EFFECT OF PLANT GROWTH REGULATORS AND IRRIGATION ON PHYSIOLOGICAL MATURITY AND SEED QUALITY OF RED CLOVER

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Introduction

Red clover is an important forage legume and is widely grown as a seed crop in western Oregon. Red clover seed crops are grown under both irrigated and non-irrigated regimes. Foliar-applied plant growth regulators (PGRs) have been widely used on temperate grass seed crops in Oregon and other parts of the world for several decades. This practice was adopted due to documented seed yield increases and reduction in lodging (Zapiola et al., 2006). Until recently, little information has been known about the effect of PGRs on crop maturation, seed quality, and yield of red clover. Currently, the acylcyclohexanedione PGRs are most commonly used in Oregon seed production. Two compounds are registered as lodging control agents in grass seed crops in Oregon: trinexapac-ethyl (TE), trade name Palisade EC® (Syngenta), and prohexadione-calcium (PC), trade name Apogee® (BASF).

A study conducted in Norway reported that seed yield was increased by 21% in red clover crops when TE (Palisade) was applied at stem elongation (Øverland and Aamlid, 2007). Anderson et al. (2012) conducted on-farm trials that revealed positive yield results with applications of TE on red clover seed in Oregon. Based on these results, TE was registered for use on red clover seed crops in Oregon in 2013. Field observation over two years of on-farm trials in Oregon indicated that in addition to increasing seed yield, TE treatments also promoted earlier crop maturation that allows more time for harvest operations. However, the effect of early maturation of red clover seed treated with TE on seed quality is unknown.

Using PGRs may alter seed development and maturation time, resulting in difficulty in determining the optimum time for harvest, but without sacrificing the quality of the seed, especially viability and vigor. Although some research looked at yield potential and lodging of red clover seed treated with PGRs, very little work has been done regarding the effects of PGRs on physiological development and maturation in relation to seed quality of red clover. However, one study investigated similar effects in canola (Elias and Copeland, 2001), and the methodology used in that study could be employed to explain the relationship between physiological maturity and seed quality in red clover.

Oliva et al. (1994) showed that a single irrigation strategically timed to coincide with flowering doubled seed yield over the non-irrigated control in red clover. The yield component most associated with the irrigation-induced increase in seed yield was the number of seeds per floret, with additional contribution from the seed weight component. However, there is no published research on the effect that a combination of PGR application and irrigation might have on physiological maturity (PM) and seed quality of red clover. Therefore, the objectives of this research were to determine the effect of irrigation, PGRs (TE and PC), and the interaction between them on (1) PM of red clover seed and (2) seed quality at different stages of maturity.

Materials and Methods

Research was conducted at Hyslop Crop Science Research Farm near Corvallis, OR. Red clover was planted in late September 2011. A randomized complete block design was used. The main plots consisted of irrigated and non-irrigated treatments. Within each main plot, eleven PGR treatments were applied to randomly selected subplots (Table 1). There were four replications of each treatment.

Table 1. Plant growth regulator treatments (trinexapac-ethyl [TE] and prohexadione-calcium [PC]) applied to each main plot (irrigated and non-irrigated) in 2012 study.

		PGR application timing	
Subplot		Stem elongation	Bud formation
1	Control (untreated)	—	—
2	Palisade (TE) 1 pt/acre	✓	—
3	Palisade (TE) 2 pt/acre	✓	—
4	Palisade (TE) 3 pt/acre	✓	—
5	Palisade (TE) 4 pt/acre	✓	—
6	Palisade (TE) 1 pt/acre	—	✓
7	Palisade (TE) 2 pt/acre	—	✓
8	Palisade (TE) 3 pt/acre	—	✓
9	Palisade (TE) 4 pt/acre	—	✓
10	Apogee (PC) 7.4 oz/acre	✓	—
11	Apogee (PC) 14.8 oz/acre	✓	—

The red clover seed crop was flail mowed in mid-May, and the residue was removed from the field. The TE treatments were applied at stem elongation and bud formation stages, while the PC treatment was applied once at stem elongation stage. Irrigation was applied at flower development stage by using a custom-designed Pierce Acre Master Micro Linear system equipped with minimum-drift Nelson sprinklers.

Physiological and visual characteristics of seeds
Inflorescences (heads) were sampled from the field plots following the protocol established by Elias and Copeland (2001). After full flowering (anthesis) and seed formation, seed and head color was recorded. Seed moisture content and dry weight of seeds were determined twice each week, from the initiation of seed development through seed harvest. Seed color, dry matter content, and seed moisture content were measured to determine the time of physiological maturity (PM, maximum seed dry weight) and harvest maturity (HM, when seeds reach proper moisture content for direct combine) of red clover seed.

Seed quality characteristics
Seed viability was determined weekly starting from the time of seed formation by the standard germination test (SGT) and the tetrazolium test (TZ). Seed vigor was ascertained by the cold test (CT) and accelerated aging tests (AAT). Germination percentage and viability by TZ percentage were recorded. All quality tests were conducted using the protocols of the Association of Official Seed Analysts (AOSA, 2009; AOSA, 2012).

Statistical analysis
The study was conducted over a two-year period. The data presented in this report are from Year 1 of the study. Analysis of variance (ANOVA) and mean separation by the Least Significant Difference test (LSD) were used to analyze the data. The MSTAT statistical package (Michigan State University) was used.

Results and Discussions

Effect of PGRs on physiological characteristics of seeds
The TE and PC application rates had no significant effects on seed moisture content (SMC). Seed moisture content of all treatments decreased rapidly after PM and was within 10% after one week. Seed dry weight (SDW) of all treatments increased gradually following seed formation, but did not change significantly after PM (Figure 1). The TE application reduced seed dry weight, while PC did not. Although TE reduced SDW and seed size, the number of seeds per head was increased over the untreated control, compensating for the decrease in SDW (data not shown). The application time of TE had no significant effect on SMC or SDW. Year 1 results did not indicate that TE treatments promoted earlier seed maturation as we expected.

Effect of irrigation on physiological characteristics of seeds
Irrigation had significant effects on SMC and SDW of red clover treated with TE and PC in Year 1. During seed development, the irrigation treatment resulted in higher SMC and SDC compared to non-irrigated seed. Irrigation resulted in a delay in PM and HM (Figure 1). The relative increase of SMC may be attributed to the delay in maturity compared to non-irrigated seeds. Irrigation also resulted in higher SDW compared to non-irrigated treatments. Consequently, seed yield from irrigated treatments was 10% higher than yields from treatments without irrigation (Figure 2). Similar results were reported by Chastain et al. (2013).

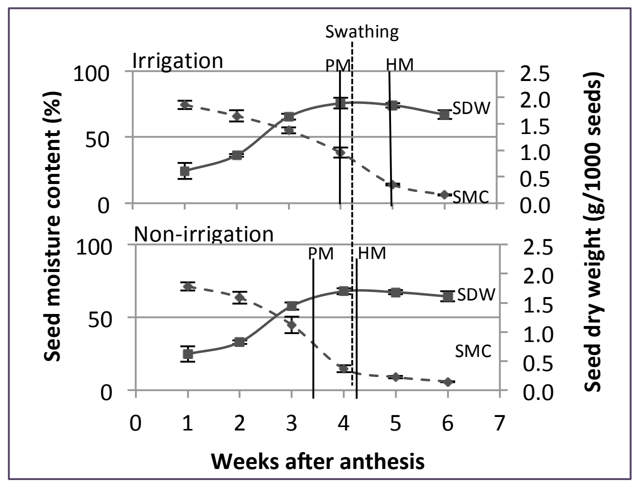

Figure 1. Seed moisture content (SMC) and dry weight (SDW) of red clover during seed development, irrigated and non-irrigated treatments. Where error bars regions do not overlap, treatments are significantly different. Curves represent the average of all PGR treatments. PM = physiological maturity; HM = harvest maturity.

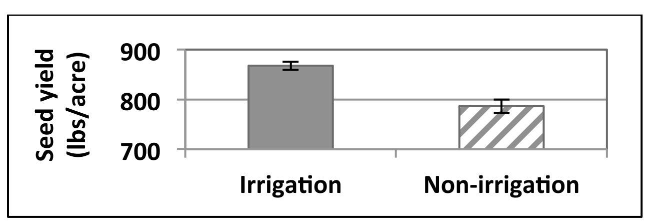

Figure 2. Seed yield of irrigated and non-irrigated red clovers, average of all PGR treatments. Statistical significance is indicated by non-overlapping error bar regions.

Table 2. Change in red clover head and seed color at different stages of seed development averaged over all treatments.

Weeks after anthesis	Seed color	Head color
1	Light green	Pale pink petals with green sepals
2	Light green	Pale to light brown petals with green sepals
3 } (PM)[1]	Light green to pale green	Light brown petals with green sepals
4	Pale green to pale yellow	Light to dark brown petals with pale green sepals
5 } (HM)[2]	Yellow or yellow-purple	Dark brown petals with light brown sepals
6	Yellow or yellow-purple	Dark brown petals with dark brown sepals

[1]PM = Physiological maturity
[2]HM = Harvest maturity

Visual indicators of physiological and harvest maturity

Changes in head and seed color were observed throughout development and maturation (Table 2). At the early stages of seed development, the heads contained pink petals with green sepals, and the seeds were tiny, soft, and translucent to light green. With further development, the heads contained pale pink petals with green sepals, and the seeds were pale green to yellow. At PM, the heads contained light brown petals with green sepals, and seeds ranged from pale green to pale yellow. The seeds were firm but not hard and could be smashed by a fingernail, mostly likely because the SMC was high, approximately 35–40%.

As head and seed color changed throughout the period of seed development and maturation, the SDW also changed (Figure 1). Therefore, the change in head and seed appearance can be indicators of PM in red clover. Visual indicators of PM have also been suggested for soybean (Gibkpi and Crookston, 1981), canola (Elias and Copeland, 2001), and cuphea (Berti et al., 2007).

Seeds reached HM about one week after PM. At HM, both petals and sepals were dark brown, and seeds were yellow or various shades of yellow-purple. The seeds were hard and could not be smashed by a fingernail or a needle because the moisture content was less than 10%. These hard seeds with low moisture content were suitable for direct combine.

Our results indicated that optimum swathing time occurs about four weeks after flowering (anthesis) for non-irrigated red clover and slightly earlier for irrigated red clover. Optimum threshing time or direct combine is one to two weeks following swathing, depending on weather conditions.

Seed quality characteristics

Seed quality from all treatments improved after PM (Table 3). Similar results were reported by Elias and Copeland (2001) in canola. In other crops, such as soybean, seeds reached maximum quality at PM (Miles et al., 1983). This difference may be the result of a different physiological change, such as the seed hormone ratio.

Irrigation, PGR application rate, seed maturity stage, and the interactions among them had no significant effects on seed quality. Seed quality at HM was not significantly different among irrigation and PGR treatments (Table 3). Seed viability as measured by the TZ test ranged from 93 to100%. Germination percentage from SGT and CT was also high and ranged from 88 to 100%. This indicates that the cold temperature stress in the CT did not affect seed vigor at HM, suggesting that maximum viability and vigor are reached at HM. However, the germination percentage from the AAT fluctuated, and results were not as consistent as those from other tests. Therefore, the AAT was not a reliable method to measure seed vigor of red clover in this study.

Seed yield

The effects of irrigation and PGR on red clover seed yield and yield component have been earlier reported by Chastain et al. (2013). Results from Year 1 of this work indicated that seed yield was increased by irrigation but was not significantly affected by PGR treatments.

This work is being carried out for a second year, and results from the two-year study period will be reported in a future article.

Table 3. Seed viability and vigor of irrigated and non-irrigated red clover at different stages of seed development and maturation and with different TE rates (pt/acre) as measured by TZ, SGT, CT, and AAT.[1]

Weeks after anthesis	Irrigated[2]					Non-irrigated[3]				
	Untreated	TE1	TE2	TE3	TE4	Untreated	TE1	TE2	TE3	TE4
Viability										
Tetrazolium test (TZ)										
	Viability (%)									
3	50	58	58	83	74	93	98	99	99	94
4	66	86	76	80	60	98	99	98	93	96
5	100	100	100	100	98	100	100	100	100	100
6	96	100	99	96	100	100	99	96	99	98
LSD (*P* = 0.05) = 7.9										
Standard germination test (SGT)										
	Germination (%)									
1	13	5	9	10	9	13	25	24	26	33
2	20	33	13	32	19	90	90	61	70	63
3	62	56	33	28	26	100	74	98	95	83
4	68	81	80	98	90	93	91	88	88	100
5	100	99	99	95	99	100	98	94	98	99
6	95	98	95	93	98	91	98	99	94	95
LSD (*P* = 0.05) = 10.3										
Vigor test										
Cold test (CT)										
	Germination (%)									
3	18	16	34	36	28	93	95	55	65	66
4	30	55	34	46	29	95	94	95	84	98
5	100	99	98	99	99	100	98	99	99	99
6	94	96	94	93	94	94	92	95	92	95
LSD (*P* = 0.05) = 10.7										
Accelerated aging test (AAT)										
	Germination (%)									
3	0	8	9	36	0	73	76	66	73	50
4	10	13	15	21	6	98	95	89	76	98
5	95	98	97	94	98	100	99	100	99	100
6	40	53	46	48	39	64	74	75	64	64
LSD (*P* = 0.05) = 13.8										

[1]Within each test, significant difference is indicated when the difference between any two means exceeds the LSD value at $P = 0.05$.
[2]In irrigated plots, physical maturity (PM) occurred about 4 weeks after anthesis. Harvest maturity (HM) occurred about 5 weeks after anthesis.
[3]In non-irrigated plots, physical maturity (PM) occurred between 3 and 4 weeks after anthesis. Harvest maturity (HM) occurred slightly more than 4 weeks after anthesis.

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