

Growth Regulation in White Clover (*Trifolium repens* L.) Grown for Seed Production

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ABSTRACT

In 1986-90 the influence of different plant growth regulators and defoliation on white clover (*Trifolium repens* L.) grown for seed was examined. Seed yield responses to the application of growth regulators were inconsistent, but paclobutrazol increased seed yield by 91-95 kg ha⁻¹ in one trial. Seed yield component responses were also inconsistent between years, but paclobutrazol increased seeds per flowerhead in one trial and in another trial Alar increased seed weight. Optimal application time and rate varied with year. Defoliation increased seed yield by increasing the percentage of ripe flowerheads at harvest in four of the five years.

Additional index words: corolla tube length, defoliation, growth regulation, seed yield components, seed yield, sugar content, visiting bees.

INTRODUCTION

In Denmark the area grown for white clover (*Trifolium repens* L.) seed production has decreased from approximately 9,000 ha in 1973 to 1,600 ha in 1991. One reason for this may be that the yield of white clover seed varies considerably between years. Standard deviation expressed as per cent of average yield for 1980-89 was 59.0% for white clover and 12.8% for spring barley. Seed yield is low when the spring/summer is cold and wet. The crop then has too strong a vegetative development, and only a few flowerheads appear.

In *Lolium perenne* L. application of growth regulators has been found to stimulate reproductive growth by increasing the production of fertile tillers and by increasing the number of seeds per spikelet (Hampton and Hebblethwaite, 1985). Research carried out in white clover in Belgium (Rijckaert, 1991) showed more ripe and nearly ripe flowerheads m⁻² in plots treated with chemical growth regulators. Marshall and Hides (1986) found that plant growth regulators reduced petiole height in white clover but did not influence peduncle height. This led to the elevation of flower heads above the leaf canopy which might improve pollination and ease harvesting. However seed yield was not increased significantly in this experiment, although in a further trial, paclobutrazol did increase seed yield (Marshall and Hides, 1991).

The present investigation was carried out in order to examine the influence of different plant growth regulators on seed yield and seed yield components in white clover (*Trifolium repens* L.) under Danish climatic conditions.

MATERIALS AND METHODS

The trials were carried out in 1986-90 at the Department of Industrial Plants and Seed Production, Roskilde on a sandy loam soil. White clover cv. Milkanova was established each year by undersowing in spring barley. Row

distance was 12 cm and the seed rate was 2 kg ha⁻¹. Every year except for 1988 smooth meadow grass (*Poa pratensis* L.) was undersown together with the white clover. The spring barley straw was removed immediately after barley harvest.

Weeds, pests and diseases were controlled as described by Nordestgaard (1986). No fertilizer was applied. The white clover was swathed approximately four weeks after peak flowering. Seed heads were combine harvested when dry. The number of days from swathing to combine harvest varied from 5-14 days according to weather conditions, but no rain fell on the swathed crop in any of the years.

The experimental design was a systematic experiment with three replicates. Plot size was 20 m². In 1986-87 the plant growth regulators Cerone (Ethephon), PP 333 (Paclobutrazol) and RSW 0411 (Triapenthenol) were used (Table 1). In 1988-90 the tested plant growth regulators were Alar (Daminozide) and Folicur (Tebuconazol) (Tables 2 and 3). In 1989 and 1990 the surfactant Li 700 was also included. The plant growth regulators were applied in 360 l water ha⁻¹. Each year there was an untreated control and a defoliated plot. Defoliation to approximately 10 cm was carried out when the first flowerheads appeared in the leaf axils. Plant growth regulators were applied at early flowering and two weeks later. Application dates and rates are presented in Tables 1, 2 and 3.

From a random subsample of twenty flowerheads, the following seed yield components were recorded in each of the three replicates: peduncle and petiole height, number of florets per flowerhead, number of seeds per flowerhead, and seed weight per flowerhead. At seed maturity (Nordestgaard, 1986), the total number of flowerheads and per cent ripe flowerheads were recorded from a 0.25 m² randomly chosen quadrat. Total seed yield was recorded from the whole plot.

Additionally, in 1989 corolla tube length was meas-

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ured in subsamples of 70 florets. Five flowerheads were centrifuged to obtain total nectar content. The total sugar content was measured and the percentage sugar was calculated. The number of bees visiting a 8 m² subplot was recorded between 10.00 and 15.00 h. Counts were made 14 times in the period 21 June to 7 July.

Data in Table 1 and 3 are means of two years whereas data from Table 2 are from only one year. All data were analysed as a systematic block design with year as a systematic factor. An analysis of variance was performed on each variable using the Statistical Analysis System (SAS Institute Inc., 1985). The main effects of treatment were tested using a General Linear Models procedure. Means of main effects were separated by a test for Least Significant

Difference with a significance level of 5%. The main effect was tested against blocks within years.

RESULTS

1986 and 1987

Subtracting the peduncle height from the petiole height gives the distance from the leaf canopy to the flowerheads. In 1986-87 spraying with PP 333 and defoliation increased the distance by 22-44 mm compared to the control (Table 1). The greatest effect was recorded at the first application. Application of Cerone and RSW 0411 did not increase the distance between the leaf canopy and the flowerheads.

Table 1. Effect of plant growth regulators on white clover seed yield and its components, mean of 1986 and 1987.

Treatment	Application rate	Application date	Peduncle - petiole height	Number of florets per flowerhead	Number of seeds per flowerhead	Seed weight	Total number of flowerheads	Per cent ripe flowerheads	Seed yield
	lha ⁻¹		(mm)			(mg)	(No. m ⁻²)	(%)	(kg ha ⁻¹)
Control			140	75	144	0.64	1012	79.3	543.7
Cerone	0.5	12 May	158	70	124	0.60	952	81.7	560.2
PP 333	2.0	12 May	184	63	150	0.69	949	90.3	634.6
RSW 0411	1.0	12 May	156	69	141	0.63	1040	79.1	560.8
Cerone	0.5	28 May	149	70	136	0.64	1239	82.5	548.9
PP 333	2.0	28 May	162	72	172	0.58	1028	83.5	638.5
RSW 0411	1.0	28 May	146	70	140	0.59	971	80.6	597.2
Defoliated		2 June	166	71	131	0.57	924	75.8	553.1
			***	***	***	***	*	ns	***
LSD P<0.05			20	5	16	0.05	198	-	33.3

* P < 0.05, *** P < 0.001, ns = non significant

All treatments except the second PP 333 application and defoliation decreased the number of florets per flowerhead compared to the control. The first Cerone application reduced the number of seeds per flowerhead by 20 whereas the second PP 333 spraying increased the number by 28 seeds. Seed weight was increased by the May 12 PP 333 application but defoliation and the May 28 RSW 0411 and May 28 PP 333 applications decreased seed weight. The total number of flowerheads was increased by 227 m⁻² by the second Cerone application but the percentage of ripe flowerheads was not significantly influenced by any treatment in either year.

In 1986-87 total seed yield was significantly increased by both applications of PP 333. In plots treated with PP 333 on 28 May yield was increased by 95 kg ha⁻¹. RSW 0411 applied at this date also significantly increased seed yield (Table 1).

1988

In 1988 all 6 June applications and defoliation reduced the distance between the leaf canopy and the flowerheads compared to the control (Table 2). Spraying with 4 kg Alar (the highest application rate) reduced the distance by 35 mm. Defoliation reduced the number of florets per flowerhead.

Seed weight was slightly increased by application of Folicur compared to the control plot, but the number of flowerheads m⁻² and seeds per flowerhead did not differ significantly. Application of Alar slightly increased the total number of flowerheads and seeds per flowerhead, but none of the effects were significant. Seed weight was higher at the highest application rate. No significant effect on the per cent ripe flowerheads was recorded. Yield was increased when plots were sprayed with Alar at the beginning of flowering (6 June), or defoliated. These treatments

increased yield by 78-105 kg ha⁻¹.

Application of 4 kg a.i. Alar ha⁻¹ at first flowering slightly reduced corolla tube length from 3.26 to 3.15 mm,

whereas application of Folicur significantly increased corolla tube length by 0.24 mm. Other treatments did not alter corolla tube length.

Table 2. Effect of plant growth regulators on white clover seed yield and its components, 1988.

Treatment	Application rate	Application date	Peduncle - petiole height	Number of florets per flowerhead	Number of seeds per flowerhead	Seed weight	Total number of flowerheads	Per cent ripe flowerheads	Seed yield
	1 ha ⁻¹		(mm)			(mg)	(No. m ⁻³)	(%)	(kg ha ⁻¹)
Control			105	82	245	0.78	919	71.8	892.8
Alar 85	2	6 June	77	81	258	0.72	1097	71.1	997.3
Alar 85	4	6 June	70	86	269	0.77	1093	73.2	970.8
Folicur	3	6 June	81	82	246	0.84	917	79.6	907.7
Alar 85	2	15 June	92	76	268	0.75	989	76.5	935.9
Alar 85	4	15 June	96	82	252	0.81	1136	76.1	932.6
Folicur	3	15 June	121	82	245	0.82	1041	77.4	927.6
Defoliated		30 May	74	75	241	0.78	1040	81.2	992.4
			***	*	ns	*	ns	ns	**
LSD P < 0.05			20	7	—	0.07	—	—	60.4

Table 3. Effect of plant growth regulators on white clover seed yield and its components, mean of 1989 and 1990.

Treatment	Application rate	Application date	Peduncle - petiole height	Number of florets per flowerhead	Number of seeds per flowerhead	Seed weight	Total number of flowerheads	Per cent ripe flowerheads	Seed yield
	1 ha ⁻¹		(mm)			(mg)	(No. m ⁻³)	(%)	(kg ha ⁻¹)
Control			60	82	191	0.70	855	85.4	494.9
Alar 85 ¹	0.5	7 June '89 21 May '90	73	80	195	0.68	895	85.9	493.9
Alar 85	1.0	7 June '89 21 May '90	52	84	205	0.70	960	88.7	512.2
Alar 85	2.0	7 June '89 21 May '90	39	77	188	0.74	870	87.1	537.0
Folicur	3.0	7 June '89 21 May '90	50	82	172	0.75	813	89.2	514.2
Alar 85	0.5	16 June '89 7 June '90	62	81	165	0.77	807	88.4	500.9
Alar 85	1.0	16 June '89 7 June '90	63	81	207	0.71	799	87.3	512.0
Defoliated		18 May '89 9 May '90	70	78	164	0.70	907	93.4	547.0
			**	ns	***	***	ns	ns	*
LSD P < 0.05			18	—	19.0	0.05	—	—	33.0

¹ All Alar treatments except Alar 2.0 l ha⁻¹ had 1.0 l LI 700 added.

1989 and 1990

In 1989-90 the highest application rate of Alar reduced the distance from the leaf canopy to the flowerheads (Table 3). The number of florets per flowerhead was not significantly influenced by any treatment. Folicur, the first application of 0.5 kg Alar ha⁻¹ and defoliation reduced the number of seeds per flowerhead. Seed weight was increased by Folicur and 0.5 kg Alar at the second application. The per cent ripe flowerheads was not significantly increased by any treatment. Defoliation and 2 kg Alar ha⁻¹ applied at first flowering significantly increased seed yield. No treatments decreased seed yield.

Folicur, defoliation and Alar applied two weeks after the beginning of flowering reduced corolla tube length by 0.25-0.47 mm compared to the untreated control. The percentage of sugar in the defoliated plot was 50.3 which was significantly lower than the control plot. However, total sugar content was not decreased by defoliation. Total sugar content was significantly higher in plots treated by Folicur and 0.5 kg ha⁻¹ Alar. The amount of sugar was increased by 14.7-22.1 mg.

In defoliated plots an average of 4464 bees ha⁻¹ were recorded, compared to only 2413 ha⁻¹ in the control plots. The number of bees was also higher in growth regulator treated plots, but differences were not significantly greater than that of the control (data not presented).

DISCUSSION

In the five years of experiments the application of growth regulators did not stabilise seed yield in white clover. Seed yield varied in relation to weather conditions (Nordestgaard and Andersen, 1991). There was no correlation between the effect of the growth regulators and the weather conditions. However, none of the treatments reduced seed yield compared to the untreated control and some positive effects were found.

Experiments carried out by Marshall and Hides (1986) showed that application of PP 333 elevated the flowerheads above the leaf canopy but no difference in seed set was observed. In 1986-87 application of PP 333 increased the distance between flowerheads and the leaf canopy by 22-44 mm compared to the control. The number of seeds per flowerhead was increased, especially by the later application where the effect was significant. The elevated flowerheads might have been more attractive to the pollinators, but this was not recorded. Rijckaert (1991) found that application of PP 333 or RSW 0411 increased the percentage of ripe or nearly ripe flowerheads. In this experiment a similar effect was seen but the effect was not significant.

In red clover (*Trifolium pratense* L.) Alar decreased corolla tube length (Svendsen, 1990). A similar effect was recorded in white clover, but the optimal application rate and time varied. In 1989, bee visits were significantly higher in the defoliated plots. In undefoliated plots, the

undersown *Poa pratensis* had established strongly and had a high number of reproductive tillers. Defoliation removed these tillers. The greater bee numbers in defoliated plots suggests therefore that bees were unwilling to visit the white clover flowerheads beneath the *Poa pratensis* tillers. However, defoliation did not result in any more seeds per flowerhead, or seeds per floret, suggesting therefore that despite differences in bee numbers, pollination *per se* was not a problem. The significant increase in seed yield (+ 100 kg ha⁻¹) in 1989 in the defoliated plots was presumably because of more flowers (+ 121 m⁻²) and a greater percentage of ripe flowerheads (81% cf 71%) at harvest, even though statistically these differences were not significant. Defoliation is recommended in Denmark in years with excessive vegetative development (Nordestgaard, 1986).

Hampton (1991) reported that PP 333 increased inflorescence number in four white clover cultivars. This response was not recorded in 1986/87, and when there was a significant increase in inflorescences (Cerone at the 28 May application), seed yield did not increase. Rijckaert (1991) also found that inflorescence numbers were not significantly increased following growth regulator application.

Hollington, Marshall and Hides (1987) showed that ripe inflorescence numbers gave a good indication of potential seed yield. Any treatment which concentrates the flowering period should increase seed yield. With the exception of the cold and wet summer of 1987 (when defoliated plots were slow to recover), defoliation was the only treatment which consistently improved the percentage of ripe inflorescences at harvest. When plant growth regulators did increase seed yield, the reason for the response was not clear, as usually differences in seed yield components were not significant. None of the plant growth regulators used consistently and significantly increased white clover seed yield under Danish conditions.

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