no indication that seed development differed over five consecutive harvests, as indicated by the fact that there were no differences in thousand seed weight over the five consecutive harvests (Table 6.2). This may be because white clover seeds do not cease further development once the crop is cut (P.T.P. Clifford, pers. comm. in Hare and Lucas, 1984). In this experiment, harvested material was allowed to dry indoors at ambient temperature before the measurements were taken. In addition, from experiments with two white clover cultivars (Grasslands Tahora and Grasslands Kopu), Barnes (1990) also reported that white clover seeds harvested at different times (15, 20, 25, 30, 35 and 40 days after peak flowering) had the same seed dry weight, although the seed dry weight was determined one day after inflorescences were harvested by drying the seeds at 103 °C for 16 hours. However, Barnes (1990) did not mention whether he used only ripe inflorescences or all inflorescences from different categories for the measurements. Despite these methodological differences for determining seed weight as an indicator for seed maturity, there is a clear indication that in order to get a high seed yield, harvesting a white clover seed crop should be based on a technique which allows the recovery of the greatest number of inflorescences rather than being solely based on the seed development process. As discussed previously, flowering pattern affected the number of inflorescences harvested. Harvest 25 days after the first flowering peak resulted in the highest inflorescence numbers gathered. Delaying harvest by five days resulted in a drop in inflorescence numbers gathered following a drop in the number of inflorescences produced (Figure 6.1). This indicates that within these five days, the number of earlier produced inflorescences,

from which seed might have been shed, were perhaps offset by later produced inflorescences, which had just ripened, and hence there had been a constant inflorescence replacement. Therefore, the number of inflorescences harvested was solely dependent on flowering pattern. However, whether the constant inflorescence replacement actually did occur is not known. In addition, how long white clover inflorescences can be retained before shedding seed after they are ripe, and to what extent seed shedding from the inflorescence is influenced by weather conditions are not known. These matters need to be further investigated.

Results from individual plant trials indicated that a reduction of seed moisture content during the ripening stage in paclobutrazol-treated plants was faster than in untreated plants (Chapter 5A). This suggests that paclobutrazol perhaps also affects ovule provisioning, which may have consequences for seed maturity, and in turn in determining different harvest times for treated and untreated plants. The seed development process following paclobutrazol application was not monitored in the present trial. Nevertheless, if paclobutrazol does speed up white clover seed maturity in a sward situation, optimum harvest for treated plants would presumably be earlier than that for untreated plants. However, there were no significant paclobutrazolXharvest time interactions for both potential harvestable seed yield and actual seed yield. Although not significant, seed yields from treated plants were consistently higher than those from untreated plants for the five consecutive harvests (Table 6.3). In addition, there was also no significant paclobutrazolXharvest time interaction for seed weight. Thousand seed weight from treated plants did not differ from the control for the five consecutive harvests (Table 6.2). These results suggest that in a sward situation, paclobutrazol probably does not affect the seed development process, either by speeding up seed maturity, as in individual plants (Chapter 5B), or by delaying seed maturity, as was reported to occur in *Lolium perenne* L. (Hampton and Hebblethwaite, 1985a). Nevertheless, this requires verification.

Seed yields from paclobutrazol treated plants were consistently higher than the controls for the five consecutive harvest times, with a mean 56 % more seed for both potential harvestable seed yield and actual seed yield (Table 6.3). However, these differences were not significant. There are several possible explanations for this. Potential harvestable seed yield may have varied among samples because of the fact that some ripe inflorescences may already have shed seed. However, the percentage increases were similar for both potential harvestable seed yield and actual seed yield, and the percentage seed yield recovery was the same for treated and untreated plants, i.e. 70 % (Table 6.3). As previously noted, there was no evidence that paclobutrazol speeded up or delayed maturity under sward conditions. Therefore, the evidence suggests that the lack of significance was due to variation caused by partly by genetic variance (Chapter 5), but most likely by the fact that the sample size was not large enough to cater for this variation, particularly in trials using an out crossing species such as white clover which has large diversity within the population.

Irrespective of treatment and harvest time effects, there were large differences (30 %) between potential harvestable and actual seed yields. Large seed losses also occurred in the previous sward trials (Chapters 3 and 4). Seed losses can occur both in the field and during processing. Since seed yield recovery for the five harvest times was similar, seed losses must be a natural phenomenon, but how or why they occur was not determined in this work. The calculation of potential harvestable seed yield assumes that all the ripe inflorescences carry the mean number of seeds per floret recorded from a subsample of 100 florets. It is possible that in some of the inflorences counted, florets and/or seeds had been shed before harvest, and that the calculation of potential harvestable seed yield was therefore an overestimate. Another assumption made is that all seeds have achieved a similar size, i.e. around the mean thousand seed weight. If this is not the case, some seeds would be lost in the cleaning process, but no record was kept of these losses.

In conclusion, inflorescence numbers are still the most important factor for determing seed yield in white clover. Although there was high variation in the field, under sward conditions paclobutrazol increased the number of inflorescences produced. The increase occurred at peak flowering, in particular at the first flowering peak. Provided harvest is conducted at an optimum time, i.e. to recover the highest number of inflorescences/unit area (which in this trial was 25 days after the first flowering peak), the increase in inflorescence numbers should result in a greater seed yield.

CHAPTER 7

EFFECTS OF RESIDUAL PACLOBUTRAZOL ON THE GROWTH, DEVELOPMENT AND SEED YIELD OF A SECOND YEAR WHITE CLOVER SEED CROP

7.1. INTRODUCTION

Paclobutrazol is relatively immobile in the soil, bound mainly by organic matter (Lever, 1986), and is therefore generally persistent. As paclobutrazol is soil active and primarily dependent on root uptake (Froggatt *et al.*, 1982; Shearing and Batch, 1982), it has been reported that in some plant species, paclobutrazol effects are carried over in the year following application. Apple trees treated with paclobutrazol in one year often show a carry over effect of reduced shoot growth during the early part of the following season (Quinlan and Richardson, 1986). This might explain why sequential paclobutrazol applications in the following season became increasingly effective in controlling apple trees' shoot growth. Hampton (1988b) also reported the residual effects of paclobutrazol applied originally to perennial ryegrass cv. Grasslands Nui. At 200 days after paclobutrazol application, the stem length or plant height in wheat (*Triticum aestivum* L.), rape (*Brassica napus* L.), turnip (*Brassica rapa* L.), Italian ryegrass (*Lolium multiflorum* Lam.)and field bean (*Vicia faba* L.), which were sown in the same field, was retarded by paclobutrazol residues. In *Lotus uliginosus* Schkuhr., Clifford and Hare (1987) and Tabora (1991) also observed a paclobutrazol carry over effect of reduced shoot growth in the following growth season. In addition, Tabora (1991) reported that seed yield was significantly increased by paclobutrazol residues. Results from both sward and individual plant trials showed that paclobutrazol affected vegetative and reproductive growth and development in white clover (Chapters 3, 4, 5 and 6). However, it was not known whether the effects would be carried over in the following growth season.

The present trial was conducted to investigate the residual effects of paclobutrazol on the growth, development and seed yield of a second year white clover seed crop.

7.2. MATERIALS AND METHODS

7.2.1. Experimental site, management and treatments

The trial was conducted in the 1989/1990 growing season. The crop of white clover cv. Grasslands Pitau described in section 3.2.1 was used. This trial was conducted adjacent to the experimental plots used to investigate the effect of plant growth regulators on a second year crop described in section 4.2.1. Thus, all management practices previously detailed apply to this experiment. The experiment utilized a three replicate randomized complete block design with a plot size of 7 x 2 m. The experiment consisted of two treatments: plots that had paclobutrazol residues from the November 1988 application at a rate of 1 kg a.i./ha (Chapter 3), and plots that did not have paclobutrazol residues as control plots.

7.2.2. Plant measurements and statistical analysis

Growth analysis was conducted using the same techniques as those described in section 3.2.2. Samples from a quadrat of 0.15 m^2 per plot were taken at four growth stages: during reproductive initiation (6 October 1989), reproductive bud visible/early flowering (7 November 1989), peak flowering (7 December 1989) and harvest (3 January 1989). Dry matter composition was determined by dissecting subsamples into four components: leaf, stolon, reproductive (reproductive buds and inflorescences) and dead material. The number of both vegetative and reproductive nodes/m^2 was also measured from the subsamples. Vegetative nodes/m^2 were measured at all four growth stages, while reproductive node numbers were measured at reproductive bud visible/early flowering, peak flowering and harvest. Stolon numbers produced per m^2 were determined by measuring the number of growing

points from the subsamples at peak flowering and harvest. Leaf score (Williams *et al.*, 1964) and petiole length were measured from 20 mature unfolded leaves/plot selected at random at all the four growth stages. Peduncle length was also measured from 20 inflorescences/plot randomly selected at peak flowering and harvest.

Similarly to the 1989/1990 plant growth regulator trial (Chapter 4), harvesting was conducted on 4 January 1990. Seed yield components (inflorescence numbers/m^2, floret numbers/inflorescence, seed numbers/floret and thousand seed weight), potential harvestable seed yield/m^2 and actual seed yield/m^2 were also measured as described for the 1989/1990 plant growth regulator trial (see section 4.2.2).

Data were subjected to a randomized complete block analysis of variance and Fisher's LSD tests at P = 0.05 to determine differences between treatment means.

7.3. RESULTS

7.3.1. Meteorological conditions

The meteorological conditions during the 1989/1990 growing season have been explained in section 4.3.1 and data are presented in Appendix 3.2.

7.3.2. Effect of paclobutrazol residues on plant growth and development

7.3.2.1. Morphological characteristics

During reproductive bud visible/early flowering (November), the leaves of the plants from the paclobutrazol plots were significantly larger (shown as leaf score in Table 7.1) than the leaves of the control plants, but there were no significant differences for any of the other growth stages (Table 7.1). In November, the petiole length of the plants from the paclobutrazol plots was 5.3 cm longer than that of the

control, but this difference was not significant. Differences at the other growth stages were also not significant (Table 7.2). There were no paclobutrazol residue effects on peduncle length at peak flowering and harvest (Table 7.2).

Table 7.1. Effects of residual paclobutrazol on the leaf score of white clover cv. Grasslands Pitau at reproductive initiation (October), reproductive bud visible/early flowering (November), peak flowering (December) and harvest (January).

Treatments	October	November	December	January
Control	19.4	21.2	20.7	19.7
Paclobutrazol	19.1	22.6	21.1	19.8
LSD (P=0.05)	NS	0.4	NS	NS
CV (%)	2.8	0.5	1.2	5.7

Table 7.2. Effects of residual paclobutrazol on the petiole and peduncle lengths of white clover cv. Grasslands Pitau at reproductive initiation (October), reproductive bud visible/early flowering (November), peak flowering (December) and harvest (January).

Treatments	Petiole length (cm)				Peduncle length (cm)	
	Oct	Nov	Dec	Jan	Dec	Jan
Control	17.3	22.3	21.1	16.7	26.2	23.9
Paclobutrazol	16.0	27.6	22.4	18.7	26.3	26.4
LSD (P=0.05)	NS	NS	NS	NS	NS	NS
CV (%)	7.7	12	14	20	9.2	16

7.3.2.2. Node numbers and growing point numbers

In October, the plants from the paclobutrazol residual plots produced 47 % more vegetative nodes/m^2 than the control, but this difference was not significant (Table 7.3). Differences at the other growth stages were also not significant. Paclobutrazol residues did not affect the number of reproductive nodes/m^2 (Table 7.3). The number of growing points/m^2 at peak flowering and harvest were also not significantly affected by paclobutrazol residues, although there was a 31 % increase in January (Table 7.3).

7.3.2.3. Plant dry weight

The leaf dry weight of the plants from paclobutrazol residual plots was consistently higher than the control at all four growth stages (Table 7.4). However, no statistically significant effects of paclobutrazol residues on dry weight/m^2 for leaves, stolons, reproductive parts (reproductive buds and inflorescences), dead parts and total dry weight were found (Table 7.4).

7.3.2. Seed yield components and seed yield

There were no statistically significant paclobutrazol residue effects on seed yield components at harvest (Table 7.5). Similarly, neither potential harvested seed yield nor actual seed yield were affected by paclobutrazol residues (Table 7.6).

Table 7.3. Effects of residual paclobutrazol on vegetative and reproductive node numbers and growing point numbers in white clover cv. Grasslands Pitau at reproductive initiation (October), reproductive bud visible/ early flowering (November), peak flowering (December) and harvest (January).

A. Vegetative nodes (no./m^2)

Treatments	October	November	December	January
Control	2889	2751	2953	3233
Paclobutrazol	4233	3258	3080	3818
LSD (P=0.05)	NS	NS	NS	NS
CV (%)	32	14	21	10

B. Reproductive nodes (no./m^2)

Control	-	402	989	1082
Paclobutrazol	-	413	998	1018
LSD (P=0.05)	-	NS	NS	NS
CV (%)	-	35	14	4

C. Growing points (no./m^2)

Control	-	-	1413	1184
Paclobutrazol	-	-	1427	1549
LSD (P=0.05)	-	-	NS	NS
CV (%)	-	-	28	16

Table 7.4. Effect of residual paclobutrazol on plant dry weight of white clover cv. Grasslands Pitau at reproductive initiation (October), reproductive bud visible/early flowering (November), peak flowering (December) and harvest (January).

A. Leaf dry weight (g/m^2)

Treatments	October	November	December	January
Control	135.36	192.71	169.08	124.34
Paclobutrazol	154.71	225.65	272.33	177.43
LSD (P=0.05)	NS	NS	NS	NS
CV (%)	29	7	17	25

B. Stolon dry weight (g/m^2)

Control	129.29	117.53	194.39	183.93
Paclobutrazol	137.53	111.05	175.35	168.45
LSD (P=0.05)	NS	NS	NS	NS
CV (%)	25	12	12	11

C. Reproductive (reproductive buds and inflorescences) dry weight (g/m^2)

Control	-	22.72	68.44	89.12
Paclobutrazol	-	14.64	77.79	98.26
LSD (P=0.05)	-	NS	NS	NS
CV (%)	-	74	12	17

D. Dead material dry weight (g/m^2)

Control	-	36.66	125.58	123.23
Paclobutrazol	-	50.30	122.90	125.39
LSD (P=0.05)	-	NS	NS	NS
CV (%)	-	11	8	24

E. Total dry weight (g/m^2)

Control	264.65	357.79	557.49	520.62
Paclobutrazol	292.24	401.64	648.36	569.53
LSD (P=0.05)	NS	NS	NS	NS
CV (%)	3	4	7	11

Table 7.5. Effects of residual paclobutrazol on seed yield components at harvest.

Treatments	Inflorescence numbers/m^2			Floret no./ Inflorescence	Seed no./ Floret	TSW (g)
	Ripe	Unripe	Total			
Control	484	238	722	68.3	4.1	0.669
Paclobutrazol	426	228	654	70.7	4.0	0.654
LSD (P=0.05)	NS	NS	NS	NS	NS	NS
CV (%)	11	11	6	6	9	1

Table 7.6. Effects of residual paclobutrazol on seed yield.

Treatments	Potential harvestable seed yield (g/m^2)	Actual seed yield (g/m^2)
Control	90.74	37.65
Paclobutrazol	77.29	33.72
LSD (P=0.05)	NS	NS
CV (%)	7.6	8.6

7.4. DISCUSSION

At this site, paclobutrazol did not show a carry over effect by reducing plant growth in white clover. This result was in contrast with results in other species e.g. apple trees (Quinlan and Richardson, 1986), potatoes (Hampton, 1988b) and *Lotus uliginosus* Schkuhr. (Clifford and Hare, 1987; Tabora, 1991). Furthermore, it is interesting to note that there appeared to be an increase in growth instead of a reduction in growth, although it was only significant for one parameter (leaf score) and at only one growth stage (Table 7.1). Although not significantly different, during reproductive initiation in October 1989 the plants from the paclobutrazol plots had more vegetative nodes (Table 7.3), and hence more leaves as indicated by higher leaf dry weight (Table 7.4), than the control plants. This was presumably a result of the increase in vegetative node numbers induced by paclobutrazol in the previous year (see Chapter 3). When the rate of growth started to increase in November 1989 following the seasonal growth pattern of white clover under the Palmerston North environment (Brougham, 1959; Chapter 4), there might have been more competition, presumably for light, in the more crowded paclobutrazol plots than in the control plots. Under such conditions, Tamaki *et al.* (1973) reported that competition for light at different degrees of mutual shading stimulates cell elongation, resulting in increased plant height in broad bean plants. In other species, plants at high density also have greater height than plants at low density, e.g. in maize (*Zea mays* L.) (Balico, 1984; Tolentino, 1985), in soybean (*Glycine max* (L.) Merrill) (Chanprasert, 1988) and in China aster (*Callistephus chinensis* (L.) Nees.) (Phetpradap, 1992). Thus, the competition in the paclobutrazol plots perhaps induced cell elongation which resulted in an increased leaf size and apparent petiole length during reproductive bud visible/early flowering in November 1989. However, these small vegetative growth differences did not continue during the following growth stages in December 1989 and January 1990 (Table 7.1 and 7.2). Paclobutrazol residues also did not significantly affect the vegetative development of white clover (the number of nodes and the number of growing points) at these stages (Table 7.3). This might be because vegetative growth stopped increasing

during flowering (see Chapter 4). This finding was in agreement with results of Tabora (1991) in *Lotus uliginosus* Schkuhr., who reported that despite reduced shoot growth by paclobutrazol residues, there was no significant alteration in the number of main and lateral shoots. The results from this trial also showed that the reproductive development of white clover (the number of reproductive nodes/m^2) was not significantly affected by paclobutrazol residues (Table 7.3). Consequently, the number of inflorescences/m^2 and in turn seed yield at harvest were not significantly affected (Tables 7.5 and 7.6).

There are two possible explanations why the carry over of paclobutrazol retardation effects were not demonstrated in white clover. Firstly, perhaps there were little or no paclobutrazol residues in the soil because paclobutrazol, which was applied to the foliage, might have had difficulty in reaching the soil directly, as the soil was well covered by the herbage at the time of application. Tabora (1991) also suggested the same reason, as in his trial the November paclobutrazol application did not have a carry over effect in *Lotus uliginosus* Schkuhr., while the October paclobutrazol application reduced shoot growth. In October the plants were still small with bare ground around them, while in November the plants had a large vegetative mass and full ground coverage which presumably prevented paclobutrazol from reaching the soil directly. Paclobutrazol levels in the field soils were not determined. However, if paclobutrazol residues were in the soil, it might have been difficult to explain why plant growth and development in white clover were not affected. The suggestion that soil residual activity may differ at different sites because of changes in soil type (Hampton, 1988b), is not an answer because this present trial was conducted in the same two growing seasons (1988/1989 - 1989/1990) and in an area adjacent to the plots used for the trial of Tabora (1991), who demonstrated a carry over effect because shoot growth was reduced in *Lotus uliginosus* Schkuhr. Therefore, the following second explanation perhaps is more likely. White clover might be genetically capable of recovering quickly from the inhibition of gibberellin biosynthesis caused by paclobutrazol (Hedden, 1990) as shown by the results in Chapters 4 and 5, which indicated that the retardation effects

of paclobutrazol lasted only two to three months in white clover. Moreover, in individual plants, the October paclobutrazol application resulted in the treated plants being taller than the control two months after paclobutrazol was applied (Chapter 4B). The shortlived and reverse responses of white clover to the retardation effects of paclobutrazol need to be further investigated.

CHAPTER 8

GENERAL DISCUSSION AND CONCLUSIONS

8.1. WHITE CLOVER SEED PRODUCTION PROBLEMS AND THE USE OF PLANT GROWTH REGULATORS

Seed yield of white clover varies from year to year because the growth and development of seed yield components are strongly affected by environmental factors as well as by genotype (Thomas, 1987c; Hampton, 1990). White clover's protracted flowering pattern also makes its difficult to optimize harvest date (see section 2.3.2). In addition, since white clover is an indeterminate plant which forms its inflorescences at nodes, white clover inflorescence production is determined by the number of potential sites, i.e. nodes, initiated in stolon apices (Thomas, 1987c). In turn, inflorescence production is also determined by the number of actively growing stolon apices and the ability of these to respond to the floral stimulus (Thomas, 1987c). However, efforts to increase stolon production may result in excessive vegetative growth which subsequently can reduce inflorescence density and fertility by the shading effect of competitive leaves (see sections 2.2.4 and 2.3.2).

Thomas (1987c) considered that there is no single most important component of seed yield in white clover. However, practice shows that the most significant component is commonly considered to be the number of inflorescences produced (Zaleski, 1970; Huxley *et al.*, 1979; Clifford, 1987; Hollington *et al.*, 1989; Marshall *et al.*, 1989; Van Bockstaele and Rijckaert, 1988; Yakuts and Kurchak, 1991). Therefore, a method which can increase and concentrate inflorescence production may be expected to improve seed yield. Besides correct crop management practices (Clifford, 1987), the use of plant growth regulators is another option for improving white clover seed yield (see Section 2.4.2). Plant growth regulators have been used

in attempts to enhance inflorescence initiation, increase and concentrate inflorescence production, increase stolon production to provide more sites for inflorescence production without necessarily producing excessive vegetative growth, and also improve other seed yield components (florets per inflorescence, seeds per floret and seed weight).

Trials using plant growth regulators to increase seed yield in white clover have been conducted by Mohamed (1981), Marshall and Hides (1986, 1987, 1989, 1991a,b), Hampton (1991), Rijckaert (1991) and Boelt (1991), but results were inconsistent, varying with the type of plant growth regulator used, rate and time of application, cultivar, site and season (see section 2.4.2). In the present study, four plant growth regulators, i.e. chlormequat chloride, daminozide, triapenthenol and paclobutrazol, were examined using white clover cv. Grasslands Pitau. Their effects on plant growth and development, and the consequences of these effects for white clover seed production are discussed in the following sections.

8.2. CHLORMEQUAT CHLORIDE EFFECTS

Results in the present study showed that chlormequat chloride did not result in growth retardation in white clover (Chapters 3 and 4). The results confirm those reported by Marshall and Hides (1986). Reports of experiments on the use of chlormequat chloride as an anti-lodging agent have often contained references to yield increases in the absence of lodging, e.g. in barley (Koranteng and Matthews, 1982). These increases are due mainly to increased tillering and tiller survival. However, the development of stolons and also nodes along stolons in white clover was not affected by chlormequat chloride (Chapters 3 and 4). Marshall and Hides (1986) reported that chlormequat chloride increased the number of inflorescences, but it reduced the number of florets per inflorescence. In the present study, chlormequat chloride reduced seed weight in a first year crop but not in a second year crop, while other seed yield components (the number of inflorescences per unit area, florets per inflorescence and seeds per floret) were not affected (Chapters 3

and 4). As a result, it is not surprising that the present study and studies by Mohamed (1981), Marshall and Hides (1986) and Boelt (1991) produced results which showed that chlormequat chloride had no effect on white clover seed yield, and thus chlormequat chloride cannot be recommended for use in white clover seed crops.

8.3. DAMINOZIDE EFFECTS

Daminozide is generally effective in controlling shoot elongation of most dicotyledonous crops (Davis and Andersen, 1989). In the present study, it also significantly retarded white clover growth (Chapter 3). Petiole length was effectively reduced. However, peduncle length was also decreased. As a consequence, peduncle and petiole length differences in daminozide treated plants did not differ with those in untreated plants, and hence no improvement in pollination efficiency occurred as indicated by the fact there was no significant increase in seeds per floret at harvest following daminozide application (Chapter 3). Daminozide also did not increase the number of nodes (Chapter 3). This means that potential sites for inflorescence production were not improved. As a result, the number of inflorescences per unit area at harvest was not increased (Chapter 3). Also, the number of florets per inflorescence was not affected, but daminozide significantly reduced seed weight (Chapter 3). Consequently, white clover seed yield in the present study was not improved following daminozide application (Chapter 3). The result confirms that reported by Mohamed (1981), but was in contrast with that reported by Boelt (1991). However, Boelt (1991) also stated that the increase of seed yield she recorded following daminozide application was not consistent from year to year, and that daminozide generally was not reliable for use in white clover seed crops. Based on these results and also concerns about possible carcinogenic effects of daminozide (Davis and Andersen, 1989), the use of daminozide in the white clover seed crops was discontinued after the first cropping year

8.4. TRIAPENTHENOL EFFECTS

Triapenthenol is a member of the triazole group, and is a potent plant growth retardant (Lürssen and Reiser, 1985; Hedden, 1990). It retards plant growth in many species (see section 2.4.1.2). In the present study, it shortened the petiole length of white clover leaves, but the effect was short lived, i.e. for only three weeks after application (Chapter 4). This explains why a retardation effect was not recorded at peak flowering, i.e. around two months after application, in a first year crop (Chapter 3). However, under Belgian conditions, Rijckaert (1991) reported that triapenthenol reduced the height of the leaf canopy at flowering. The present study used rates of 0.5 and 1.0 kg triapenthenol/ha. These are lower than the rates used by Rijckaert (1991) which ranged from 0.7 to 2.8 kg a.i/ha. It is possible that the rates used in the present study may have been too low to maintain triapenthenol's retardation effect for longer than three weeks.

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 (Chapters 3 and 4). Reasons for this might be either that triapenthenol is species specific or that the rates used in the present study were too low to show its effects. Because the number of nodes and stolons was not increased, a potential for increasing the number of inflorescences produced through increasing sites for inflorescence production was not apparent in triapenthenol treated plants, as shown by the fact there were no significant increases in the number of inflorescences at harvest in both first and second year crops (Chapters 3 and 4). However, when measured at peak flowering, the application of triapenthenol at the reproductive buds visible stage increased the number of reproductive nodes. This was presumably reflecting the increase in reproductive node development along main stolons, although this only occurred in a second year crop (Chapter 4). Surprisingly, triapenthenol applied at the same rate and stage of plant development as in a second year crop did not affect the number of reproductive nodes at peak flowering in a first year crop (Chapter 3). These differences might be caused by different climatic

conditions between years. However, Rijckaert (1991) also found that inflorescence production in a first year white clover cv. Merwi crop was not affected following triapenthenol application in three experimental years, in which yearly climatic conditions varied considerably. There is therefore a possibility that second year crops respond better to triapenthenol application than first year crops. However, whether this is consistent is not known and needs to be further investigated.

Triapenthenol also did not affect the number of florets per inflorescence or seeds per floret, but it did reduce seed weight (Chapters 3 and 4). As a result, white clover seed yields in the present study were not improved following triapenthenol application (Chapters 3 and 4). On the other hand, Rijckaert (1991) reported that although the number of florets per inflorescence and seed weight were not affected, triapenthenol tended to increase the number of seeds per floret, and thereby increase seed yield. This increase in seeds per floret was presumably as a result of improved pollination following reduction of plant height as discussed above. Inconsistent white clover seed yield responses after triapenthenol application were also reported by Boelt (1991). Because the rates used in the present study perhaps were low, a further study of the effects of triapenthenol applied at high rates would be useful to verify this matter. In addition, Wiltshire and Hebblethwaite (1990) found that harvest time affected the effect of triapenthenol on *Lolium perenne* L. seed yield, as triapenthenol delays crop maturity. Thus, there is a possibility that the inconsistent effects of triapenthenol on white clover seed yield may be partly because harvest was not conducted at the optimum time. This matter also needs to be further investigated.

8.5. PACLOBUTRAZOL EFFECTS

Paclobutrazol was the only plant growth regulator which showed potential value in white clover seed production in this study. It increased both potential harvested seed yield and actual seed yield, although the increases were not usually statistically

significant (Chapters 3, 5 and 6). The effect, limitations and future of paclobutrazol for use in white clover seed crops are discussed in more detail in the following sections.

8.5.1. Plant growth retardation and assimilate supply maintenance

As reported in other herbage legume species, e.g. *Lotus uliginosus* Schkuhr. (Tabora, 1991) and *Lotus corniculatus* L. (Li and Hill, 1989; Supanjani, 1991), paclobutrazol reduced white clover growth by a reduction in leaf size, petiole length and stolon length (Chapters 3, 4 and 5). This reduction in leaf size may have a deleterious effect on the maintenance of adequate assimilates for plant growth and development. However, the plant dry weight results suggested that there was no significant difference in whole plant photosynthetic performance between treated and untreated plants (Chapters 3, 4 and 5). Several possible explanations have been discussed in Chapter 5. However, a further study is required to verify these. Nevertheless, based on all the results found in the present study, paclobutrazol generally does not seem to have had a detrimental effect on whole plant assimilate production to support plant growth and development, although the details of its effect on photosynthesis in white clover still need to be further investigated. In addition, it was found that the reduction of plant growth following paclobutrazol application disappeared within two to three months after application. This transient effect of paclobutrazol also occurred in China aster (Phetpradap L., 1992) and dahlia (Phetpradap S., 1992). The reason for this is not known and also needs further investigation.

8.5.2. Plant development and its relation with seed production

Under sward conditions, the number of nodes and stolon apices in white clover increased following paclobutrazol application (Chapters 3 and 4). However, it is interesting to note that paclobutrazol generally did not affect the number of nodes developed along individual stolons (Chapters 4 and 5). The increase in node

production following paclobutrazol application was simply because of increased stolon production, not the number of nodes/stolon. From the individual plant trial (Chapter 5B), it was found that paclobutrazol increased stolon production by increasing secondary, tertiary and to a lesser extent quaternary branches from main stolons, while the number of quinary branches was not affected. This increase in branching is presumably the result of apical dominance removal. Paclobutrazol has been shown to reduce apical dominance, and hence increase branching or tillering, for example, in *Lotus uliginosus* Schkuhr. (Clifford and Hare, 1987; Tabora, 1991) and *Lolium perenne* L. (Hampton and Hebblethwaite, 1985a). As mentioned previously, inflorescences form at nodes (Thomas, 1987c). Thus, any increase in node or stolon production creates more sites for inflorescence production. However, whether this results in increased inflorescence production depends on the response of these nodes and stolons to the floral stimulus. This matter is further discussed in the next section.

8.5.3. Inflorescence initiation and production

In the present study, paclobutrazol increased inflorescence production (Chapters 3, 4, 5 and 6). This is in agreement with results reported by Marshall and Hides (1989, 1991b) and Hampton (1991). However, there was no evidence that paclobutrazol was a direct cause of inflorescence initiation or that it altered the pattern of inflorescence development along stolons (Chapter 5B). The effect of plant hormones on inflorescence initiation in white clover is not fully known. However, there is an indication that gibberellins promote white clover inflorescence initiation (Cohen and Dovrat, 1976; Thomas, 1987c; see also section 2.1.2). As the mode of action of paclobutrazol is to inhibit gibberellin biosynthesis (Schott *et al.*, 1984; Burden *et al.*, 1987; Hedden, 1990), it is unlikely that it would act as a direct cause of inflorescence initiation. Paclobutrazol also generally had no effect on the rate of node initiation or the number of nodes produced on stolons (Chapters 4 and 5B). Thus, if paclobutrazol is related to increased inflorescence production, it seems probable that it acts by increasing branching, i.e. by providing more sites for

inflorescence development, and so in turn increasing inflorescence production, as discussed in the previous section. However, results in the present study showed that not all genotypes increased their inflorescence production in response to paclobutrazol, even though they all increased their branching following paclobutrazol application (Chapter 5B). The differences might be because of different cold temperature requirements between genotypes for inflorescence initiation (Thomas, 1987c). Paclobutrazol increased secondary, tertiary and to a lesser extent quaternary branches from main stolons (Chapter 5B). However, it might be that temperatures at the time these branches were developed were above the critical low temperatures required for inflorescence initiation in some genotypes, and hence the newly developed branches of these genotypes failed to produce inflorescences. Nevertheless, the results can help to explain how paclobutrazol increases inflorescence production, i.e. provided that individual genotypes are capable of floral initiation, paclobutrazol can increase the number of inflorescences formed by increasing the number of stolon branches.

8.5.4. Florets per inflorescence, seeds per floret and seed weight

Under sward conditions, paclobutrazol generally did not affect the number of florets per inflorescence, seeds per floret or seed weight (Chapters 3, 4 and 6). In fact, it reduced seed weight in a second year crop (Chapter 4). These results differed from those from an individual plant trial (Chapter 5B), in which paclobutrazol reduced seed abortion and increased seed weight. The different results obtained with sward and individual plants might have arisen because competition for light and nutrients in the sward was higher than competition between individual plants, which experienced unrestricted growth. Both sward and individual plants might have had a similar improvement of assimilate translocation into inflorescences following paclobutrazol application (Hampton, 1983; Hampton and Hebblethwaite, 1984b). Under sward conditions, however, because the total assimilate production in whole plants was not affected by paclobutrazol, as shown by a similar dry weight for both treated and untreated plants (Chapters 3 and 4), and the number of inflorescences

was increased, such an improvement of assimilate translocation was perhaps only sufficient to offset higher competition for assimilates among inflorescences and allowing them to maintain floret and seed growth and development at the same level as in the control. In fact, maintaining higher numbers of florets and seeds to maturity with the same amount of assimilate production will presumably lead to greater competition and can commonly result in reduced seed weight, as reported in *Lolium perenne* L. (Hampton and Hebblethwaite, 1985b) and *Lotus uliginosus* Schkuhr. (Tabora, 1991). This may help to explain why paclobutrazol reduced seed weight in a second year crop

8.5.5. Harvest timing in paclobutrazol treated plants

Results in the present study using individual plants showed that paclobutrazol speeded up the ripening stage of the seed development process (Chapter 5A). Therefore, this may affect the optimum harvest time of paclobutrazol treated plants which presumably would be earlier than the optimum harvest time of untreated plants. However, whether this also happened in the sward situation was not recorded but seems unlikely as there were no significant interactions between paclobutrazol and harvest time for seed yield components and seed yield (Chapter 6). Highest seed yields for both treated and untreated plants were recorded when harvest was conducted 25 days after the first peak flowering, i.e. when the highest number of inflorescences could be gathered (Chapter 6). Seed yield gradually decreased in the following weeks. Although not statistically different, when harvest was delayed for five days, i.e. harvest at 30 days after the first peak flowering, a considerable amount of seed was lost (Chapter 6). This suggests that some earlier produced inflorescences had shattered and shed seed, and that the seed losses could not be offset by increasing maturity of seed from newly ripened inflorescences. Holloway (1987) also reported that the best seed yield was obtained from harvesting 28 days after peak flowering, and that harvest delay resulted in a linear decline in seed yields. These results imply that the decision on when to harvest should be based on a method that allows recovery of the highest number of ripe inflorescences, i.e. by

calculating the time from the first peak flowering. The results suggest that 25 days after the first peak flowering is a safe time to obtain high seed yield since the majority of seeds are mature and inflorescences have not started to shed seed. Precisely when inflorescences start to shed seed after they are ripe, and to what extent seed shedding from the inflorescence is influenced by weather conditions is not known. As climatic conditions can vary from year to year, this matter needs to be further investigated for improving optimum harvest timing in white clover seed crops.

8.5.6. The future of paclobutrazol for use in white clover seed crops

Paclobutrazol successfully increased the number of stolons without necessarily producing excessive vegetative growth (Chapters 3, 4 and 5). However, as discussed previously, not all stolons produced inflorescences either because the stolons originated from genotypes with a poor capacity to produce inflorescences, or despite the fact that stolons originated from genotypes which flowered profusely they were produced at a time when the temperatures exceeded the critical low temperatures for inflorescence initiation (Chapter 5). Nevertheless, results from sward trials show that paclobutrazol consistently increased the number of inflorescences/m^2 (Chapters 3, 4 and 6). Although not always significant, these increases produced increases in both potential harvestable and actual seed yields in first year crops (Chapters 3 and 6) but not in a second year crop (Chapter 4). The reason for the latter might be because seed weight was reduced following paclobutrazol application due to presumably higher competition for assimilates among and within inflorescences (Chapter 4). Similar inconsistent results have also been reported by Marshall and Hides (1986, 1989, 1991b), Boelt (1991) and Rijckaert (1991). Rijckaert (1991) suggested that different climatic conditions between years affected the response of white clover to paclobutrazol application. Under bad climatic conditions (high rainfall and low sunshine), paclobutrazol performed well in increasing seed yield, but under more favourable conditions for seed production (dry, warm and sunny), its effect was less pronounced. This is probably due to the activation of paclobutrazol

in the soil. Paclobutrazol is more active when rainfall is high (Shearing and Batch, 1981; Hampton and Hebblethwaite, 1984b). However, results in the present study show that paclobutrazol also increased seed yield when the weather conditions were favourable for white clover seed production (Chapter 3). Thus, as mentioned above, genetic factors might be another important contributor to these inconsistent results. Different rates and times of application produced only small effects on the effectiveness and duration of paclobutrazol activity in retarding plant growth and developing stolons, and on subsequent inflorescence production and seed yield (Chapters 3, 4 and 5). There was no evidence that paclobutrazol directly promoted inflorescence initiation (Chapter 5B). Thus, it is unlikely that a different rate and time of paclobutrazol application would have any major effects. Considering the inconsistent results found in the present study and the previous studies by the other investigators mentioned above, for the time being, paclobutrazol cannot be currently recommended for commercial use in white clover seed production. In the future, however, if a plant growth regulator which can substitute the effect of the environment for inflorescence initiation becomes available, paclobutrazol could perhaps be recommended for use in combination with it for consistent increased seed yield, since it greatly increased stolon production in all genotypes (Chapter 5B).

8.6. VARIATION IN HERBAGE LEGUME SEED PRODUCTION TRIALS

Results in the present study and also those reported by Hampton (1991) and Marshall and Hides (1989, 1991b) have shown that under sward conditions, paclobutrazol significantly increases the number of inflorescences/m^2. In turn, it is expected that this increase should produce increased seed yield. In the present study, both potential harvestable seed yield and and actual seed yield, particularly for a first year crop, were increased (Chapters 3 and 6). However, these increases were not always statistically significant, although the average increase was high (Table 8.1). Supanjani (1991) similarly reported that although seed yield in *Lotus corniculatus* L. was increased by up to 70 % following paclobutrazol application,

this increase was also not statistically significant. These non significant results were most likely due to high variation in the field, i.e. CV of 30 % or greater. Such variation can be caused by several factors. It is likely that genetic variability is involved, particularly in an outcrossing species such as white clover. This may result in some genotypes being superior to others in terms of vegetative growth and development, and hence they will dominate during establishment. However, the capability of these superior vegetative genotypes to produce inflorescences and their responses to paclobutrazol application may differ, as shown in Chapter 5B. In addition, the proportion of genotypes which ranges from those which have capabilities for mainly vegetative growth but produce few inflorescences, to those which flower profusely, is not known. This possibility may create a situation in which the number of inflorescences produced is uneven and results in a lack of uniformity within and among plots. Reducing genetic variation, particularly for inflorescence production capacity, can indirectly be achieved during seed multiplication through the genetic drift process, i.e. genotypes which produce only a few inflorescences may be eventually eliminated from a population, as the number of seeds from these genotypes gradually becomes less from one generation to the next one. However, this process has a limitation. A study on genetic selection within white clover cv. S.184 over three generations of seed multiplication (Ennos and Ollerenshaw, 1990) showed only a few genetic changes, which were relatively insignificant from a seed production perspective. Also, the number of generations permitted in seed certification for maintaining stability of cultivars in terms of herbage production is limited, e.g. only four generation from breeders seed under the New Zealand (Anon., 1988) and British (Ennos and Ollerenshaw, 1990) seed certification schemes.

A second possible factor is sample size. Sample sizes used in the present study were probably too small to recover inflorescences produced from many different genotypes, raising the possibility that some samples may have been taken from areas dominated by genotypes which produced few inflorescences and were not affected by paclobutrazol application. This could also contribute to high variation, which

resulted in non significant seed yield increases, although mean differences were high, particularly in first year crops (Table 8.1). Statistically, reducing high variation in a field trial can be achieved by increasing the number of replicates and selecting an appropriate size and shape of the experimental units (Steel and Torrie, 1980). In the 1989/1990 trial (Chapter 4), samples per plot were doubled and variation was reduced, i.e. CV < 20 % (Table 8.1). However, seed yield was not increased, although the number of inflorescences/m^2 was increased. This suggests that high field variation was not solely caused by the uneven numbers of inflorescences produced within and among plots. It is also possible that harvest was not carried out at the optimum time. Seed shedding might have occurred when harvest was late (Chapter 3), and there is a possibility that the capability of genotypes to retain seeds in inflorescences after they are ripe may be different. This again could create a lack of uniformity within and among plots.

Table 8.1. Effect of paclobutrazol on potential harvestable seed yield and actual seed yield during the three years of sward trials.

A. Potential harvestable seed yield (g/m^2)

Year[1)]	Control	Paclobutrazol[2)] Oct	Nov	LSD[3)] (P=0.05)	CV (%)	Increases (%) Oct	Nov
1988/1989	59.3	101.3	92.8	37.7 (*)	32	71	57
1989/1990	73.4	72.6	82.5	22.8 (NS)	18	-1	12
1990/1991	23.1	-[4)]	36.0	34.2 (NS)	33	-	56

B. Actual seed yield (g/m^2)

1988/1989	39.4	49.1	49.8	27.8 (NS)	41	25	26
1989/1990	33.5	33.4	31.6	9.3 (NS)	17	0	-6
1990/1991	16.7	-	26.0	32.7 (NS)	44	-	56

Note: 1. 1988/1989 = a first year crop, 1989/1990 = a second year crop and 1990/1991 = a first year simulated sward crop.
2. Paclobutrazol applied at a rate of 1 kg a.i/ha.
3. * = significant and NS = non significant.
4. - = not applied.

8.7. CONCLUSIONS

Based on results in the present study and the subsequent discussion, several conclusions can be drawn:

1. Chlormequat chloride and daminozide were not effective in increasing white clover seed yield, and hence are not recommended for use in white clover seed crops.
2. Although triapenthenol did not show a positive effect on white clover seed yield in the present study, further work using a higher rate of application is recommended to verify its effects, as other studies, e.g. Rijckaert (1991), have shown that triapenthenol applied at higher rates does increase white clover seed yield.
3. Paclobutrazol was the only plant growth regulator used in this study which had a potential for increasing seed yield through increased inflorescence production. Although paclobutrazol did not directly affect inflorescence initiation at stolon apices, it increased inflorescence number by providing more reproductive sites as a result of increased stolon production. However, this effect was limited by the different response of genotypes to the floral stimulus, and hence may be inconsistent.
4. Although paclobutrazol increased stolon production in all genotypes, its future use in white clover seed crops may depend on the availability of other plant growth regulators which can substitute for the effect of the environment on inflorescence initiation, to eliminate differences between genotypes in their environmental requirements for the flowering stimulus. This aspect is open for further exploration, as only a few investigations on the effect of plant growth regulators on inflorescence initiation in white clover have been reported. In addition, apart from chlorophyll content, studies on paclobutrazol effects on photosynthesis and respiration, and changes in hormonal balance in plants following paclobutrazol application were not conducted in the present study. Further knowledge of these aspects may help to explain the changes in white

clover growth pattern following paclobutrazol application and also the short duration of its primary effects on plant growth and development, as discussed in Chapters 4 and 5.

5. Irrespective of the use of plant growth regulators, harvest timing is still one of the more important aspects to be further explored for achieving consistently high white clover seed yield. Wrong harvest timing can result in low seed yield. In the present study harvesting 25 days after the first peak flowering resulted in the highest seed yield. Yield was decreased when harvesting was delayed. Thus, a greater knowledge of flowering duration, the timing and extent of inflorescence shattering, pod shattering and seed shedding, and the role of the environment is important for better determination of optimum harvest time. This matter needs to be further investigated.
6. Results from the present study also emphasize the importance of genetic factors, particularly inflorescence production capacity, in white clover seed production. Cultural or chemical manipulation for increasing inflorescence production by increasing stolon production is limited by the response of different genotypes within the population to the floral stimulus. The incorporation of reproductive aspects into white clover plant breeding programmes is desirable to achieve improved and consistent seed yield.
7. Conventional sample sizes and numbers of replicates result in high variation and the non significance of biologically consistent results when dealing with an outcrossing species such as white clover. Further research is required to determine the sample size, subsampling techniques and replicate number which will allow the statistical significance of what appear to be biological increases in seed yield.

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APPENDICES

Appendix 3.1A. Soil analysis of the Frewin's block.

Growing season	pH	Olsen P	Exch. K	Exch. Ca	Exch. Mg	Exch. Na	SO_4
1986/1987[1)]	5.8	5	0.26	4.5	1.08	0.05	na[3)]
1989/1990[2)]	5.8	11	0.27	7.5	0.82	0.1	4.4
1990/1991[2)]	5.0	26	0.30	6.2	1.18	0.07	11.0

- Phosphate and sulphate values are expressed as micrograms per gram (air-dry).
- Exchangeable cations are expressed as meq/100 g (air-dry).

Note:
1. Analyzed by Analytical Services Limited (extracted from Frewin's Farm Development Proposal by M.J. Hill 1987).
2. Analyzed by the Fertilizer and Lime Research Centre, Massey University.
3. na = not available.

Appendix 3.1B. TOKOMARU SOIL SERIES

Tokomaru silt loam soil. It is classified as an aeric fragiaqualf (gleyed yellow-grey earth) and is part of the rolling country at the foot of the western Tararua ranges, New Zealand. Below is its technical description:

Soil name: Tokomaru silt loam; Tokamaru silt loam, rolling phase.

Parent material or rock: Loess.

Topography and Physiography: Flat, flat to undulating, and rolling. High terrace.

Elevation above sea level: 30-150 m

Mean annual rainfall: 890-1140 mm

Brief description of soil profile:

A_1	20 cm	dark greyish brown to brown (10YR-7.5YR 4/2) silt loam; few brown mottles, nut structure.
A_{2gc}	18 cm	greyish brown to light brownish grey (2.5Y 5/2 - 6/2) heavy silt loam; few red mottles; many iron/ manganese concretions; nut structure.
$B_{21}G$	20 cm	pale olive (5Y 6/3) clay loam; abundant brown mottles; thin clay coatings; weak prismatic breaking to blocky structure.
$B_{22}G_{cy}$	18 cm	pale olive (5Y 6/3) clay loam; many brown mottles; moderately thick clay coatings; weak prismatic breaking to blocky structure.
C_1+C_2	on	mottled light grey (5Y 7/2) and strong brown (7.5 YR 5/6) sandy clay loam; upper part compact and with vertical grey veins.

Distinguishing features of soil and environment:

On higher terraces from silty to fine sandy loess under low to moderate rainfall (890-1 140 mm) with summer dry. Poorly drained with pale olive compact clay loam B horizon with many to abundant brown mottles and moderately thick clay coatings in lower part. At about 76 cm has a compact fragipan with vertical grey veining. Differs from Milson and Marton (12-12a) series in being formed from thicker and lighter-textured loess and has lighter-textured B horizon with less development of clay coatings.

Overall drainage (class): Poorly drained.

Internal drainage (class): Moderate: low P and Ca, medium K.

Present land use: Fattening, dairying, cereal cropping.

Potential land use: Fattening, dairying, cropping.

Limitations for intensive soil use: Poor winter drainage; dries out in summer.

Pasture responses to topdressing: Phosphate, lime, potash.

Soil erosion: Nil. Slight sheet on rolling phase if cultivated.

Soil Classification:

Common Name: Weakly leached moderately to stongly gleyed yellow-grey earths.

Technical Name: Weakly enleached, moderately clay-illuvial, net-gammate pseudo-madenti-pallic soils with fragipan.

Soil set reference: Tokomaru soils.

Reference:

Cowie, J.D. 1978. Soil and agriculture of Kairanga country. Soil Bureau Bulletin 33, Soil Bureau, DSIR.

Appendix 3.2. The characteristic of white clover cv. Grasslands Pitau.
(Source: DSIR Grasslands' Promotional Leaflet, New Zealand)

White clover cv. Grasslands Pitau was first released and certified in 1975, and became commercially available in 1978. It was bred by crossing New Zealand white clover, known as cv. Grasslands Huia, with a winter active Spanish ecotype adapted to warmer climates. Cv. Grasslands Pitau is a more erect and open-growing plant than cv. Grasslands Huia (longer stems with fewer stolons), with markedly larger leaves in winter.

cv. Grasslands Huia cv. Grasslands Pitau

The benefits:

1. Offers greater annual production than other white clovers, especially in the North Island of New Zealand.
2. Offers more cool season clover growth than other white clover cultivars.
3. It produces well on lowland fertile soils and has an important potential role as an improved pasture legume in dairy areas.
4. Increased white clover content in pastures provides a more nutritive diet promoting better animal performance.

The weakness: Cv. Grasslands Pitau has a slower recovery from hard grazing, particularly under drier conditions. Therefore, it is best suited to rotational grazing.

Seed production: The average seed yield of cv. Grasslands Pitau is 300 kg/ha.

Appendix 3.3. Sixty year averages for temperature (minimum and maximum), sunshine hours and rainfall at Palmerston, and deviations from these averages during 1988/1989 and 1989/1990[1)].

	September		October		November		December		January	
					Temperature °C					
	min	max	min	max	min	max	min	max	min	max
Average 60 Yrs	6.6	14.7	8.3	16.6	9.8	18.5	11.6	20.6	12.8	29.1
1988/1989	2.8	0.9	1.9	0.5	1.1	1.3	1.8	2.3	2.4	-5.0
1989/1990	1.7	1.8	1.3	1.4	2.2	1.5	-0.3	-0.5	0.2	-6.1

			Number of sunshine hours		
Average 60 Yrs	133.0	158.0	177.0	193.0	209.0
1988/1989	-65.0	-20.4	4.5	31.9	14.1
1989/1990	18.0	-28.0	14.5	-35.2	-8.1

			Rainfall (mm)		
Average 60 Yrs	75.0	88.0	78.0	94.0	79.0
1988/1989	69.0	10.0	-14.7	-37.0	13.8
1989/1990	-49.7	35.0	-55.0	-35.2	25.2

Note: 1. Data (obtained from AgResearch Grasslands) were recorded at the station 1 km from the trial area.

Appendix 3.4. Effect of plant growth regulators on the germination of 1988/1989 harvested seed following scarification.

Treatments	Normal seedlings (%)	Abnormal seedlings (%)	Hard seed (%)	Fresh un-germinated (%)	Dead seed (%)
Control	92	7	0	0	1
Paclobutrazol 0.5 kg Oct	94	4	0	1	1
Paclobutrazol 1.0 kg Oct	96	4	0	0	0
Paclobutrazol 0.5 kg Nov	97	3	0	0	0
Paclobutrazol 1.0 kg Nov	94	5	0	1	0
Triapenthenol 0.5 kg Oct	94	6	0	0	0
Triapenthenol 1.0 kg Oct	91	8	0	0	1
Triapenthenol 0.5 kg Nov	91	8	0	0	1
Triapenthenol 1.0 kg Nov	94	6	0	0	0
Chlormequat chloride 1.5 kg Oct	96	4	0	0	0
Chlormequat chloride 3.0 kg Oct	93	7	0	0	0
Chlormequat chloride 1.5 kg Nov	93	7	0	0	0
Chlormequat chloride 3.0 kg Nov	90	10	0	0	0
Daminozide 2.0 kg Nov	94	6	0	0	0
Daminozide 4.0 kg Nov	94	5	0	1	0
LSD (P=0.05)	NS	NS	NS	NS	NS
CV (%)	3	44	0	180	176

Appendix 4.1. Effect of plant growth regulators on dry matter distribution (g/m^2) for a second year crop of white clover cv. Grasslands Pitau at reproductive initiation (October), reproductive buds visible/early flowering (November), peak flowering (December) and harvest (January).

Treatments	October		November				December				January			
	LDM	SDM	LDM	SDM	RDM	DDM	LDM	SDM	RDM	DDM	LDM	SDM	RDM	DDM[1)]
Control	170*a*	128	251	136	11	35*bc*	231	174	58*cd*	113	185	142*b*	117*ab*[2)]	136
Paclobutrazol Sept	114*b*	147	222	150	10	30*c*	188	193	68*bcd*	102	167	215*a*	70*c*	123
Paclobutrazol Oct	-	-	206	148	10	41*ab*	219	163	66*bcd*	95	122	167*ab*	82*bc*	130
Paclobutrazol Nov	-	-	-	-	-	-	236	193	52*d*	103	156	222*a*	129*a*	127
Triapenthenol Sept	136*ab*	155	248	171	16	41*ab*	227	184	61*bcd*	135	168	164*ab*	99*abc*	132
Triapenthenol Oct	-	-	265	127	12	32*c*	243	182	79*b*	132	150	169*ab*	93*abc*	135
Triapenthenol Nov	-	-	-	-	-	-	277	159	99*a*	103	153	159*ab*	121*ab*	131
Chlormequat chloride Sept	146*ab*	122	232	134	21	44*a*	241	172	74*bc*	103	179	169*ab*	125*a*	129
Chlormequat chloride Oct	-	-	233	129	9	37*abc*	270	224	64*bcd*	116	190	185*ab*	103*abc*	150
Chlormequat chloride Nov	-	-	-	-	-	-	248	205	67*bcd*	108	168	163*ab*	129*a*	133
Mean	142	138	237	142	13	37	238	185	69	111	164	176	107	133
LSD (P=0.05)	39	NS	NS	NS	NS	8	NS	NS	20	NS	NS	67	39	NS
CV (%)	14	11	15	24	80	12	14	19	17	20	28	22	21	19

Note: 1. LDM = leaf dry matter, SDM = stolon dry matter, RDM = reproductive dry matter and DDM = dead dry matter.
2. Values in the same column followed by the same letter are not significantly different according to Fisher's LSD test at P=0.05.

Appendix 4.2. Effect of plant growth regulators on the germination of 1989/1990 harvested seed following scarification.

Treatments	Normal seedlings (%)	Abnormal seedlings (%)	Hard seed (%)	Fresh un-germinated (%)
Control	90*d*	6*a*	0*d*	4*a*[1)]
Paclobutrazol Sept	96*ab*	2*b*	1*c*	1*b*
Paclobutrazol Oct	95*bc*	2*b*	2*b*	1*b*
Paclobutrazol Nov	96*ab*	2*b*	2*b*	0*b*
Triapenthenol Sept	95*bc*	1*b*	4*a*	0*b*
Triapenthenol Oct	93*c*	1*b*	5*a*	1*b*
Triapenthenol Nov	98*a*	1*b*	1*c*	0*b*
Chlormequat chloride Sept	98*a*	1*b*	1*c*	0*b*
Chlormequat chloride Oct	97*ab*	2*b*	1*c*	0*b*
Chlormequat chloride Nov	97*ab*	2*b*	0*d*	1*b*
LSD (P=0.05)	3	2	1	1
CV (%)	2	59	58	94

Note: 1. Values in the same column followed by the same letter are not significantly different according to Fisher's LSD test at P=0.05.

Appendix 5A.1. Soil fertility analysis of the nursery block (the 1990/1991 trial).

Growing season	pH	Olsen P	SO_4	Exch K	Exch Ca	Exch Mg	Exch Na	CEC
1990/1991			Prior to the trial					
	6.7	93	2.0	1.05	-	-	-	-
			End of the trial (harvest)					
Control	6.4	90	2.0	0.52	10.7	1.00	0.13	13
Paclobutrazol	6.4	90	3.0	0.75	10.4	1.08	0.20	15

- Phosphate and sulphate values are expressed as micrograms per gram (air-dry).
- Exchangeable cations and CEC values are expressed as meq/100g (air-dry).
- This soil analysis was conducted by the Fertilizer and Lime Research Centre, Massey University.

Appendix 5A.2. Sixty year averages for temperature (minimum and maximum), sunshine hours and rainfall at Palmerston North, and deviations from these averages during 1990/1991 and 1991/1992[1)].

	August		Sept		Oct		Nov		Dec		Jan		Feb		March	
	Temperature (^{o}C)															
	min	max	min	max	min	max	min	max	min	max	min	max	min	max	min	max
Ave. 60yrs	5.0	13.1	6.6	14.7	8.3	16.6	9.8	18.5	11.6	20.6	12.8	21.9	12.8	22.3	11.7	20.9
1990/1991	1.4	0.6	-0.9	-0.3	1.1	0.8	1.0	0.3	0.7	-0.1	0.1	0.3	0.3	-0.6	0.7	0.1
1991/1992	1.7	1.2	0.7	1.1	-0.2	-0.6	-1.5	-2.3	-0.3	-1.3	0.1	0.0	-0.4	-1.4	-2.2	-2.3
	Number of sunshine hours															
Ave. 60yrs	132.0		133.0		158.0		177.0		193.0		209.0		186.0		170.0	
1990/1991	-29.1		-0.7		-4.6		-11.4		12.1		-22.6		-33.5		27.0	
1991/1992	-34.4		-7.0		0.9		-42.2		-74.4		-36.6		-18.8		-33.7	
	Rainfall (mm)															
Ave. 60yrs	89.0		75.0		88.0		78.0		94.0		79.0		67.0		69.0	
1990/1991	22.2		-58.1		-4.3		20.3		-43.3		41.2		64.5		-40.3	
1991/1992	9.7		-9.9		-7.1		3.0		-12.8		-1.8		88.2		19.9	

Note: 1. Data (obtained from AgResearch Grasslands) were recorded at the station 1 km from the trial area.

Appendix 5B.1. Soil fertility analysis for the 1991/1992 trial.

Growing season	pH	Olsen P	SO_4	Exch K	Exch Ca	Exch Mg	Exch Na	CEC
1991/1992	6.7	51	9.5	0.37	14.1	0.61	0.32	17

- Phosphate and sulphate values are expressed as micrograms per gram (air-dry).
- Exchangeable cations and CEC values are expressed as meq/100g (air-dry).
- This soil analysis was conducted by the Fertilizer and Lime Research Centre, Massey University.

Appendix 5B.2. Effect of paclobutrazol on stolon length for quaternary and quinary branches in two different genotypes.

A. Quaternary branch length (cm)

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	48.7	45.7	36.7	43.7
Genotype II	38.7	43.3	37.3	39.8
Treatment means	43.7	44.5	37.0	

LSD (P=0.05) : genotype means = NS, treatment means = NS, genotypeXtreatment = NS.
CV : main plots = 30 %, sub plots = 40 %

B. Quinary branch length (cm)

Genotype I	14.8	14.0	6.7	11.8
Genotype II	3.3	3.8	8.3	5.1
Treatment means	9.1	8.9	7.5	

LSD (P=0.05) : genotype means = NS, treatment means = NS, genotypeXtreatment = NS.
CV : main plots = 70 %, sub plots = 115 %

Appendix 5B.3. Effect of paclobutrazol on % light penetration at peak flowering for three different genotypes during full sun.

A. 8 am - Full sun

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	0.78	0.29	0.20	0.42
Genotype II	2.25	1.12	0.72	1.36
Genotype III	0.89	0.30	0.10	0.43
Treatment means	1.31	0.57	0.34	

LSD (P=0.05) : genotype means = 0.37, treatment means = 0.31, genotypeXtreatment = NS.
CV : main plots = 29 %, subplots = 49 %

B. 1 pm - full sun

Genotype I	0.76	0.83	0.40	0.66
Genotype II	3.25	1.63	1.02	1.97
Genotype III	1.22	0.77	0.35	0.78
Treatment means	1.74	1.08	0.59	

LSD (P=0.05) : genotype means = 0.51, treatment means = 0.27, between treatments for the same genotype = 0.48 between treatments for different genotypes = 0.64
CV : main plots = 26 %, subplots = 28 %

C. 6 pm - full sun

Genotype I	1.04	0.39	0.38	0.60
Genotype II	2.79	0.86	0.84	1.50
Genotype III	0.46	0.30	0.22	0.33
Treatment means	1.43	0.52	0.48	

LSD (P=0.05) : genotype means = 0.20, treatment means = 0.19, between treatments for the same genotype = 0.34 between treatments for different genotypes = 0.34
CV : main plots = 14 %, subplots = 28 %

Appendix 5B.4. Effect of paclobutrazol on % light penetration at peak flowering for three different genotypes during overcast.

A. 8 am - Overcast

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	4.34	2.18	1.16	2.56
Genotype II	8.88	4.81	3.08	5.59
Genotype III	2.50	0.89	0.44	1.28
Treatment means	5.24	2.63	1.56	

LSD (P=0.05) : genotype means = 2.43, treatment means = 0.86, between treatments for the same genotype = 1.49, between treatments for different genotypes = 2.72
CV : main plots = 45 %, sub plots = 32 %

B. 1 pm - Overcast

Genotype I	2.44	0.81	0.65	1.30
Genotype II	9.04	4.26	2.58	5.29
Genotype III	1.88	1.01	0.42	1.10
Treatment means	4.45	2.03	1.22	

LSD (P=0.05) : genotype means = 1.10, treatment means = 0.79, between treatments for the same genotype = 1.37 between treatments for different genotypes = 1.56
CV : main plots = 25 %, sub plots = 36 %

C. 6 pm - Overcast

Genotype I	3.15	0.85	0.64	1.55
Genotype II	9.05	5.18	4.53	6.25
Genotype III	1.61	1.00	0.80	1.14
Treatment means	4.60	2.34	1.99	

LSD (P=0.05) : genotype means = 1.38, treatment means = 1.10, genotypeXtreatment = NS
CV : main plots = 27 %, sub plots = 43 %

Appendix 5B.5. Effect of paclobutrazol on the number of nodes formed along quaternary branches in two different genotypes.

A. Vegetative nodes (genotypeXtreatment = NS)

Genotypes	A[1)] Paclobutrazol		Genotype means	B Paclobutrazol		Genotype means
	Control	October	(for A)	Control	November	(for B)
Genotype I	15.9	16.9	16.4	10.1	11.2	10.7
Genotype II	15.7	18.2	17.0	9.9	10.9	10.4
Treatment means	15.8	17.6		10.0	11.1	

LSD (P=0.05) for A : genotype means = NS, treatment means = NS,
CV : main plots = 19 %, sub plots = 25 %
LSD (P=0.05) for B : genotype means = NS, treatment means = NS
CV : main plots = 20 %, sub plots = 37 %

B. Reproductive nodes (genotypeXtreatment = NS)

Genotype I	2.4	2.6	2.5	0.9	1.1	1.0
Genotype II	2.9	3.4	3.2	1.7	2.3	2.0
Treatment means	2.7	3.0		1.3	1.7	

LSD (P=0.05) for A : genotype means = NS, treatment means = NS,
CV : main plots = 39 %, sub plots = 31 %
LSD (P=0.05) for B : genotype means = NS, treatment means = NS
CV : main plots = 71 %, sub plots = 53 %

C. Total nodes (genotypeXtreatment = NS)

Genotype I	18.3	19.5	18.9	11.0	12.3	11.7
Genotype II	18.6	21.6	20.1	11.6	13.2	12.4
Treatment means	18.5	20.6		11.3	12.8	

LSD (P=0.05) for A : genotype means = NS, treatment means = NS,
CV : main plots = 22 %, sub plots = 23 %
LSD (P=0.05) for B : genotype means = NS, treatment means = NS
CV : main plots = 25 %, sub plots = 36 %
Note: 1. A and B are analysed separately (details in section 5B.2.2).

Appendix 5B.6. Effect of paclobutrazol on the number of nodes formed along quinary branches in two different genotypes.

A. Vegetative nodes

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	7.7	7.9	6.3	7.3
Genotype II	2.9	4.4	6.7	4.7
Treatment means	5.3	6.2	6.5	

LSD (P=0.05) : genotype means = NS, treatment means = NS, genotypeXtreatment = NS.
CV : main plots = 60 %, subplots = 79 %

B. Reproductive nodes

Genotype I	0.4	0.8	0.8	0.7
Genotype II	0.4	0.5	1.2	0.7
Treatment means	0.4	0.7	1.0	

LSD (P=0.05) : genotype means = NS, treatment means = NS, genotypeXtreatment = NS.
CV : main plots = 81 %, subplots = 117 %

C. Total nodes

Genotype I	8.1	8.7	7.1	8.0
Genotype II	3.3	4.9	7.9	5.4
Treatment means	5.7	6.8	7.5	

LSD (P=0.05) : genotype means = NS, treatment means = NS, genotypeXtreatment = NS.
CV : main plots = 61 %, subplots = 80 %

Appendix 5B.7. Effect of paclobutrazol on the number of quinary branches/ quaternary branch for two different genotypes.

A. Vegetative branches

Genotypes	Paclobutrazol treatments			Genotype means
	Control	October	November	
Genotype I	4.9	7.1	7.2	6.4
Genotype II	3.7	5.2	4.4	4.4
Treatment means	4.3	6.2	5.8	

LSD (P=0.05) : genotype means = NS, treatment means = NS, genotypeXtreatment = NS.
CV : main plots = 38 %, subplots = 65 %

B. Fertile branches

Genotype I	0.6	1.3	0.9	0.9
Genotype II	0.7	1.0	2.4	1.4
Treatment means	0.7	1.2	1.7	

LSD (P=0.05) : genotype means = NS, treatment means = NS, genotypeXtreatment = NS.
CV : main plots = 112 %, subplots = 122 %

C. Total branches

Genotype I	5.5	8.4	8.1	7.3
Genotype II	4.4	6.2	6.7	5.8
Treatment means	5.0	7.3	7.4	

LSD (P=0.05) : genotype means = NS, treatment means = NS, genotypeXtreatment = NS.
CV : main plots = 46 %, subplots = 65 %

Appendix 6.1. Inflorescence numbers/m^2 on 3, 8, 14 and 19 January 1991 for the control plants.

Date	inflorescences (no/m^2)
3 January 1991	34.7
8 January 1991	28.0
14 January 1991	29.3
19 January 1991	41.3
LSD (P=0.05)	NS
CV (%)	30.8

Appendix 6.2. Analysis of variance for actual seed yield data from three harvests (25, 30 and 35 days after peak flowering).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	3112.469444	345.829938	6.55	0.0072
Error	8	422.495556	52.811944		
Corrected Total	17	3534.965000			

R-Square	CV	Root MSE	ASY Mean
0.880481	25.6338	7.267183	28.35000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	2	1256.443333	628.221667	1.20	0.4555
Treatment	1	627.7605556	627.760556	1.19	0.3885
Treatment*Block	2	1050.881111	525.440556	9.95	0.0068
Time	2	134.063333	67.031667	1.27	0.3321
Treatment*Time	2	43.321111	21.660556	0.41	0.6768

Appendix 6.3. Analysis of variance for actual seed yield data from two harvests (40 and 45 days after peak flowering).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	424.1325000	60.5903571	1.11	0.4875
Error	4	217.9766667	54.4941667		
Corrected Total	11	642.1091667			

R-Square	CV	Root MSE	ASY Mean
0.660530	68.2993	7.382016	10.808333

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	2	149.4116667	74.7058333	3.13	0.2424
Treatment	1	86.9408333	86.94083333	3.64	0.1968
Treatment*Block	2	47.8116667	23.90583333	0.44	0.6726
Time	1	120.9675000	120.96750000	2.22	0.2105
Treatment*Time	1	19.0008333	19.00083333	0.35	0.5866

Appendix 6.4. Analysis of variance for the number of ripe inflorescences/m^2 from three harvests (25, 30 and 35 days after peak flowering).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	112224.0000	12469.3333	4.33	0.0255
Error	8	23047.1111	2880.8889		
Corrected Total	17	135271.1111			

R-Square	CV	Root MSE	ASY Mean
0.829623	24.20166	53.67391	221.777778

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	2	32876.44444	16438.22222	1.68	0.3732
Treatment	1	40707.55556	40707.55556	4.16	0.1782
Treatment*Block	2	19575.11111	9787.55556	3.40	0.0855
Time	2	14535.11111	7267.55556	2.52	0.1414
Treatment*Time	2	4529.77778	2264.88889	0.79	0.4878

Appendix 6.5. Analysis of variance for the number of ripe inflorescences/$m^2$from two harvests (40 and 45 days after peak flowering).

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	7	31262.66667	4466.09524	1.54	0.3555
Error	4	11621.33333	2905.33333		
Corrected Total	11	42884.00000			

R-Square	CV	Root MSE	ASY Mean
0.729005	46.87056	53.90114	115.00000

Source	DF	Anova SS	Mean Square	F Value	Pr > F
Block	2	9672.00000	4836.00000	5.64	0.1506
Treatment	1	833.33333	833.33333	0.97	0.4281
Treatment*Block	2	1714.66667	857.33333	0.30	0.7594
Time	1	18881.33333	18881.33333	6.50	0.0634
Treatment*Time	1	161.33333	161.33333	0.06	0.8253