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Title: Trinexapac-ethyl Timing and Rate Effects on Crimson Clover (*Trifolium incarnatum* L.) Seed Yield and Seed Yield Components.

Abstract approved: ____

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Oregon is the world's leading producer of crimson clover (*Trifolium incarnatum* L.) seed. Recent research has reported that seed yield has been increased in cool-season grass and red clover (*T. pratense* L.) seed crops with the application of trinexapac-ethyl, a gibberellic acid biosynthesis inhibiting plant growth regulator (PGR) and lodging-control agent. There is very little readily accessible information on the management of crimson clover seed crops, and no work has been done that determines the effect of trinexapac-ethyl on seed production in crimson clover. The objectives of this study were: (i) to create a resource document for production of crimson clover seed crops, (ii) to determine the effect of trinexapac-ethyl on seed yield and seed yield components in crimson clover, and (iii) establish a recommendation for usage of trinexapac-ethyl in crimson clover to maximize yield for seed growers in the Willamette Valley.

Field trials were conducted at OSU's Hyslop Experimental Farm, Corvallis, Oregon, in two growing seasons (2014-15 and 2015-16). Trinexapac-ethyl PGR application rates (140, 280, 420, and 560 g a.i. ha^{-1}) and timing [Biologische

Bundesanstalt, Bundessortenamt und CHemische Industrie (BBCH) stages 32 and 50] treatments and an untreated control were investigated. Seed yield was not significantly affected by PGR treatments, while seed weight was reduced in both growing seasons. Seed number m^{-2} was significantly increased in the second growing season when mowing occurred. Both seed number and seed weight contributed to the stability in seed yield over years. Seed crops in both years were produced under extreme weather conditions where the first growing season was dry, while parts of the field in the second growing season were flooded. The number of inflorescence and florets m^{-2} was higher in the second growing season, while the seed set was lower, thus, seed yields in the second year were lower. Under the extreme conditions of this study, trinexapac-ethyl was not beneficial for crimson clover seed yields but was effective as a lodging control agent.

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Trinexapac-ethyl Timing and Rate Effects on Crimson Clover (*Trifolium incarnatum* L.) Seed Yield and Seed Yield Components

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Mohammed Morad, Author

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1.1. What is Crimson Clover?

Crimson clover (*Trifolium incarnatum* L.) is a winter annual species that is native in Europe and was introduced to the United States in 1818 (Knight, 1970). Crimson clover takes its name from distinctive scarlet red inflorescences. The plant's root system is characterized by a central taproot and fibrous root branches. The above-ground portion of the plant has lower and median pubescent leaves with long petioles and cuneate-obovate emarginate sessile leaflets. However, variations in morphology are observed with inbreeding. Different leaf and stem size, shape, and pubescence are observed among crimson clover genotypes. Trifoliolate leaves, sessile leaflet attachment, and pubescent leaf traits are dominant to multifoliolate leaves, petiolulate leaflet, and glabrous leaves, respectively.

The initial use for crimson clover was as a green manure crop and as a forage crop in 18th century Europe. Crimson clover was introduced as a forage crop and was spread throughout the southeastern United States where it is grown as a winter annual, while it is grown as a summer annual in Maine (Hollowell, 1947). Usage of legumes for green manure declined in the 1960s, nevertheless, crimson clover is still used as a self-reseeder in orchards, and also used in crop rotations. The crop is desirable for its ability to grow under a wide range of climatic conditions, nitrogen-fixing efficiency as a cover crop, large seed yield potential, and its important and effective association with other crops in bicultural practices. Cultivars that produce more hard seed and increased reseeding rates were developed to overcome stand losses. Crimson clover is also used as an ornamental for roadside beautification. Nitrogen can be supplied from crimson clover residue;

bermudagrass has been shown to receive between 120 and 240 kg N per hectare from crimson clover (Knight, 1970).

1.2 Crop Growth and Development

Crimson clover grows on both sandy and clay soils and tolerates mild soil acidity, but thrives best in well-drained fertile soils. Soil moisture and tilth are important to root development. Calcareous or high-lime soils limits growth by iron deficiency (Knight, 1970).

Crimson clover seed germination is influenced by temperature, moisture, and storage conditions; seed germinated at 20˚C within a 36 hr period, while germination at 10˚C was delayed for an additional 24 hr (Ching, 1975). Only 22% of crimson clover seed germinated at 30˚C. No effect was recorded on water uptake after 24 hr of treatment. However, protein and enzyme synthesis is regulated by temperature in the seed, thus affecting seed germination (Ching, 1975). Rincker and Rampton (1969) indicated that large-seeded cultivars produced 40% more dry forage than small-seeded cultivars.

Growing degree-days (GDD), or average heat accumulation, can be used for predicting and monitoring plant growth and development. The GDD upper threshold for crimson clover is 30˚C, while the base temperature is 0˚C (Butler et al., 2002). A legume seedling goes through three stages: heterotrophy, transitional stage, and autotrophy, where the seedling is fueled initially by stored substances in the cotyledon and later by photosynthesis (Cooper, 1977). Crimson clover seedlings grow rapidly under favorable conditions and form rosettes. The number of stems per plant is determined by stand density, where plants usually compensate for thin stands with larger rosettes and more stems. Stem and petiole elongation are also influenced by stand density.

Flowering in crimson clover is primarily dependent on day length instead of temperature (Butler et al., 2002). Photoperiodism is a plant response to the duration of daylight. This mechanism enables flower stems to elongate with many leaves and nodes when day length exceeds 12 hours. Elongation of the stem is terminated with the formation of flower heads composed of 75 to 125 florets. The corolla is usually deep red or scarlet, and extends beyond the calyx. Florets open in succession from the bottom to the top of the inflorescence. The fruit, known as a legume, is usually one-seeded.

Seed yield components are species-specific. The seed yield components for crimson clover are seed weight and seed number. Thick plant stands had a positive significant correlation with crimson clover seed yield (Iannucci et al., 1998). Seed number and inflorescence number also had a positive correlation with seed yield. However, seed weight had a negative correlation with seed number per inflorescence, indicating the action of seed yield component compensation (Iannucci et al., 1998).

Sod seeding is used to introduce crimson clover into dense grass sods in establishment years. Mowing and close grazing of the grass late in summer is required for reseeding stands in bermudagrass or other warm-season grasses. Light disk-tillage before frost may reduce competition for nutrients, light, and moisture. Seeding rates usually range from 12 to 33 kg ha^{-1} depending on the desired grazing amount.

1.3 Crimson Clover Seed Production

1.3.1 Seedbed Preparation and Stand Establishment

Inoculation of seed with nitrogen-fixing bacteria (*Rhizobium trifolii*) is important for vigorous growth and good stand establishment. Before stand establishment, a well-prepared, fine and firm seedbed is recommended for all clover crops. The seedbed should be free from air pockets and clods for best establishment results. The seedbed must be smooth and leveled for surface irrigation if applied. Planting between September and mid-October is necessary to establish a vigorous crimson clover seed crop in Western Oregon (Youngberg and Hickerson, 1975). This planting time allows for seedlings to produce four to six true leaves prior to the start of harsh winter conditions. Fall planting extends the growing season and increases production (Butler et al., 2002). Soil pH must be higher than 5.5 to avoid Al toxicity. Seedling nodulation decreased with decreased pH while decreased nodulation activity and N_2 fixation after reaching soil Al toxicity. Shoot protein percentage is highest between 5.5 to 6.0 pH (Evers, 2003). Crimson clover showed Fe-deficiency chlorosis symptoms in high pH, calcareous soils (Gildersleeve and Ocumpaugh, 1988). A suitable pH for the field of crimson clover would range from 6.0 to 7.0 (Gardner et al. 2000).

1.3.2 Nutrient Management

Nutrient management is important in crimson clover seed production. Boron (B) is known to increase seed yield in coarse-textured soil in Alabama. Water-soluble boron accumulation in the sandy coarse soil did not increase with boron annual application so it does not affect rotating boron-sensitive crops such as soybean (*Glycine max* L.) and cotton (*Gossypium spp.*). No significant increase was recorded with B application higher than 11 kg ha^{-1}. Annual application of B did not increase in fine-textured soils where water-soluble B was found (Wear, 1956). In Western Oregon, B application didn't increase seed yield in red clover, while soil tests showed B deficiency. Red clover seed crops are not affected by B soil test levels in the range of 0.3 to 0.4 ppm. B application is not economically recommended (Anderson et al., 2013). Nitrogen (N) can be obtained from symbiotic N fixation, so only an establishment application is recommended. N application rates are 22 kg/ha. Applications of nutrients should be based on soil tests; phosphorus (P) application rates range from 45 to 90 kg/ha. Phosphate applications are most effective when applied 2.5 cm to the side or below the seed (Gardner et al., 2000). Potassium (K) is broadcast into the seedbed as potash prior to seeding. K application rates range from 67 to 112 kg/ha. Plants absorb sulfur (S) as sulfate from fertilizer applications. Warm and moist soils are preferred for elemental S to sulfate conversions. Responses to other nutrients such as zinc or copper have not been observed in Western Oregon. Sulfate recommended rates range from 17 to 22 kg/ha. Lime application should be incorporated prior to planting when pH is 5.5 or lower.

1.3.3 Pollination

Crimson clover is both an outcrossing and self-fertile species, but self-incompatibility occurs in plant populations (McGregor, 1976). The stigma extends beyond the stamens and is held by the keel under tension. Pollen is transferred to the stigma by tripping. When the inflorescence is pollinated, the floret withers within 18 hours of fertilization, while florets remain fresh-looking when not pollinated. The floret senesces after seed maturity, which occurs in 24 to 30 days. Wind and rain are responsible for 13 to 20 percent of the total pollination, so insects are required to maximize pollination and profitable seed production. The honeybee is crimson clover's primary pollinator, whereas wild bees such as bumblebees are responsible for two percent of insect pollination.

Several studies have shown that the presence of honeybee hives in the field is relevant to significant seed yield differences in the southern states (McGregor, 1976). Under optimal agronomic practices, harvested seed yield was 1,142 kg ha^{-1} in crimson clover with three honeybee hives per acre, an increase of more than 897 kg ha^{-1} without bees. Caged plots where crimson clover heads were isolated from bees produced 5.08 seeds head^{-1}, while 69.2 seeds head^{-1} were obtained in an open field. Studies reported different recommendations for the number of bees required for highest yields, where it was reported that one hive per acre is sufficient when there is no presence of other competing crop adjacent to the crimson clover field.

Bee pollination and tripping are not effective in increasing seed yield in Western Oregon. Crimson clover bloom during unfavorable conditions for honeybee flights in the Willamette Valley, while native bumblebees are not affected by the same conditions making bumblebees the primary tripping agents during crimson clover bloom (Anderson et al., 2010). Anderson et al. (2010) indicated that low abundance of working bees didn't affect crimson clover seed yield since there was no positive correlation between presence of bee hives and seed yield, therefore, bee pollinators absence doesn't affect seed yield in the northern part of the Willamette Valley.

1.3.4 Pest Management

Insect pests are not problematic in crimson clover crops in every growing season unlike in white and red clovers. Most damage in crimson clover seed crops is caused by gray field slugs (*Deroceras reticulatum* M.) during the seedling stage. Metaldehyde and iron-based granule baits are used to manage slug infestations between September and November following warm and overcast weather conditions to maximize slug mortality. Sodium ferric EDTA is applied at 280 to 1,121 g a.i. ha^{-1}, iron phosphate is applied at 1,121 to 1,681 g a.i. ha^{-1}, and 4% metaldehyde is applied at 448 to 1,345 g a.i. ha^{-1}. Metaldehyde granule baits have 24c Special Local Need (SLN) registrations, and lack legal tolerances established for pesticide residues that may be necessary for the harvested hay, grazing, or seed screenings. Therefore, crimson clover seed crops that were exposed to metaldehyde-based granule baits are considered nonfood crops. (Pacific Northwest Pest Management Handbook, 2016).

Weeds compete with crops for nutrients, light, and water resources, so their increased incidence in the crimson clover seed crop field can lower seed yield. The most common weed problems in crimson clover seed crops are annual weeds. Weed control is important for economical seed yields as uncontrolled weeds interfere with harvest, and cleaning of the seed to remove weed seed, further reduce seed yield. Weeds can interfere with uniform herbicide and desiccant applications (Rincker and Rampton, 1985). Weed seed can affect seed crop certification, crimson clover seed crop can be certified with a maximum of 0.5% weed seed (Oregon Seed Certification Service Handbook, 2013). Common weeds occurring in crimson clover seed crop fields in Western Oregon are mayweed chamomile (*Anthemis cotula*), prickly lettuce (*Lactuca serriola*), pineapple weed (*Matricaria matricarioides*), wild carrot (*Daucus carota*), Italian ryegrass (*Lolium multiflorum*), small vetches (*Vicia spp.*) (tiny, narrowleaf, sparrow, four seed), cutleaf geranium (*Geranium dissectum*), catchweed bedstraw (*Galium aparine*), nipplewort

(*Lapsana communis*), and wild brassicas (Anderson and Hulting, 2013). Isolation distances between seed fields to grow foundation, registered, and certified seed in two-hectare or less fields are 402, 201, and 101 meters, respectively, while the distance is 402, 101, 49 meters for fields larger than two hectares. Usage of clean seed at planting is important for weed control. Also, crop rotation can be introduced to control perennial and annual weeds (Lee, 1985).

Benefin and EPTC may be used as preplant herbicides. Benefin is applied from 1345 to 1681 g a.i. ha^{-1} depending on the soil texture. Applying benefin to a flooded field could result in poor weed control, and rotation with grass, or cereal crops may cause crop injury due to herbicide carry-over. EPTC is applied at rates from 2.2 to 4.5 kg a.i. ha^{-1}, and may be used as a preplant herbicide application but must be applied before final harrowing or disking. MCPA Amine is applied when the established crops are showing two true leaves. The application rate ranges from 258 g a.e. ha^{-1} to 516 g a.e. ha^{-1} to control broadleaf weeds. Application of imazamox and bentazon are other herbicides that may be used to control grass and broadleaf weeds. Imazamox and bentazon application rates are 45 g a.i. ha^{-1} and range from 280 to 560 g a.i. ha^{-1}, respectively. Applications must be made when weeds are 2.5 to 7.6 cm tall and before seed crop bloom. Clethodim rates from 0.11 kg a.i. ha^{-1} to 0.28 kg a.i. ha^{-1} may be applied to control annual and perennial grasses if these are a problem in the crimson clover seed field (Pacific Northwest Pest Management Handbook, 2016).

Few plant diseases affect crimson clover seed crops. In early-planted crops having more foliage, the incidence of sclerotinia crown and root rot caused by *Sclerotinia trifolium* may be increased. The sclerotinia infestation can cause defoliation of the crop canopy and reduce seed yield (Aldrich-Markham, 2012). Symptoms of this disease include appearance of brown spots in late fall, and appearance of fungal bodies on the crown and the root. Methods of cultural control include crop rotation, planting pathogen-free seed, deep plowing to bury the fungus bodies, and avoiding over-fertilization. White mold (*Sclerotinia* sp.) and black stem (*Phoma* sp.) infestations can decrease yield significantly with lack of control (Leffel et al., 1993). Although Rovral and Ronilan are not currently labeled, applications decrease the severity of infestations while significantly affecting seed yield. Rovral and Ronilan applications provided an increase of 620 kg ha^{-1} and 450 kg ha^{-1} seed yield, respectively. Nevado 4F and Quadris Flowable are both currently labeled fungicides that control black stem and white mold. (Pacific Northwest Pest Management Handbook, 2016).

1.3.5 Irrigation

Little or no irrigation is applied on crimson clover in the Willamette Valley. Irrigation may be applied to offset drought when autumn rainfall is limited after planting of the crop. There is no study reporting the effects of irrigation on crimson clover seed yield in Western Oregon. Under stressful water deficit in Southern Italy, crimson clover seed yield was reduced by 24% (Iannucci et al., 1998). Martineillo (1999) demonstrated that crimson clover seed yield may be increased with irrigation in Mediterranean environments. This seed yield increase resulted from greater numbers of seeds inflorescence^{-1}, but unlike other annual clovers, the seed yield increase is negligible if forage harvest was absent. When applying irrigation after crimson clover forage harvest and regrowth, seed yield is significantly increased by 86% over forage harvested nonirrigated crimson clover seed crops. Irrigation was found to consistently increase seed yield in red clover in the Willamette Valley (Anderson et al., 2016).

1.3.6 Forage Management for Seed Production

Mowing is done to reduce lodging and remove excess vegetative growth of the crop, and promote seed yield. Rampton (1969) demonstrated that mowing crimson clover when stems reached a maximum height of 20 cm to 23 cm decreased lodging, increased seed yield, and decreased seed weight. Applying mowing at a 13.4 kg ha^{-1} planting rate treatment had the highest seed yield increase compared to other planting rates, showing an interaction between the two treatments. When grazing is applied to terminate growth, grazing in mid-April is preferred for higher seed production, and higher percentage of volunteer seedling establishment (Evers et al., 2006).

1.3.7 Seed Harvest

Direct combining is difficult during harvest because of lodged stems, a mixture of green and ripe flower heads, green weed contamination, and shattering problems with matured heads (McDonald and Copeland, 1996). Efficient harvest with direct combining may be achieved with a uniform mature field and low weed infestation. Crimson clover is typically harvested in Oregon by swathing the crop into windrows. Swathing should be done at night or early mornings when the crop is damp. Windrows must be uniform and small to enable efficient stubble feed into the combine. Commercial combines are usually 4 to 5 meters wide.

Threshing windrows is the second-step in harvest of the crimson clover seed crop. The combine must be adjusted to move the plant material into the draper with a pick-up header (McDonald and Copeland, 1996). Combine adjustments for dry, cured material include the following: cylinder speeds of 1,500 to 1,600 rpm, closed spacing between concaves and cylinder, closed slots between full set of concaves, chaffer, adjustable shoes sieve, special adjustable chaffer extension, and tailboard adjusted so unhulled seed drop to the tailing auger and are carried back to cylinder. The use of air is important to keep unhulled seeds from passing through the shoe sieve.

Other adjustments for improperly cured material include, reducing forward speed of combine to keep a minimum amount of material on the chaffer and prevent clogging of elevator, reduction of cylinder speed from 1,000 to 1,100 rpm, cylinder clearance of 0.32 to 0.48 cm with normal number of concaves, and enough air to blow chaff of the chaffer (McDonald and Copeland, 1996). This way of threshing would have one-third of the seed hulled. Threshing crimson clover seed is considered a specialized job due to numerous ways to adjust the combine.

Crimson clover seed is harvested at 35% seed moisture. Threshed seed is usually high in moisture, so spreading, stirring, and airing frequently is required to prevent heating. Harvested immature seed must not be stored until seed moisture is 12% or below. Seed cleaning is not necessary if combines were adjusted to hull all seed. After threshing hulled and unhulled seed, unhulled seed can be cleaned after running through a hammermill while hulled seed are screened out in a screening mill (McDonald and Copeland, 1996).

1.4 Plant Growth Regulators

Plant growth regulator (PGR) are chemical compounds that affect metabolic and developmental processes in plants (Rademacher, 2015). PGRs affect the balance of plant hormones by inhibiting the translocation or the biosynthesis of plant hormones, including auxins, gibberellins, cytokinins, abscisic acid, and ethylene. True PGRs are compounds that inhibit hormonal functions, while atypical PGRs are phytotoxic and thus affect the hormonal system indirectly.

Gibberellins (GA) are plant hormones involved in longitudinal growth, bolting in long-day plants, induction of enzymes during seed germination, and promotion of fruit development (Rademacher, 2015). GA-inhibiting PGRs can be categorized into three function-based groups. The first group is based on blocking GA metabolism by inhibiting cyclases in early stages of GA biosynthesis such as chlormequat chloride and mepiquat chloride. Triazoles, such as paclobutrazol and uniconazole-P, and pyrimidines, such as ancymidol and flurprimidol are examples of the second group inhibiting cytochrome P450-dependent monoxygenases which serve as a catalyst of ent-kaurene oxidation into GA12-aldehyde. The last group which inhibit dioxygenase reaction by blocking 2-oxoglutaric acid as a catalyst includes daminozide, prohexadione-calcium, and trinexapac-ethyl (TE). TE is a GA-inhibiting PGR that inhibits biosynthesis because of the structural similarity to 2-oxoglutaric acid, a co-substrate in a dioxygenase catalysis in the late stages of GA biosynthesis. The third group is more precise in GA inhibition than PGRs in the other two GA-inhibiting groups.

Several studies reported TE effects on grass seed crops and the PGR has been widely adopted as a result of this work. Ryegrass (*Lolium spp.*), and tall fescue [*Schedonorus arundinaceus* (Shreb.) Dumort.] seed yield increased by 50% and 67% on average with different TE applications at BBCH 32 in New Zealand because of increased floret site utilization (Chastain et al., 2003; Rolston et al., 2004). Chynoweth et al. (2010) reported the highest seed yield increase in perennial ryegrass (*Lolium perenne* L.)

by applying 400 g ai ha^{-1} TE rate. Seed yield increase was a result of seed number spikelet^{-1} increase. Less seed abortion and seed development retention was caused by reduced lodging and better solar radiation use. Perennial ryegrass seed yields increased proportionally with seed number m^{-2} with similar TE rate (Chastain et al., 2014). A one-day delay in reaching 50% lodging in perennial ryegrass increased seed yield on average 24 kg ha^{-1} (Rolston et al., 2010).

PGRs have been tested in clover seed crops but not in crimson clover. Chlormequat chloride, paclobutrazol, and ethephon, an ethylene releaser, were applied at three rates on white clover (*Trifolium repens* L.), but did not affect seed yield (Marshal and Hides, 1986). However, paclobutrazol applied at 0.5 and 1.0 kg a.i. ha^{-1} and ethephon applied at 1.92 kg a.i. ha^{-1} significantly reduced petiole height. Ethephon and chlormequat chloride applied at the highest application rates, 1.92 kg a.i. ha^{-1} and 1.28 kg a.i. ha^{-1}, respectively, increased flower number and ripe inflorescences number at harvest.

Paclobutrazol applications on white clover in New Zealand increased inflorescence number at inflorescence initiation (BBCH 32) and flower bud emergence (BBCH 50) (Hampton, 1991). The application at BBCH 32 hastened flowering, while the application at BBCH 50 delayed flowering in white clover. Petiole length reduction was significantly higher at BBCH 50 than BBCH 32; however, peduncle length was only affected by PGR application at BBCH 50. Paclobutrazol application at BBCH 32 elevated the inflorescences above the canopy by 0.5 to 5 cm. In general, paclobutrazol increased seed yield; however, some cultivars tested were not significantly affected by applications at BBCH 32. Seed yield increase was correlated with the increase of seeds inflorescence^{-1} with the exception of one cultivar where seed yield was increased correlating to harvested inflorescences m^{-2} increase. Seed weight was not affected by the PGR treatments.

Paclobutrazol, ethephon, and triapenthenol applications at early flowering decreased floret number per head, while paclobutrazol and defoliation increases the distance between inflorescence and leaf canopy in white clover in Denmark (Boelt and Nordestgaard, 1993). Paclobutrazol increased seed yield in both early application (early flowering stage) and late application (two weeks later). The distance between inflorescences and the leaf canopy was decreased with daminozide while increasing white clover seed yield compared to the control and tebuconazole application in the first year of the three trial years. Daminozide and defoliation were less significantly effective in the last two years. Daminozide application increased the number of flower heads which correlated with increased seed yield. Boelt and Nordestgaard (1993) reported seed yield variability which could be caused by weather conditions in the five trial years; however, weather conditions were not collated with PGR effects. Leaf size was not affected by PGRs, while daminozide applied at 2.0 kg a.i. ha^{-1} when reproductive buds were visible (BBCH 50) significantly reduced petiole and peduncle lengths (Budhianto et al., 1994a). Dry matter was affected by PGR application rates, while timing can affect dry matter accumulation and specific partitioning. Paclobutrazol application at 1.0 kg a.i. ha^{-1} during reproductive bud initiation (BBCH 32) increased reproductive dry matter while application at BBCH 50 increased vegetative dry matter. In the second year, triapenthenol applied at 1.0 kg a.i. ha^{-1} in both timings increased reproductive dry matter. PGR had no effect on stolon length; however, paclobutrazol and triaphenthenol applications at BBCH 50 reduced vegetative nodes while increasing reproductive nodes. Node number and stolon length was not affected by chloremequat chloride applications. Paclobutrazol applied at 1.0 kg a.i. ha^{-1} in at BBCH 32 and 50 increased inflorescences m^{-2} (Budhianto et al., 1994b). Pactobutrazol application at 1.0 kg a.i. ha^{-1} increased potential seed yield, while potential seed yield was not significantly affected by other PGR treatments. All treatments increased levels of white clover seed germination over the control when scarification was used. Paclobutrazol showed more consistency in increasing seed yield than other PGRs used, however, some studies showed that weather conditions such as rainfall may affect the soil-applied paclobutrazol root uptake. Paclobutrazol application increased the number of inflorescences m^{-2} 25-35 days after peak flowering stage (Budhianto et al., 1995). Seed yield was higher 25 days after peak flowering compared to 45 days after peak flowering. Potential and actual harvest seed were lower when harvest was delayed 35 days after peak flowering. Overall, paclobutrazol treatments did not significantly increase potential and actual seed yield in white clover. There was a strong correlation between white clover seed yield, leaf area index (LAI) below 3, and total dry matter. Chakwizira et al. (2011) reported no significant PGR effect on seed yield and LAI compared to control, however, mowing increased stolon and inflorescences number.

PGRs were tested in red clover (*Trifolium pratense* L.) seed crops. Silberstein et al. (1996) reported paclobutrazol and uniconazol applications increased red clover seed yield by 38 and 11%, respectively. The seed yield increase was correlated with the increase of inflorescence number stem^{-1}, and inflorescence number m^{-2}. Øverland and Aamlid (2007) reported seed yield increase in red clover with TE application, accompanied by reduction in plant height and seed weight. Ethephon had negative effects on seed yield, while not affecting seed weight and plant height. Red clover seed yield was increased significantly with 500 g TE ha^{-1} during BBCH 32 (two internodes elongation), but increased significantly with split application of the same rate at BBCH 32 and BBCH 51 (10% flower bud emergence) in New Zealand and Oregon (Anderson et al., 2015). Seed weight was not affected with PGR treatments in New Zealand, while decreased by most treatments in Oregon. The seed yield in red clover was positively correlated with an increase in open florets and number of florets $inflorescence^{-1}$. TE applications did not affect the quality of red clover seed crops nor delayed physiological maturity in seed (Angsumalee, 2016). Anderson et al. (2016) reported that there was no interaction between TE and irrigation applications on red clover seed yield, while both treatments can increase seed yield independently. Strategically-timed irrigation applied at 100 mm at BBCH 55 increased seed weight and seed number in two trials. Crop height was generally reduced with all TE treatments while above-ground biomass was not affected. While there is no published work for TE effects on crimson clover, preliminary on-farm trials showed an increased seed yield by TE application at BBCH 32 (Anderson et al, unpublished).

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Chapter 2: Crimson Clover Seed Production in Western Oregon

Oregon State University Extension Publication

2.1 Introduction

Crimson clover is one of the important forage legume seed crops grown in the Willamette Valley of Oregon. The Willamette Valley produces about 95% of the total annual US crimson clover seed crop and the value of the production reached an all-time high of $20 million in 2014.

Crimson clover has been one of the most widely grown forage legume seed crops in the valley and is experiencing a resurgence in acreage in recent years after a protracted period of decline (Fig. 2.1). The long-term average acreage since 1975 to the present is 8185 acres with a high of 12777 acres in 2014 and a low of 3580 in 2007.

Crimson clover seed yields have more than doubled since the mid-1970s when average yields were as low as 300 lbs/acre in 1978 (Fig. 2.2). The long-term average seed yield for crimson clover in the region since 1975 is 708 lbs/acre with a high yield of 1126 lbs/acre in 2015.

The price of crimson clover seed for Oregon seed growers has varied in a repeating cyclical pattern with a peak and trough in price reached once every 3-5 years (Fig. 2.3). The price reached in the peaks have been progressively increasing over time but the troughs in price have remained somewhat consistent in averaging about $0.33/lb. The long-term average seed price for crimson clover is $0.57 per pound. The highest prices were in 2014 at $1.60 per pound and the lowest prices were in 1987 at $0.25 per pound.

2.2 Description

Crimson clover grows on both sandy and clay soils and can tolerate medium soil acidity, but thrives best in well-drained fertile soils. Soil moisture and tilth are important for root development. The plant is mainly characterized by its scarlet red inflorescences. The root system is characterized by a central taproot and fibrous root branches, while the above-ground portion of the plant has lower and median pubescent leaves with long petioles and cuneate-obovate emarginate sessile leaflets. Different leaf and stem size, shape, and pubescence are observed among crimson clover genotypes. Elongation of the stem is terminated with the formation of flower heads composed of 75 to 125 florets. The corolla is usually deep red or scarlet, and extends beyond the calyx. Florets open in succession from the bottom to the top of the inflorescence. The fruit, known as a legume, is usually one-seeded. Trifoliolate leaves, sessile leaflet attachment, and pubescent leaf traits are dominant to multifoliolate leaves, petiolulate leaflet, and glabrous leaves, respectively.

2.3 Seedbed Preparation and Stand Establishment

Before planting, a well-prepared, fine seedbed with a moist zone of 2 to 3 inches free from air pockets is recommended for crimson clover seed crops. The seedbed must be smooth and leveled on the surface if irrigation is to be applied. Crimson clover seed must be inoculated with nitrogen-fixing bacteria (*Rhizobium trifolii*) prior to planting for good stand establishment. Planting in September and mid-October is necessary to establish a vigorous crimson clover seed crop in Western Oregon (Youngberg and Hickerson, 1975). Soil pH must be maintained above 5.5 to avoid Al toxicity. Seedling nodulation decreased with decreased pH while decreased nodulation activity and N_2

fixation after reaching soil Al toxicity (Evers, 2003). Crimson clover showed Fe-deficiency chlorosis symptoms in high pH calcareous soils (Gildersleeve and Ocumpaugh, 1988). A suitable pH for the field of crimson clover would range from 6.0 to 7.0 (Gardner et al., 2000).

2.4 Nutrient Management

Nutrient management is important in crimson clover seed production. In Western Oregon, Boron (B) application didn't increase seed yield in red clover, while soil tests showed B deficiency. Red clover seed crops are not affected by B soil test levels in the range of 0.3 to 0.4 ppm. B application is not economically recommended (Anderson et al., 2013). Nitrogen (N) can be obtained from symbiotic N fixation, so only establishment application is recommended. N application rates are 20 lbs/a. Applications of nutrients should be based on soil tests; phosphorus (P) application rates range from 40 to 80 kg/ha. Phosphate applications are most effective when applied one inch to the side or below the seed (Gardner et al., 2000). Potassium (K) is broadcast into the seedbed as potash prior to seeding. K application rates range from 60 to 100 lbs/a. Plants absorb sulfur (S) as sulfate from fertilizer applications. Warm and moist soils are preferred for elemental S to sulfate conversions. Responses to other nutrients such as zinc or copper have not been observed in Western Oregon. Sulfate recommended rates range from 15 to 20 lbs/a. Lime application should be incorporated prior to planting when pH is 5.5 or lower.

2.5 Pollination

Crimson clover is an outcrossing and self-fertile plant, but self-incompatibility can be observed in plant populations. The stigma extends beyond the stamens and is held by the keel under tension. Pollen is transferred to the stigma by the process of tripping. When the inflorescence is pollinated, the floret withers within 18 hours during fertilization, while the floret remains fresh-looking when not pollinated. The plant senesces after seed maturity which occurs in 24 to 30 days.

Bee pollination and tripping are not effective in increasing seed yield in Western Oregon. Crimson clover bloom during unfavorable conditions for honeybee flights in the Willamette Valley, while native bumblebees are not affected by the same conditions making bumblebees the primary tripping agents during crimson clover bloom (Anderson et al., 2010). Anderson et al. (2010) indicated that low abundance of working bees didn’t affect crimson clover seed yield since there was no positive correlation between presence of bee colonies and seed yield; therefore, bee pollinators absence doesn’t affect seed yield in the northern part of the Willamette Valley.

2.6 Pest Management

Insect pests are usually not problematic in crimson clover seed crops in Western Oregon, damage by cutworm or symphylan may occur. Most damage in crimson clover seed crops is caused by gray field slugs (*Deroceras reticulatum* M.) during the seedling stage. Metaldehyde and iron-based granule baits are used to manage slug infestations between September and November following warm and overcast weather conditions to maximize slug management. Sodium ferric EDTA is applied at 4 to 16 oz ai/a, iron phosphate is applied at 16 to 24 oz ai/a, metaldehyde 4% is applied at 6.4 to 19 oz ai/a. Metaldehyde granule baits have 24c Special Local Need (SLN) registrations, and lack legal tolerances established for pesticide residues that may be on the harvested hay, seed, or screenings. Therefore, crimson clover crops that were exposed to metaldehyde-based granule baits are considered nonfood crops. Crimson clover seed production is dependent on bee pollination, so pesticide labels must be examined carefully to reduce bee poisoning while managing slugs (Pacific Northwest Pest Management Handbook, 2016).

Weeds compete with crops for nutrients, light, and water resources, so their presence in the crimson clover seed crop field can reduce seed yield. The most common weed problems in crimson clover seed crops are annual weeds. Weed control is important for economical seed yields as uncontrolled weeds interfere with harvest, and cleaning as seed to remove weed seed and further reduce seed yield. Weeds can interfere with uniform herbicide and desiccant applications (Rincker and Rampton, 1985). Weed seed can affect seed crop certification – the crimson clover seed crop is classified as certified with a maximum of 0.5% weed seed (Oregon Seed Certification Service Handbook, 2013). Common weeds occurring in crimson clover seed crop fields in Western Oregon are mayweed chamomile (*Anthemis cotula*), prickly lettuce (*Lactuca serriola*), pineapple weed (*Matricaria matricarioides*), wild carrot (*Daucus carota*), Italian ryegrass (*Lolium multiflorum*), small vetches (*Vicia spp.*) (tiny, narrowleaf, sparrow, four seed), cutleaf geranium (*Geranium dissectum*), catchweed bedstraw (*Galium aparine*), nipplewort (*Lapsana communis*), and wild brassicas (Anderson and Hulting, 2013). Most crimson clover seed produced in Oregon is not certified. The isolation distances for five-acre or smaller seed fields to grow foundation, registered, and certified seed are 1320, 660, and 330 feet, respectively, while the distance is 1320, 330, 165 feet for fields larger than five acres. Usage of clean seed is important for weed control. Also, crop rotation can be introduced to control perennial and annual weeds (Lee, 1985).

S-ethyl dipropylthiocarbamate (EPTC) may be used as a pre-plant herbicide (31 to 64 oz ai/a) but must be applied before final harrowing or disking. This herbicide cannot be used if the clover is planted with a cereal or grass nurse crop. Stunted growth can occur when crimson clover is grown under optimum conditions. MCPA amine is applied when established crimson clover and showing two true leaves. The application rate for MCPA amine is from 3.7 oz ae/a to 7.4 oz ae/a to control broadleaf weeds. Application of imazamox and bentazon are used to control grass and broadleaf weeds. Imazamox and bentazon are both applied at rates 0.6 oz ai/a and from 4 to 8 oz ai/a, respectively. Applications must be made when weeds are 1 to 3 inches tall and before seed crop bloom. Clethodim rates from 1.6 oz ai/a to 4 oz ai/a may be applied to control annual and perennial grasses (Pacific Northwest Pest Management Handbook, 2016).

Few plant diseases affect crimson clover seed crops. In early planted crimson clover with high amounts of foliage may result in the incidence of sclerotinia crown and root rot (*Sclerotinia trifolium*). The crown and root rot infestation can cause defoliation and reduce seed yield (Aldrich-Markham, 2012). Symptoms of this disease include the appearance of brown spots in late fall, and appearance of fungal bodies on the crown and the root. Methods of cultural control include crop rotation, planting pathogen-free seed, deep plowing to bury fungus bodies, and avoiding over-fertilization. Nevado 4F and Quadris Flowable are both currently labeled fungicides that control black stem and white mold. Nevado 4F is applied twice at most at 1.5 to 2 pints/a with a 24-hr reentry interval. Quadris Flowable is applied at 10 fl oz/a with a four-hour reentry. The fungicide must not be applied within 14 days of harvest for forage. (Pacific Northwest Pest Management Handbook, 2016).

2.7 Irrigation

There is no work to date reporting the effects of irrigation on crimson clover seed yield in Western Oregon; however, under stressful water deficit in southern Italy, crimson clover seed yield was reduced by 24% (Iannucci et al., 1998). Martineillo (1999) demonstrated that crimson clover seed yield may be increased with irrigation in Mediterranean environments. This seed yield increase resulted from increased seeds/inflorescences, but unlike in other annual clovers, the seed yield increase is negligible when applying irrigation alone. Harvesting crimson clover forage by cutting one inch from ground level coupled with irrigation increased seed yield by 86% compared to forage harvesting without irrigation.

2.8 Forage Management for Seed Production

Mowing can be applied to reduce lodging, remove excess growth, and control weeds; however, mowing is not commercially used in Oregon. Rampton (1969) demonstrated that mowing crimson clover when the stems reached a maximum height of 8 to 9 inches decreased lodging, increased seed yield, and decreased seed weight. The greatest seed yield increases were observed when the crop was planted at a 12 lbs/a rate and mowed showing an interaction between the two treatments; however, higher planting rates had lower seed yields. Grazing is not a commercial practice in Oregon, but when grazing was applied in Texas to terminate growth in mid-April, both higher seed production and higher percentage of volunteer seedling establishment were achieved over non-grazed the non-grazed crop (Evers and Smith, 2006).

2.9 Seed Harvest

Direct combining is difficult during seed harvest because of lodged stems, a mixture of green and ripe flower heads, green weed contamination, and shattering problems with matured heads (McDonald and Copeland, 1996). Efficient harvest with direct combining may be achieved with a uniform, mature field and low weed infestation. Crimson clover is typically harvested by swathing the crop into windrows at 35% seed moisture. Swathing should be done at night or early morning when the crop is damp with dew. Windrows must be uniform to enable efficient stubble feed into the combine. Commercial combines range from 14 to 16 ft wide.

Threshing windrows is the second-step in seed harvest - the combine must be adjusted to move the material into the draper without picking or pulling (McDonald and Copeland, 1996). Combine adjustments for dry, cured material are cylinder speeds of 1,500 to 1,600 rpm, closed spacing between concaves and cylinder, closed slots between full set of concaves, chaffer, adjustable shoes sieve, special adjustable chaffer extension, and tailboard adjusted so unhulled seed drop to the tailing auger and are carried back to cylinder, use of air is important to keep unhulled seeds from passing through the shoe sieve, and round hole 0.36 cm screen in lower part of shoe to sieve out unhulled seed.

Other adjustments for improperly cured material include, reducing forward speed of combine to keep a minimum amount of material on the chaffer and prevent clogging of elevator, reduction of cylinder speed from 1,000 to 1,100 rpm, cylinder clearance of 0.14 to 0.2 inches with normal number of concaves, enough air to blow chaff of the chaffer (McDonald and Copeland, 1996). This way of threshing would have one-third of the seed hulled. Threshing crimson clover seed is considered a specialized job due to numerous ways to adjust the combine.

Threshed seed may be high in seed moisture content, so spreading, stirring, and airing frequently is required to prevent heating. Harvested immature seed must not be stored until seed moisture is 12% or below. Extensive seed cleaning might not be necessary if combines were adjusted to hull all seed. After threshing, hulled and unhulled seed, unhulled seed can be cleaned after running through a hammermill while hulled seed are screened out in a mill (McDonald and Copeland, 1996).

2.10 Plant Growth Regulators

Under the right conditions, trinexapac-ethyl (TE) can be applied to increase seed production in crimson clover seed crops (Anderson et al, unpublished). Preliminary on-farm trials showed increased seed yields were possible with 1 pint/acre and 2 pints/acre TE applied at stem elongation. Yield increases ranged from 10 - 24% over the untreated control. Extreme weather conditions such as drought and heat or excessive rain and flooding during the cropping season can negate the benefits of TE application on seed yield of crimson clover although canopy height is reduced (Morad et al., 2017 – see Chapter 3 this thesis).

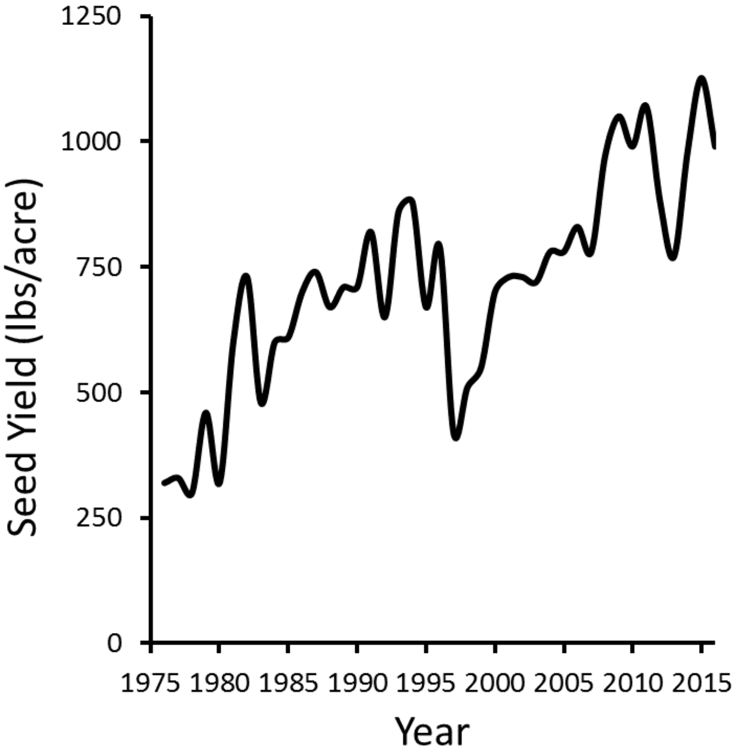

Figure 2.1. Crimson clover seed yields in Oregon.

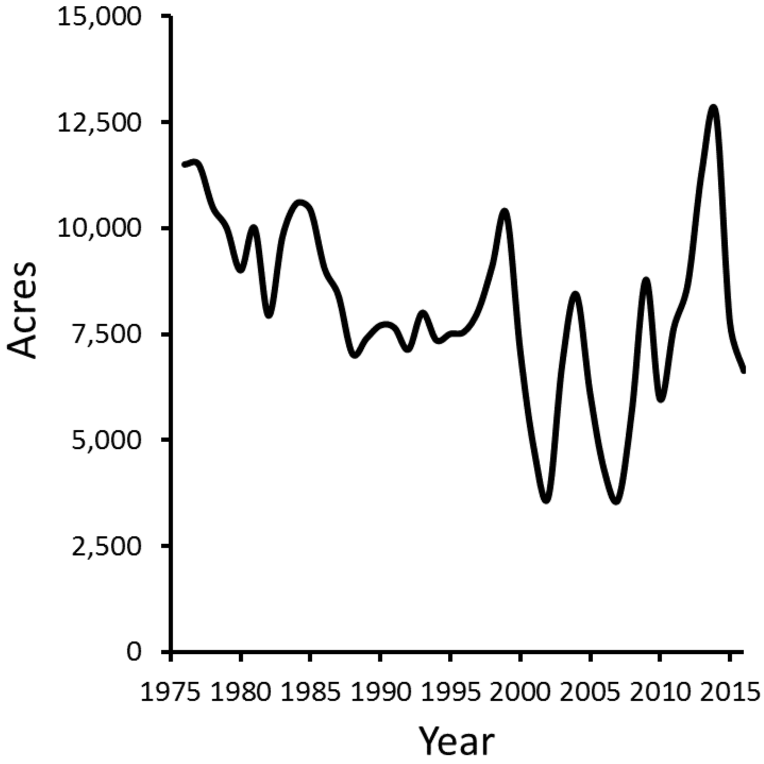

Figure 2.2 Crimson clover seed production acreage in Oregon.

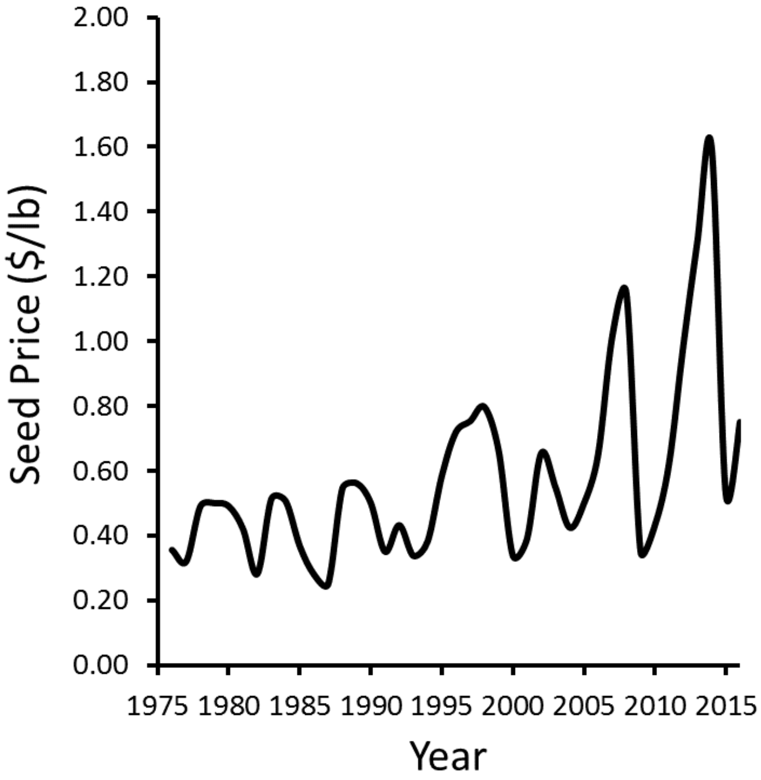

Figure 2.3. Variation in crimson clover seed price at the farm gate.

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Chapter 3: Trinexapac-ethyl Timing and Rate Effects on Crimson Clover (*Trifolium incarnatum* L.) Seed Crops in Extreme Weather Conditions.

Abstract

Oregon is the world's leading producer of crimson clover (*Trifolium incarnatum* L.) seed. Recent research reports that seed yield was increased in cool-season grass and red clover (*T. pratense* L.) seed crops with trinexapac-ethyl (TE), a gibberellic acid biosynthesis inhibiting plant growth regulator (PGR) and lodging-control agent. Field trials were conducted at Hyslop Experimental Farm near Corvallis, OR in two crop years. TE PGR application rates (140, 280, 420, and 560 g a.i. ha^{-1}) and timing [Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie (BBCH) stages 32 and 50] treatments and an untreated control were investigated. Seed yield was not affected by TE applications in general, while seed number m^{-2} and seed weight were affected. Seed weight was decreased with increased TE rate and this was most evident in the later stage application (BBCH 50). Seed yields were variable and low as a result of extreme drought and high temperature conditions in the 2014-15 crop year and extremely wet conditions in the 2015-16 crop year. There was no effect of TE PGR on seed yield at either application timing or for any of the four rates tested in both crop years. Seed weight was reduced with all TE application treatments and seed weight generally declined with increasing rate of TE and the BBCH 50 application. Seed number was not affected by TE in 2015 but was increased in 2016 with 280, 420, and 560 g a.i. ha^{-1} TE at BBCH 32 and by 420, and 560 g a.i. ha^{-1} TE at BBCH 50. Canopy height of the crop, a major factor in lodging, was consistently reduced with TE applications in both years. Poor growing conditions in these two crop years resulted in lower than expected seed yields for crimson

clover. Under these extreme conditions, TE consistently reduced height of the crimson clover seed crop canopy but this reduction did not affect seed yield.

3.1 Introduction

Oregon is the world's leading producer of crimson clover (*Trifolium incarnatum* L.) seed with production centered in the Willamette Valley of the western part of the state (Ball and Lacefield, 2000). The Willamette Valley accounts for 85% of crimson clover seed production in the U.S (Oregon Department of Agriculture, 2016), and the value of the production of seed reached $20 million in 2015. Crimson clover is a winter annual species that was introduced as a cover crop to the U.S. in the 1818 (Knight, 1985).

Crimson clover seed yields in Oregon have more than doubled since the mid-1970s, and recent research in red clover (*T. pratense* L.) seed crops suggests that further improvement of crimson clover seed yield may be possible (Anderson et al., 2015; Anderson et al., 2016).

Plant growth regulators (PGR) are chemical compounds that affect metabolic and developmental processes in plants (Rademacher, 2015). PGRs affect the balance of plant hormones by inhibiting the translocation or the biosynthesis of plant hormones, including auxins, gibberellins, cytokinins, abscisic acid, and ethylene. True PGRs are compounds that inhibit hormonal functions, while atypical PGRs are phytotoxic and thus affect the hormonal system indirectly.

Gibberellins (GA) are plant hormones involved in longitudinal growth, bolting in long-day plants, induction of enzymes during seed germination, and promotion of fruit development (Rademacher, 2015). GA-inhibiting PGRs can be categorized into three function-based groups. The first group is based on blocking GA metabolism by inhibiting cyclases in early stages of GA biosynthesis such as chlormequat chloride and mepiquat chloride. Triazoles, such as paclobutrazol and uniconazole-P, and pyrimidines, such as ancymidol and flurprimidol are examples of the second group inhibiting cytochrome P_{450}-dependent monoxygenases which serve as a catalyst of ent-kaurene oxidation into GA_{12}-aldehyde. The last group (acyclohexanediones) inhibit dioxygenase reaction by blocking 2-oxoglutaric acid as a catalyst, which includes daminozide, prohexadione-calcium and trinexapac-ethyl (TE). Trinexapac-ethyl [4-(cyclopropyl- α-hydroxymethylene)-3, 5-dioxo-cyclohexanecarboxylic acid ethylester] is a lodging control agent that belongs to this third group of GA-inhibiting PGRs and inhibits GA biosynthesis because of the structural similarity to 2-oxoglutaric acid, a co-substrate in a dioxygenase catalysis in the late stages of GA biosynthesis.

Considerable work has been done with PGRs in white clover (*Trifolium repens* L.) but results have been mixed with regard to efficacy as seed yield enhancing agents. In white clover, PGRs such as chlormequat chloride, paclobutrazol, and ethephon, an ethylene releasing compound, were tested but they did not affect seed yield (Marshall and Hides, 1986). Hampton (1991) found that seed yield responses to application of paclobutrazol were mixed among white clover cultivars tested and results dependent on application timing. Boelt and Nordestgaard (1993) reported that paclobutrazol increased seed yield in both early applications (early flowering stage) and late applications (two weeks later). Budhianto et al. (1995) showed that paclobutrazol treatments did not affect seed yield in white clover. Application of TE had no significant effect on seed yield in white clover (Chakwizira et al., 2011).

More positive seed yield responses to PGRs have been observed in red clover seed crops. Paclobutrazol and uniconazol PGR applications increased red clover seed yield by 38 and 11%, respectively (Silberstein et al., 1996). The increase in seed yield was correlated with the increase of inflorescence number stem^{-1}, and inflorescence number m^{-2}. Øverland and Aamlid (2007) reported that red clover seed yield was increased up to 34% when TE PGR was applied at BBCH 32 and that this was accompanied by reduction in plant height and seed weight. Ethephon had negative effects on seed yield, while not affecting seed weight and plant height. Anderson et al. (2015) reported a 9 to 16% seed yield increase in red clover across New Zealand and Oregon environments when 500 g TE ha^{-1} was applied at BBCH 32. Red clover seed yield was increased when applied at BBCH 32 in second-year stands, but not in first-year stands (Anderson et al., 2016). There was no interaction between TE and irrigation applications on red clover seed yield.

No research on application of TE or other PGRs has been conducted in crimson clover seed crops until recently. Preliminary on-farm trials have shown that TE can increase crimson clover seed yield by 10–24% over the untreated control (Anderson et al., unpublished). The objective of this study was to examine the effects of TE timing and application rate on crimson clover seed crops in the Willamette Valley of Oregon.

3.2. Materials and Methods

3.2.1 Overview

Field trials were conducted in two crop years (2014 – 15 and 2015 – 16) at Oregon State University’s Hyslop Experimental Farm (44° 40’ N, 123° 11’ 36’’ W) near Corvallis, Oregon, to quantify the effect of trinexapac-ethyl timing and rate treatments on seed yield and yield components in crimson clover. The farm site was flat and the soil was Woodburn silt loam (Fine-silty, mixed, mesic, Aquultic Argixeroll). The experimental site was 0.4 ha and each plot was 51.1 m^2 in the first growing season, while the northern plots of the experimental site was reduced to 40.8 m^2 due to flooding.

The experimental design was a randomized block design with four replications. Applications of TE were made at the following rates: 140, 280, 420, and 560 g a.i. ha^{-1}. The applications were made in two timings and were compared to an untreated control. The BBCH scale was used to characterize crop development and to schedule the two application timings at stem elongation (BBCH 32) and bud emergence (BBCH 50). The TE application rates were applied using a bicycle-type boom sprayer with a flat spray nozzles operated at 138 kPa in 26 March 2015 and 17 April 2016, at stem elongation (BBCH 32) and in 18 April 2015 and 21 April 2016 at bud emergence (BBCH 50).

The seed bed was prepared in September 2014 and 2015 by first plowing to 30-cm deep, and then followed with disk, and harrow. The crimson clover seed was planted on 2 October 2014, and 8 October 2015 by using a Nordsten drill set at a 15-cm row spacing. The seeding rate was 19 kg/ha. A pre-plant application of 20-0-0-24 fertilizer was made and was incorporated into the seedbed. Growing degree days (GDD) were calculated from the date of seeding and were based on air temperatures observed at

Hyslop Farm. The base temperature used in the GDD calculations was 0°C (Butler et al., 2002).

MCPA (2-Methyl-4-chlorophenoxyacetic acid) and clethodim ((E)-2-[1-[[(3-chloro-2-propenyl)oxy]imino]propyl]5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one) herbicides were applied at 350 g a.e. ha^{-1} and 103 g ai/ha at BBCH 12 and BBCH 17 in November 2014 and February 2016. Mowing was applied to control wild garlic (*Allium vineale*) on 11 April 2016.

3.2.2. Seed Yield and Seed Yield Components

Seed yield, seed yield components, and soil moisture content were determined in both trials. Soil moisture content was recorded in early May. Three measurements were recorded from each plot by a Spectrum time domain reflectometer (FieldScout). Above-ground biomass was determined by sampling fresh crimson clover stems, leaves, and inflorescences by cutting two adjacent 30 cm^2 quadrats and drying at 38° C in 15 May 2015 and 12 May 2016. Canopy height measurements were taken at mid-May using a measuring meter, an instrument that can be used to measure physical length of the crops.

The crop was harvested by swathing, followed by combine threshing on 10 June and 23 June 2015, and 18 June and 28 June 2016, respectively. The crop was swathed by a plot swather (Modified John Deere 2280), and was dried in windrows, seed in dried windrows were later threshed by a plot combine (Hege 180). Dirt seed yield was determined after threshing on the farm site. Sub samples were collected for further analysis of seed weight and seed number. Dirt yield samples were cleaned by M2B Clipper Cleaner and clean marketable seed yield was determined. Cleanout represents the non-seed material harvest and was determined to calculate clean seed yield. Seed samples used for seed number m^{-2} and seed weight were cleaned with screens and a blower, and were counted into two 1000 seed samples using an electric seed counter (The Old Mill Company, Model 850-2). The 1000 seed samples were weighed by micro scale (Mettler AE 160). Seed number m^{-2} was determined by dividing the clean seed yield harvested from each plot by individual seed weight. Harvest index was calculated as the ratio of clean seed yield to above-ground biomass.

3.2.3. Statistical Analysis

Statistical analysis for the trials was conducted using the generalized linear model procedure in SAS 9.3. Trinexapac-ethyl application timing and rates were considered fixed effects in the model. A co-variance test revealed that error variances were not homogenous across crop years (2014-15 and 2015-16), so data were analyzed separately. Treatment effects on canopy height, soil moisture content, seed yield, and seed yield components were analyzed by ANOVA and separation of means was conducted by Fisher’s protected LSD tests at 5% significance. Regression analysis was used to study the relationship between the TE application treatments and seed yield.

3.3. Results

3.3.1 Growing Season Environment

Western Oregon’s climate is classified as a Mediterranean warm summer type (Csb) and is marked by dry summers and mild, wet winters (Peel et al., 2007). Annual precipitation in the region is 1015 mm, with 90% recorded between October and April. Very dry conditions prevailed in the spring of 2015, with only 58% of normal rainfall occurring April through June, and these dry conditions likely influenced the results. Extremely wet conditions were prevalent in the 2015-16 crop year, especially in fall 2015 (132% of normal rainfall) and March 2016 (183% of normal rainfall). As a consequence of the wet fall and late spring conditions in the 2015-16 crop year, stands were uneven and poor in flooded parts of the experimental site. Temperatures also varied among the two growing seasons leading to differences in crop growth and development (Table 3.1).

The number of GDD (base temperature = 0°C) accumulated from the date of planting until early stem elongation ranged from 1568 GDD in 2015 to 1648 GDD in 2016. From planting until bud emergence (BBCH 50) was reached, the number of GDD accumulated was 1784 in 2015 and 1715 in 2016. Butler et al. (2002) reported that 1688 to 1999 GDD were accumulated between October planting dates and BBCH 50 so the values reported in this study are similar. To reach flowering (BBCH 60), Butler et al. (2002) found that between 2050 and 2100 GDD were required from October planting dates. Results were similar in this study for the number of GDD needed to reach BBCH 60 with 2107 GDD in 2015 and 2002 GDD in 2016. From planting, crimson clover seed crops required 2544 GDD in 2015 and 2572 GDD in 2016 to be accumulated at harvest of the crop by swather.

3.3.2 Growth Characteristics and Harvest Index

The number of stems m^{-2} produced by crimson clover was not significantly affected by TE application timings and rates in both crop years (Table 3.3). Nevertheless, canopy height was consistently reduced with PGR applications in crimson clover across years. Canopy height reduction increased with the increased TE application in both years; however, early timing application decreased canopy height to a greater extent and this effect was more evident in 2015 (Table 3.3). Biomass and harvest index were not significantly affected by TE application in both crop years.

3.3.3 Inflorescence Characteristics

Application of TE in crimson clover had no significant effect on inflorescence number m^{-2} in both years (Table 3.4). Florets m^{-2} was increased with TE application in in 2015; highest floret number was observed at 140 and 420 g a.i. ha^{-1} at BBCH 32, and with 420 g a.i. ha^{-1} at BBCH 50. No effects of TE on florets m^{-2} were evident in 2016. The number of florets inflorescence^{-1} was also affected by TE application in 2015. Florets inflorescence^{-1} in crimson clover was increased by TE at both BBCH 32 and BBCH 50 timings with the 420 g a.i. ha^{-1} rate.

3.3.4 Soil Water Content

Soil water content varied among TE PGR rate and timing treatments in 2015 while there were no significant differences observed in 2016 (Table 3.5). Soil water content was significantly lower compared to the control in 2015 for several TE rate and treatment timing combinations. The soil water content was lower than the untreated control when TE was applied at the 140, 420 and 560 g a.i. ha^{-1} rates at BBCH 32, and at the 280 g a.i. ha^{-1} rate when applied at BBCH 50.

3.3.5 Seed Yield, Seed Weight, and Seed Number

The ANOVA revealed that most characteristics of seed production in crimson clover including seed yield, stems m^{-2}, inflorescence number m^{-2}, biomass, and harvest index were not significantly affected by PGR treatments in both crop years (2014-15 and 2015-16) (Table 3.2). Seed yields were variable and lower than the ten-year average yield of 1,019 kg ha^{-1} for the Willamette Valley as a result of extreme drought and high temperature conditions in the first crop year, and extremely wet conditions in the second crop year. There was no significant difference among PGR timing and rate treatments on seed yield in both years (Table 3.6).

Seed weight in crimson clover was affected by TE in the same way in both crop years despite the lack of influence on seed yield (Table 3.6). In general, seed weight was significantly reduced by TE PGR application at both timings and that effect was most pronounced at high TE rates.

Seed number in crimson clover was not affected by TE rate and timings of application in 2015 (Table 3.6). Seed number was increased in 2016 by TE with rates between 280-560 g a.i. ha^{-1} at the BBCH 32 timing and by the 420 and 560 g a.i. ha^{-1} rates at the BBCH 50 application timing.

3.3.6 Cleanout and Seed Set

Cleanout represents the quantity of non-seed material harvested. Cleanout of the crimson clover seed crop increased with 420 and 560 g a.i. ha^{-1} TE applied at the BBCH 50 timing in 2015 (Table 3.7). There was no effect of TE application timing or rate on cleanout in 2016. Seed set was not significantly affected by TE rate or application timings in both years.

3.4 Discussion

There was no effect of TE PGR on crimson clover seed yield at either application timing or for any of the four rates tested in the study (Table 3.2). These results were not consistent with the preliminary on-farm trials conducted with crimson clover seed crops in prior years, which showed a seed yield increase with TE applications (Anderson et al., unpublished). There are several possible reasons for the differences in results with TE.

Both crop years were characterized for having unfavorable weather conditions, where the first crop year was very dry, warm and the second crop year was cold, very wet, and some flooding of the site occurred. Thin poor stands and increased branching caused by flooding in the second year likely contributed to the lower seed yield observed in the study. Leffel et al. (1993) reported crimson clover seed yields of 1896 kg ha^{-1} in the northern part of the Willamette Valley. Seed yields in this trial are lower than those experimental results and the long-term average for Willamette Valley seed growers.

Anderson et al. (2010) reported an average seed set of 74.4% in northern part of the Willamette Valley for crimson clover seed crops, while seed set for crimson clover grown in this study never exceeded 9.2% (Table 3.7.). Weather conditions can be responsible for affecting crimson clover pollination, where cool wet conditions can reduce the abundance of pollinators during bloom (Anderson et al., 2010). The wet and cool conditions that were prevalent in the 2015-16 crop year might have contributed to the low seed set and seed yields through a reduction in pollinators incidence and pollinator activity in the seed fields.

Seed weight was reduced in crimson clover with all TE PGR rate and time of application treatments (Table 3.2). The reduction in seed weight resulting from application of TE PGR on clover seed crops has been reported previously in red clover (Anderson et al., 2016), but not in cool-season grass seed crops where TE PGR has gained world-wide acceptance (Chastain et al., 2014).

There was no effect of TE on seed number m^{-2} in crimson clover in 2015 (Table 3.2), which was the primary factor responsible for the seed yield increase by TE PGR in red clover seed crops (Anderson et al., 2015; Anderson et al., 2016). Unlike in 2015, seed number m^{-2} in crimson clover was increased by TE PGR at certain high rates of application. This increase in seed number was unable to offset the loss in seed weight experienced when the crop was treated with TE, thereby resulting in no seed yield increases by TE.

The lack of effect of TE on stem number in crimson clover is not unexpected as the TE was applied after stem number was set during growth of the crimson clover seed crop and TE did not cause an observed mortality of stems (Table 3.3). Stem number was not affected by TE in red clover seed crops (Anderson et al., 2016).

Canopy height of the crimson clover seed crop was consistently reduced with TE applications, but biomass was not affected by TE treatment (Table 3.3). Anderson et al. (2016) found that crop height was generally reduced with all TE treatments in red clover seed crops while above-ground biomass was not affected. Silberstein et al. (1996) reported a decrease in stem height and the mean number and length of internodes from paclobutrazol PGR application. Iannucci et al. (1998) indicated that seed yield in crimson clover is positively correlated with dry biomass, number of seeds per inflorescence, and number of inflorescences per stem.

Harvest index (HI) was not affected by applications of TE PGR (Table 3.3). This result is to be expected since both seed yield and biomass were not influenced by TE. Anderson et al. (2016) reported that HI was increased by TE in red clover seed crops. Harvest index in crimson clover was low in this study, ranging from 2.18 to 3.45, while Anderson et al. (2016) found that HI ranged from 5.2 to 12.3. Harvest index is a measure of the preferential partitioning of dry matter to seed production rather than vegetative biomass, and these results suggest that not much in the way of seed was produced in relation to crop biomass under these extreme weather conditions.

The reduction in canopy height by TE most likely opened up the canopy, thereby allowing a greater loss of soil water through evaporation in 2015 (Table 3.4). Coupled with the abnormally dry and hot conditions in 2015, the reduction in canopy coverage with TE reduced the amount of soil water available for seed filling, and as a result seed weight was also reduced more than previously noted. Height reduction of the crop canopy increased transmission of photosynthetic active radiation (PAR) through the canopy in red clover seed crops (Anderson et al., 2015). With very wet conditions in March and April 2016, no effects of TE on soil water content were detected in early May 2016.

The number of inflorescences m^{-2} in crimson clover was not affected by TE PGR in either year and could be an important reason for the lack of seed yield response to TE in this study (Table 3.6). Paclobutrazol PGR applications increased inflorescence number in white clover seed crops (Hampton, 1991; Budhianto et al., 1995). Boelt and

Nordestgaard (1993) found that higher rates of daminozide PGR increased the number of inflorescences in white clover seed crops which was correlated with increased seed yield. Increases in inflorescences m^{-2} with TE PGR applications in red clover ranged from 26% to 62% (Anderson et al., 2015), and these increases contributed to greater seed yield with TE.

Poor growing conditions in these two crop years resulted in lower than expected seed yields for crimson clover. Under these extreme weather conditions, TE PGR consistently reduced height of the crimson clover seed crop canopy but this reduction did not affect seed yield.

Table 3.1. Seasonal temperature and precipitation, and departures from the 122-year means at Corvallis Oregon during crimson clover trials.

Year	Season	Temperature	Departure	Precipitation	Departure
		°C		mm	
2014-15	Fall	9.9	1.8	497	69
	Winter	8.4	2.4	332	-75
	Spring	14.1	1.0	85	-62
2015-16	Fall	9.2	1.1	564	136
	Winter	7.7	1.7	520	113
	Spring	14.9	1.8	116	-32

Table 3.2. ANOVA for trinexapac-ethyl treatment effects on crimson clover seed yield and seed yield components.

Characteristics	Treatment significance	
	2015	2016
Seed yield	ns	ns
Seed weight	***	***
Seed number	ns	***
Cleanout	***	ns
Biomass	ns	ns
Harvest Index	ns	ns
Stems/m^2	ns	ns
Heads/m^2	ns	ns
Florets/m^2	*	ns
Florets/inflorescence	*	ns
Canopy Height	***	***
Soil water content	*	ns
Seed set	ns	ns

* $P \leq 0.05$

*** $P \leq 0.001$

ns = Not significant

Table 3.3. Trinexapac-ethyl timing and rate effects on crop growth characteristics and harvest index in crimson clover.

Treatment		Stem Number		Canopy Height		Biomass		Harvest Index	
Timing	Rate	2015	2016	2015	2016	2015	2016	2015	2016
	g ai/ha	stems m^{-2}		cm		kg m^{-2}		%	
Untreated Control		373 a †	690 a	71.1 a	69.1 a	1.23 a	1.44 a	3.29 a	2.18 a
BBCH 32	140	404 a	603 a	61.1 bc	61.1 bc	1.41 a	1.18 a	2.76 a	2.70 a
	280	444 a	592 a	58.3 cd	54.1 de	1.24 a	1.13 a	3.45 a	2.75 a
	420	432 a	603 a	55.7 d	56.7 cd	1.35 a	1.10 a	3.19 a	3.36 a
	560	363 a	545 a	53.6 d	51.7 e	1.03 a	1.04 a	3.28 a	3.05 a
BBCH 50	140	379 a	472 a	65.2 b	63.1 b	1.26 a	1.09 a	2.51 a	2.53 a
	280	394 a	500 a	63.9 b	57.3 cd	1.29 a	1.18 a	2.71 a	2.64 a
	420	418 a	506 a	63.8 b	56.8 cd	1.32 a	1.05 a	2.48 a	3.09 a
	560	379 a	601 a	62.4 bc	53.4 de	1.17 a	1.21 a	2.76 a	2.53 a
CV* (%)		19.8	23.0	5.5	5.6	17.6	18.6	23.5	22.5

† Means within each column are not significantly different by Fisher’s protected LSD values (*P* = 0.05) if followed by the same letter.

*Coefficient of variation

Table 3.4. Trinexapac-ethyl timing and rate effects on inflorescence characteristics in crimson clover.

Treatment		Inflorescence Number		Floret Number			
Timing	Rate	2015	2016	2015	2016	2015	2016
	g ai/ha	Inflorescence m^{-2}		Floret m^{-2}		Floret inflorescence^{-1}	
Untreated Control		655.3 a	1240.5 a	78668 a	146599 a	120 a	117 a
BBCH 32	140	845.0 a	1205.6 a	100903 bc	138682 a	119 a	115 a
	280	789.8 a	1161.2 a	99025 ab	137656 a	126 abc	117 a
	420	924.4 a	1167.9 a	121244 c	134793 a	131 c	116 a
	560	648.5 a	1134.2 a	78888 a	135148 a	122 ab	118 a
BBCH 50	140	695.6 a	963.4 a	85909 ab	107238 a	123 abc	112 a
	280	791.2 a	1124.8 a	93744 ab	131404 a	119 a	116 a
	420	807.3 a	982.2 a	105232 bc	110840 a	130 bc	112 a
	560	753.5 a	1229.8 a	93250 ab	144967 a	125 abc	118 a
CV* (%)		16.2	19.5	15.6	22.9	4.8	7.5

† Means within each column are not significantly different by Fisher's protected LSD values (*P* = 0.05) if followed by the same letter.

*Coefficient of variation

Table 3.5. Trinexapac-ethyl timing and rate effects on volumetric soil water content in crimson clover.

Treatment		Soil Water Content	
Timing	Rate	2015	2016
	g ai/ha	%	
Untreated Control		23.0 a†	22.6 a
BBCH 32	140	19.4 b	23.4 a
	280	20.8 ab	23.7 a
	420	19.4 b	25.3 a
	560	19.9 b	23.4 a
BBCH 50	140	23.1 a	22.3 a
	280	20.0 b	24.4 a
	420	21.7 ab	24.9 a
	560	20.8 ab	25.2 a
CV* (%)		8.7	10.0

† Means within each column are not significantly different by Fisher's protected LSD values ($P = 0.05$) if followed by the same letter.

*Coefficient of variation

Table 3.6. Effect of trinexapac-ethyl timing and rate on seed yield, seed weight, and seed number in crimson clover.

Treatment							
		Seed Number		Seed Weight		Seed Yield	
Timing	Rate	2015	2016	2015	2016	2015	2016
	g ai/ha	seeds m^{-2}		mg $seed^{-1}$		kg ha^{-1}	
Untreated Control		7443 a	6145 ab	5.67 a	5.24 a	421 a†	322 a
BBCH 32	140	7523 a	6864 abc	5.38 b	4.98 b	404 a	341 a
	280	8162 a	7260 cd	5.17 c	4.48 d	422 a	326 a
	420	8812 a	8389 e	5.05 cd	4.42 de	444 a	371 a
	560	7497 a	7890 de	4.79 e	4.24 e	356 a	335 a
BBCH 50	140	6386 a	6012 a	5.11 c	4.98 b	326 a	299 a
	280	7227 a	6943 bc	4.88 de	4.74 c	352 a	329 a
	420	7617 a	7873 de	4.49 f	4.37 de	340 a	344 a
	560	7423 a	7427 cd	4.38 f	4.36 de	325 a	323 a
CV* (%)		17.1	8.3	2.6	2.6	16.8	10.1

† Means within each column are not significantly different by Fisher's protected LSD values (*P* = 0.05) if followed by the same letter.

*Coefficient of variation

Table 3.7. Trinexapac-ethyl timing and rate effects on cleanout and seed set in crimson clover.

Treatment		Cleanout		Seed Set	
Timing	Rate	2015	2016	2015	2016
	g ai/ha	%		%	
Untreated Control		2.23 bc †	2.87 a	9.15 a	4.43 a
	140	1.86 bc	3.33 a	7.29 a	5.00 a
BBCH 32	280	1.74 bc	2.86 a	8.12 a	5.60 a
	420	1.72 c	2.60 a	6.95 a	6.77 a
	560	1.87 bc	3.14 a	9.06 a	6.07 a
	140	1.87 bc	2.50 a	7.27 a	5.61 a
BBCH 50	280	2.29 b	2.35 a	7.44 a	5.42 a
	420	3.03 a	2.30 a	6.95 a	7.37 a
	560	2.96 a	4.00 a	7.96 a	5.30 a
CV* (%)		17.3	29.8	24.0	25.1

† Means within each column are not significantly different by Fisher's protected LSD values (*P* = 0.05) if followed by the same letter.

*Coefficient of variation

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4. Conclusion and Recommendations

In summary, crimson clover has been used in forage, soil maintenance, and as a cover crop. Crimson clover is one of the most important seed crops in Oregon. Trinexpac-ethyl, a lodging-control plant growth regulator, was successful in increasing seed yield in various seed crops from grass seed crops and legumes.

Following preliminary studies in Western Oregon by Anderson et al. (unpublished), trinexapac-ethyl has shown potential for increasing seed yield in crimson clover seed crops. Due to extreme weather conditions in both years at the experimental site, seed yield was low and not affected by TE. TE reduced seed weight in both trial years and increased seed number in the second year. Canopy height, an important factor that leads to lodging, was reduced with TE applications. Applying 140 grams ai/ha is recommended to increase seed yield in crimson clover seed crop, without decreasing seed weight in the northern part of the Willamette Valley.

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