Application of Growth Regulators in Seed Crops of White Clover (*Trifolium repens* L.) Under Belgian Climatic Conditions

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ABSTRACT

Field trials were conducted in 1987, 1989 and 1990 to assess the effects of the growth regulators paclobutrazol (PP333) and triapenthenol (RSW0411) on seed yield and yield components of white clover cv. Merwi. Application rates were 0.25, 0.5 and 1.0 kg a.i. ha^{-1} for paclobutrazol and 0.7, 1.4 and 2.8 kg a.i. ha^{-1} for triapenthenol.

The mean seed yield increases for paclobutrazol treatments in the three experiments (271, 22 and 20% against the control), were associated with a large reduction of vegetative crop growth without decreasing peduncle length. Triapenthenol increased yield in 1987 and 1990 by an average of 145 and 11% respectively.

Both growth regulators were identified as useful chemicals for obtaining higher and more profitable white clover seed yields, particularly when climatic conditions in Belgium do not favour seed production.

***Additional index words:* seed production, growth retardants, paclobutrazol, triapenthenol.**

INTRODUCTION

Seed yields of white clover (*Trifolium repens* L.) crops fluctuate widely from year to year under North-West European climatic conditions. In Denmark, the principal white clover seed producer within the EC, the average yield was 357 kg ha^{-1} during the period 1979-88 but ranged from 43 to 634 kg ha^{-1} (Anon., 1989). Similarly in the UK, yields ranged from 40 to 400 kg ha^{-1} with an average yield of 159 kg ha^{-1} (Anon., 1990).

In Belgium it was also impossible to achieve consistently high white clover seed yields between 1971-85. The average yield was 127 kg ha^{-1}, but yields ranged from 5 to 450 kg ha^{-1} (Rijckaert, 1988). Prebasic seed production of RvP white clover cultivars was therefore often disappointing, and could not be estimated accurately, especially in wet seasons.

Poor seed yields of white clover produced in NW-Europe are mainly due to adverse weather conditions during the critical periods of crop growth (Vyncke, 1982; Boelt, 1989). In particular, rainfall and low temperature before and during flowering can result in a leafy seed crop which can reduce flowering and bee activity, and impair pollination. Moreover, wet weather during seed development, maturation and harvesting can often lead to poor seed yields (Marshall, Hides and Evans, 1987).

Although some management techniques, such as defoliation, can change the crop into a more favourable open sward, the effect on seed yield is rather limited and variable. Vyncke (1963) and Nordestgaard (1986) did not obtain consistently higher seed yields following defoliation treatments.

A white clover seed crop growing during rather wet weather conditions usually produces excessive leaf growth, with poor reproductive growth and seed yield. Increased seed yields, therefore, might be expected from the use of plant growth regulators. Preliminary experiments conducted in 1985 (Rijckaert, 1988), indicated that the growth regulators PP333 (Parlay, paclobutrazol) and RSW0411 (Baronet, triapenthenol) could manipulate white clover growth by i) increasing the number of flower heads, the ratio of ripe heads to total flower heads, and seed yield, and ii) by reducing the dry matter proportion of leaf mass in relation to total dry matter.

The present paper reports the results of three field experiments in 1987, 1989 and 1990 respectively, designed to evaluate the effect of paclobutrazol and triapenthenol on seed yield and its components in white clover cv. Merwi.

MATERIALS AND METHODS

Three experiments were conducted at the Government Plant Breeding Station (RvP), Merelbeke on a light sandy-loam soil. All trials were sown with white clover cv. Merwi, a medium large leaved and medium late RvP-cultivar. Details of mangement and treatments are presented in Table 1. A randomised complete block design with four replicates of each treatment was used.

During the establishment years, plots were regularly cut as required to control broadleaved weeds and received 2 kg ha^{-1} carbetamide to control grass weeds. Plots were defoliated to about 5 cm from ground level in spring and the cut swath was removed from the field. All growth regulator treatments were applied in 900-1000 l water ha^{-1}, using a small scale experimental sprayer. Applications took place about two weeks after spring defoliation. The application dates coincided with the appearance of the first green flower buds. Honey bees (3 hives per ha) were provided for optimal pollination during flowering.

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Table 1. Crop management and treatments.

Experiment number	1	2	3
Harvest year	1987	1989	1990
Sowing date	July 1986	June 1988	July 1989
Row distance (cm)	15	15	15
Seed rate (kg ha^{-1})	3.5	2.7	4.6
Plot size			
- gross plot	2 x 7 m	2.3 x 10 m	2.25 x 10 m
- net plot (harvest area)	1.5 x 7 m	1.5 x 10 m	1.5 x 10 m
Fertiliser (kg ha^{-1})			
P_2O_5	75	38	112
K_2O	150	75	-
Herbicide application rate and date	bentazon (1.5)[1] Sept. '86 paraquat (0.6) Oct. '86 carbetamide (2.0) Feb. '87	paraquat (0.6) Aug. '88 carbetamide (2.0) Dec. '88 diquat (0.5) Jan. '89 dinoseb (2.0) Feb. '89	triallaat (1.4) July '89 paraquat (0.6) Oct. '89
Time of defoliation	6 May 87 2 June 87	27 May 89	15 May 90
Harvest date	31 August	9 August	10 August
Growth regulator treatments			
- Application date	16 June 87	12 June 89	28 May 90
- Crop height (cm)	8.3	10	10.3
- Treatments (a.i. kg ha^{-1})			
Control	0	0	0
Paclobutrazol	0.25	0.25	0.25
	0.5	0.5	0.5
	1.0	1.0	-
Triapenthenol	0.7	0.7	-
	1.4	1.4	-
	2.8	2.8	2.8

[1]a.i. ha^{-1}

At flowering, leaf canopy height was measured in Expt 1 (1987) and leaf mass quantity was recorded by visual observation on a 1-5 scale (1 = very low; 5 = very high) in Expt 2 (1989). Just prior to harvest, seed yield components were assessed by taking crop samples from quadrats (625 cm^2) from each plot (three replicates in Expt 1 and four replicates in Expt 2 respectively). In Expt 3 the sample size was increased to 2500 cm^2 per replicate, in order to decrease the variance of results. All plant material was cut just above ground level within these quadrats and inflorescences were classified in the following ripeness categories:

(a) ripe : brown head - brown peduncle
(b) nearly ripe : brown head - green peduncle
(c) unripe : green head - green peduncle
(d) other heads : head with white florets

The number within each group was counted. Five ripe heads (ten heads in Expt 3) from group (a) were dissected to give the number of florets per head. Ten ripe heads from groups (a) + (b) (twenty heads in Expt 3) were rubbed out by hand and cleaned to obtain mean seed yield per head. Seed set per floret was determined from the seed weight per head, the number of florets per head and the 1000 seed weight. The length of ten peduncles was also measured in experiments 1 and 2.

Diquat (0.8 kg ha^{-1}) as a pre-harvest desiccant was applied 2-3 days prior to harvest. Plots were harvested by cutting with a forage plot harvest (Hege) designed for efficient bagging. The harvested swath was cool air dried in jute bags to a seed moisture content of 10-12% before threshing, rubbing and seed cleaning. Cleaned seed was weighed and the yield per ha was calculated. Yields are expressed at 0% moisture content.

Subsamples were tested for germination and thousand seed weight using the rules of the International Seed Testing Association (ISTA, 1985).

RESULTS

Seed Yield

Both plant growth regulators significantly increased seed yield at all the rates used in 1987 and 1990 (Tables 2 and 4) but only paclobutrazol at the two highest rates was effective in 1989 (Table 3). In all three experiments paclobutrazol was more effective than triapenthenol in increasing seed yield and maximum yields were always obtained using the highest application rate of paclobutrazol. Maximum seed yield increases were 350, 32 and 22% in 1987, 1989 and 1990 respectively. However, the control plot yields also varied markedly producing 124, 909 and 886 kg ha^{-1} seed in consecutive seasons.

There was a general trend of increased seed yield with higher rates of plant growth regulators used. This trend was not consistent in all years. Increasing the rate of paclobutrazol increased seed yield in 1987 and 1989 but not in 1990 and for triapenthenol in 1987 but not in 1989 (Tables 2, 3 and 4). Furthermore, paclobutrazol at the highest rate produced significantly more seed than the lowest rate in 1987 and 1989 but not in 1990, and triapenthenol at the highest rate was significantly more effective than the lowest rate in 1987.

Table 2. Effect of growth regulators on seed yield and yield components of white clover cv. Merwi - Experiment 1 - harvest 1987.

Treatment (kg a.i. ha^{-1})	Total ripe heads at harvest[1] (m^{-2})	Total flowers at harvest (m^{-2})	Florets per head	Seed weight per 10 heads (mg)	Seed set per floret	Harvested seed yield (kg ha^{-1})
Control	811	923	67.2	820 ab	2.0 a[3]	124 a
Paclobutrazol						
0.25	811	917	70.4	867 ab	2.0 a	385 cd
0.5	1211	1328	71.7	1002 bc	2.3 a	439 d
1.0	1333	1397	63.1	1234 c	3.2 b	558 e
Triapenthenol						
0.7	944	1077	68.7	625 a	1.7 a	243 b
1.4	1248	1344	68.5	862 ab	2.2 a	298 bc
2.8	976	1061	65.1	847 ab	2.3 a	372 cd
LSD ($P < 0.05$)[2]				($P < 0.01$)		
Chemical	NS	NS	NS	195.7	NS	55.9
Rate	NS	NS	NS	239.6	0.63	68.4
Chemical x rate	NS	NS	NS	NS	NS	NS

[1]Total ripe heads includes heads from groups (a) + (b) : i.e. brown head + brown or browngreen peduncle.
[2]Significance level is 0.05, unless otherwise stipulated.
[3]Values followed by the same letter do not differ significantly.

Table 3. Effect of growth regulators on seed yield and yield components of white clover cv. Merwi - Experiment 2 - harvest 1989.

Treatment (kg a.i. ha^{-1})	Total ripe heads at harvest (m^{-2})	Nearly ripe heads group b (m^{-2})	Total flowers at harvest (m^{-2})	Florets per head	Seed weight per 10 heads (mg)	Seed set per floret	Harvested seed yield (kg ha^{-1})
Control	976	696	1084	69.1	1033	2.1	909 a
Paclobutrazol							
0.25	828	548	1036	69.1	1195	2.5	1019 ab
0.5	1024	712	1212	74.2	1299	2.5	1115 bc
1.0	1004	768	1244	67.1	358	2.9	1200 c
Triapenthenol							
0.7	632	436	752	63.6	1239	2.8	977 a
1.4	892	608	1016	64.5	1069	2.4	993 ab
2.8	696	444	860	64.1	1218	2.7	1018 ab
LSD (P < 0.05)			(P < 0.01)				(P < 0.01)
Chemical	NS	131.3	278.6	NS	NS	NS	72.3
Rate	NS	NS	NS	NS	NS	NS	88.6
Chemical x rate	NS	NS	NS	NS	NS	NS	NS

Table 4. Effect of growth regulators on seed yield and yield components of white clover cv. Merwi - Experiment 3 - harvest 1990.

Treatment (kg a.i. ha^{-1})	Total ripe heads at harvest (m^{-2})	Total flowers at harvest (m^{-2})	Florets per head	Seed weight 10 heads (mg)	Seed set per floret	Harvested seed yield (kg ha^{-1})	Straw yield (kg ha^{-1})
Control	845 b	1391 b	74.3	1612	3.0	886 a	5166 b
Paclobutrazol							
0.25	559 a	1090 a	70.0	1561	3.2	1039 bc	4711 a
0.5	821 b	1358 b	68.0	1548	3.1	1084 c	4593 a
Triapenthenol 2.8	789 b	1404 b	68.0	1486	3.0	989 b	5127 b
LSD (P < 0.05)	205.9	242.8	NS	NS	NS (P < 0.01)	80.1	364.7

Seed yield components

No consistent relationships were established between seed yield and the components of yield over the three experimental years. In 1987 increasing the application rate of both growth regulators appeared to have a positive effect on seed set per floret (Table 2) but the increases were not significant. However, there was a positive correlation between the number of seeds set per floret and harvested seed yield ($r = 0.59$ - $P < 0.01$).

There were no differences in the seed weight per 10 heads except for the paclobutrazol treatment at the highest

rate. There was a positive correlation between the seed weight per 10 heads and harvested seed yield ($r = 0.69$ - $P < 0.01$).

Other seed yield components were unaffected except for a trend of an increased number of ripe heads (group (a): brown heads + brown peduncles) with higher application rates of paclobutrazol. This was also reflected in the total number of ripe heads (groups (a) + (b)) but the differences were not significant (Table 2).

In Experiment 2 (1989) significant differences in the number of nearly ripe heads and total flowers per m^2 were evident. Paclobutrazol increased these components when compared with triapenthenol, but both chemical treatments were not significantly different from the control (Table 3). Other yield components did not differ.

Despite significant seed yield increases following chemical treatments in 1990, yield components were little affected. The reduced number of total ripe heads and total flowers at harvest after paclobutrazol was applied at 0.25 kg a.i. ha^{-1}, could not be explained (Table 4). Only the straw yield was a decisive factor for actual seed yield. Linear regression analysis showed a negative correlation between actual seed yield (= y) and straw yield (= x); i.e. $y = 1.83 - 0.17x$; $r = -0.73$ ($P < 0.01$). Straw yield was calculated from the difference between gross quantity (straw + seed) after harvest and quantity of uncleaned seed after threshing.

Further analysis of the number of seeds per head for the three experiments (Table 5) showed significant differences for Expt 1 (1987) only; i.e. paclobutrazol at the highest rate significantly increased seeds per head by 54% over the control. This trend of a higher number of seeds per head was also present in Expt 2 (1989), but it was not significant.

Peduncle length

Peduncle lengths did not differ significantly between treated and control plots (Table 6). However, there was a tendency to reduced peduncle length with an increasing rate of paclobutrazol (Expt 1). The reason for extended peduncles with paclobutrazol at 0.5 kg a.i. ha^{-1} in Experiment 2 (1989) was not apparent.

Vegetative crop material

Leaf canopy (height and quantity) at flowering was significantly reduced by paclobutrazol treatments in 1987 and 1989. This shortening was more distinct as application rate increased, but significant in 1987 only (Expt 1). Triapenthenol reduced leaf canopy in 1987 only (Table 6).

Straw yields at harvest in Experiment 3 (1990) partly reflect the effect of the respective chemical treatments on vegetative crop growth at flowering, as observed in Experiments 1 and 2 (Tables 4 and 6).

Germination and thousand seed weight (TSW)

Growth regulator treatments did not significantly affect either germination or thousand seed weight of the harvested seed in any year. The mean TSW was 0.59, 0.70 and 0.69 respectively in 1987, 1989 and 1990.

DISCUSSION

Annual seed yields of white clover in Belgium are strongly related to weather conditions during flowering, ripening and harvesting. The results of the three experiments therefore have to be considered in relation to the climatic conditions (Table 7).

Table 5. Effect of growth regulators on number of seeds per head of white clover cv. Merwi.

Treatment (kg a.i. ha^{-1})		Seeds per flower head		
		Expt 1 (1987)	Expt 2 (1989)	Expt 3 (1990)
Control		131.2 ab	147.2	221.1
Paclobutrazol	0.25	139.0 ab	173.7	221.3
	0.5	164.1 bc	184.2	206.2
	1.0	201.6 c	193.7	-
Triapenthenol	0.7	113.2 a	174.8	-
	1.4	152.0 ab	151.3	-
	2.8	150.6 ab	175.3	200.6
LSD ($P < 0.05$)				
Chemical		27.9	NS	NS
Rate		34.2	NS	NS
Chemical x rate		NS	NS	NS

Table 6. Effect of growth regulators on peduncle length at harvest and quantity of leaf mass at flowering of white clover cv. Merwi: Expt 1 - 1987; Expt 2 - 1989.

Treatment (kg a.i. ha^{-1})		Peduncle length Expt 1 (cm)	Peduncle length Expt 2 (cm)	Height of leaf canopy Expt 1 (cm)	Evaluation of leaf mass quantity Expt 2 (scale 1-5)[1]
Control		16.4ab	19.2a	26.6e	5.0b
Paclobutrazol	0.25	19.4b	19.2a	18.7b	3.7a
	0.5	16.7ab	22.6b	14.7a	2.5a
	1.0	13.3a	20.7ab	13.8a	1.0a
Triapenthenol	0.7	20.5b	20.6ab	23.4d	5.0b
	1.4	16.6ab	19.8a	21.6cd	5.0b
	2.8	18.6b	19.7a	20.2bc	5.0b
LSD (P < 0.05)		3.85	2.09 (P < 0.01)	2.51 (P < 0.01)	0.58

[1] 1 = very low; 5 = very high

The very low seed yield of the control plot in 1987 (124 kg ha^{-1}) was mainly due to wet weather at the most critical periods of development. The 1987 crop produced excessive vegetative growth following defoliation (1st and 2nd decade of June). Flowering which occurred in a very dense sward was followed by unfavourable weather during the seed development stage (2nd and 3rd decade of July) and rain at harvest time (3rd decade of August). In fact, this was an ideal season to test plant growth regulators in a white clover seed crop.

Table 7. Meteorological data per decade during May, June, July and August at Melle (50° 59' N, elevation 11 m) - (Behaeghe *et al.*, 1990).

Mean temperature (°C)	May 1	May 2	May 3	June 1	June 2	June 3	July 1	July 2	July 3	August 1	August 2	August 3
1987	9.7	8.6	12.6	13.7	12.6	16.7	17.7	18.1	15.8	14.5	18.9	17.0
1989	12.3	14.2	16.4	11.1	18.6	15.9	18.8	15.4	19.6	17.5	18.9	16.4
1990	16.0	13.0	12.4	13.9	14.0	17.1	14.3	17.5	18.3	19.8	17.8	19.1
1962-1980	11.3	12.4	13.1	14.4	15.3	15.8	16.5	16.7	17.1	17.8	17.3	15.8
Rainfall (mm)												
1987	9.7	35.9	10.9	64.4	46.8	22.9	0.0	87.3	45.5	12.2	3.2	71.9
1989	1.0	4.1	0.0	39.7	0.0	13.4	27.8	0.0	39.9	4.8	2.6	35.3
1990	6.3	19.3	0.0	42.8	0.5	43.0	14.3	0.0	0.5	5.4	14.1	12.1
1962-1980	19.7	16.9	23.9	17.3	22.4	27.8	26.6	27.2	21.3	23.0	17.0	18.4
Number of days with rain												
1987	5	10	8	10	10	8	0	7	10	9	3	9
1989	5	4	0	9	0	7	6	0	5	4	3	7
1990	3	4	0	9	2	7	9	0	1	2	6	7

Paclobutrazol increased seed yield by 271% on average, while triapenthenol resulted in a mean increase of 145% over the control. PGR-application resulted in a large decrease in vegetative growth, without any decrease in peduncle length. In this way flowers were elevated upwards through the leaf canopy, presumably resulting in a more favourable micro-environment for good pollination, easy ripening and harvesting. Significant reductions in petiole height but not peduncle height have also been recorded by Marshall and Hides (1986, 1987). In the poor weather conditions of 1987, the more favourable micro-environment in the PRG-treated plots was obvious. The reduced leaf canopy (Table 6) resulted in higher seed set data, namely a better seed set per floret (Table 2) and more seeds per head (Table 5). However, the better seed set per floret only accounted for 35% of the variance in harvested seed yields, and other factors must be involved.

Experimental years 1989 and 1990 gave exceptional seed yields in the control plots; i.e. 909 and 886 kg ha^{-1} respectively. Paclobutrazol increased seed yields in both years, by an average of 22 and 20% respectively. This relatively low seed yield response to growth regulators in both years compared with Experiment 1 (1987), can be explained by the excellent weather conditions in 1989 and 1990. Growth regulators may not alter reproductive growth under such dry weather conditions. Triapenthenol did not increase seed yield in 1989. This could be because of low rainfall after application (Table 7) and the less aggressive dwarfing effect on plant growth as also noticed in our PGR-trials on perennial ryegrass (Rijckaert, unpub. data).

High seed yields were obtained when reproductive growth exceeded vegetative growth. A significant positive effect of both regulators on the total number and percentage of ripe inflorescences could not be derived from the statistical analysis. This is in contrast with the findings of Marshall and Hides (1989) and with the results from screening trials (Rijckaert, 1988). However, from visual observation of the individual plots, it appeared that growth regulators had a distinct positive effect on the number of ripe seed heads per unit area, especially in Experiment 1 during the wetter season of 1987 and in Experiment 2 and 3 for the paclobutrazol treatments.

Clearly, seed yield increases in these experiments can be explained firstly by an increase in seed head number per unit area and secondly by reduced vegetative growth which improves the micro-environment for good pollination, even ripening and easy harvesting. Potential seed yield data were calculated but were considered irrelevant in order to evaluate the seed recovery at harvest.

Although both growth regulators can be used successfully to obtain consistently high white clover seed yields, attention should be given to the residual effect of these chemicals on succeeding crops, especially that of paclobutrazol (Hampton, 1988).

CONCLUSIONS

The application of paclobutrazol in white clover consistently results in high seed yields. A rate of 0.5 kg a.i. ha^{-1} seems to be sufficient.

The positive yield response of triapenthenol is less consistent, particularly when there is a shortage of rainfall after application.

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