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The Use of Seed Pelleting in Order to Delay Germination of *Trifolium repens* L.

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Abstract: In the seed industry, seed pelleting is a method used for better crop establishment. Due to problems with the cultivation of *Trifolium repens* seeds in cold regions, seed pelleting was used. In the first stage of experiment, the effect of some allelopathic compounds including Ephedrine, Vanillin, Caffeine, ABA, extracts of *Eucalyptus camadulensis* leaf, *Onobryichis sativa* seed and *Juglans regia* leaf on germination indicators (percentage germination, germination start, coefficient of velocity and coefficient of allometry) of *Trifolium repens* seeds were tested. Results showed that among tested allelochemicals Vanillin, ABA and *Eucalyptus camadulensis* leaf extract were suitable allelochemicals for seed pelleting. In the second stage, seeds were coated with those allelochemicals using seed pelleting method, with no negative effect on seedling growth or on percentage germination. Germination of seeds coated with these allelochemicals was delayed with respect to control but seeds pelleted with *Eucalyptus camadulensis* leaf extract exhibited delayed germination for longer time than the other treatments.

Key words: *Trifolium repens* L., allelopathic compounds, seed pelleting, seeding growth, germination

INTRODUCTION

Seed coating methods act as efficient carriers of chemicals, which can be applied on the seed surface. The chemicals involved are mostly fungicides and insecticides (Huijbregts *et al*., 1995), hormones (Tonkin, 1984), peroxidase (Dadlani *et al*., 1992), hydrophilic and hydrophobic compounds (Baxter and Waters, 1986; Hwang and Sung, 1991; Klein and Sachs, 1992). One of the seed coatings methods is seed pelleting (Taylor *et al.,* 1998; McDonald and Copeland 1997, 1999). Pelleting is defined as the deposition of a layer of inert materials that obscures the original shape and size of the seed, resulting in a substantial weight increase and improved plant ability (Taylor *et al*., 1998). This method is used to protect rhizobia, increase in seed weight or size (Halmer, 1988), attract moisture (Scott, 1989), stimulate germination, delay germination, supply oxygen and attract moisture (Scott, 1989). Several reports describe the use of hydrophobic seed coatings to delay seed germination for specific purposes. Chemicals with allelopathic potential are released from leaves, flowers seeds, stems and roots of living or decomposing plant material (Weston, 1996) and these compounds can positively or negatively affect the growth and development of vegetation (Rice, 1984). Nowadays, allelochemicals are used in agricultural practices such as weed control, intercropping, nutrient recycling and suppressing the activity of several pets and disease (Kruse *et al*., 2000). Studies also indicate that some plant species releases allelochemicals, which delay germination (Kruse *et al*., 2000). Legumes such as White clover (*Trifolium repens* L.) are usually cultivated in the spring but there are some problems about the time of cultivation in cold regions. First, at the beginning of the spring pastures are not ready for the entry of workers and machinery to cultivate seeds, although in the middle of spring there is insufficient rainfall for seedling stabilisation. Second, seedlings cannot stabilize in cold weather. Therefore seeding must be done when there is enough moisture and a suitable temperature. In the present study seeds were coated by seed pelleting together with selected allelopathic compounds. These had no negative effects on seedling growth or percentage germination to delay in germination start in order to cultivate them in the fall but their germination were delayed until the temperature became suitable.

MATERIALS AND METHODS

In order to consider the effect of coated allelopathic compounds around *Trifolium repens* seeds on germination start, experiments were performed in two stages. In the first stage, the effect of some allelopathic compounds including Ephedrine, Vanillin, Caffeine, ABA, 40% weight-volume extracts of *Eucalyptus camadulensis* leaf and *Onobryichis sativa* seed (40 g of powdered eucalyptus leaf and onobrychis seed in 100 mL of distilled water) and 30% weight- volume extract of Walnut (*Juglans regia*) leaf (30 g of powdered walnut leaf in

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100 mL of distilled water) were tested on germination indicators of *Trifolium repens* seeds. By doing preliminary experiments, suitable concentrations for each allelopathic compound were selected as below. Concentrations of 333, 250 and 200 mM for Ephedrine and Vanillin, 55.5, 47.6, 43.4 and 40 mM for Caffeine, 400, 700 and 1000 μM for ABA and 80 and 100% *Eucalyptus* and *Juglans* leaf extracts and Onobrychis seed extract. For each allelopathic compound four replicates of 20 seeds were placed in 9 cm diameter petri dishes lined with one layer of Whatman filter paper No.1 wetted with 3 mL of distilled water or solutions of the given allelopathic compounds. Petri dishes were placed in a germination chamber with germination conditions of 20°C, darkness and relative humidity of 90%. Germination was evaluated every two days. A seed was considered germinated when the radicle protruded ≥ 2 mm (Hou and Romo, 1998). Indicators of Percentage Germination (PG), germination start, Coefficient of Velocity (CV) and Coefficient of Allometry (CA) were calculated according to the following formulas:

- Percentage germination (PG) = 100 (n/N)

Where n is the number of seed germinated and N is the number of sowed seeds.

- Germination start = long of time between seeds sowing and beginning of germination is consider as germination start.
- Coefficient of Velocity (CV) = 100 ($\sum$Ni / $\sum$Ni Ti)

Where N is the number of seeds germinated on day i and T is the number of days from sowing.

- Coefficient of allometry (CA) =Ls/Lr

Where Ls is shoot length and Lr is root length.

Among considered allelopathic compounds those with no negative effect on the PG, CA and also those, which delayed the germination start in comparison with control, were selected as the suitable allelopathic compounds.

In the second stage of experiment, selected allelopathic compounds from the first stage were pelleted around *Trifolium repens* seeds. There are two components to a seed pellet: coating material and binder. The coating material is a mixture of several different mineral and/or organic substances and also selected allelopathic compounds. These allelopathic compounds were used with two different amounts (5.04 and 10.14 g of Vanillin in 1000 g of coating material, 0.264 and 0.528 g of ABA in 1000 g of coating material and 40 and 50 g of *Eucalyptus camadulensis* leaf in 100 g of coating material). They were used in two different levels (two-fold and three-fold of thousand-grain weight). The binder holds the coating material together. Binder concentration is critical because too much binder would decrease PG. Also too little binder would cause chipping and cracking of pellets. Glue (1% Solution) was used as binder. Three replicates of 10 coated seeds containing allelopathic compounds, coated seeds with no allelopathic compounds and non-coated seeds were separately cultivated in plastic pots. Pots were placed in a germination chamber with germination conditions of 20°C, darkness and relative humidity of 90%. Germination was recorded every two days and indicators of percentage germination, germination start and coefficient of velocity were calculated.

Statistical analyses of data: Data were analysed using SAS method and the design was a Randomized Complete Block. Also Duncan's Multiple Range Test was used to determine significant difference among mean value at the 0.01 probability levels.

RESULTS AND DISCUSSION

The effect of different concentrations of allelopathic compounds on Percentage Germination (PG) of *Trifolium repens* seeds (not shown) indicate that none of treatments significantly reduced germination except concentrations of 55.5 mM caffeine, 333 and 250 mM ephedrine and 100% *Juglans regia* leaf extract. Findings show that several allelopathic compounds are structurally similar to plant hormones (Olofsdotter, 1998). Also some mechanisms of action of allelopathic compounds seem to resemble those of plant hormones (Kruse *et al*., 2000). Thus the allelopathic compounds used here probably affect inducible hormones of germination such as gibberellin (Rice, 1984; Kruse *et al*., 2000) or activity of specific enzymes such as amylases and proteinases, which are necessary for seed germination (Rice, 1984). Therefore PG decreases in treated seeds with allelochemicals. The effects of allelopathic compounds on the activity of hormones are showed by experiments done on phenolic growth inhibitors from *Salix rubra* and Apple tree which prove to suppress the activity of IAA and gibberellin (GA) (Rice, 1984). Results for PG also show that the effect was not significant at level 0.01 between other testedconcentrations of allelochemicals and control. The effects of allelochemicals on Germination Start (GS) of *Trifolium repens* seeds (not shown) indicate that all

allochemicals delay germination start with respect to the control but among considered compounds, ABA was most effective and delayed germination start for a longer time than the control. Experiments done on several plant species show that ABA plays a key role in induction and maintenance of dormancy (Le Page-Degivry and Garello, 1992). Also among considered different concentrations of allelopathic compounds, germination start in treated seeds with concentrations of 400, 700 and 1000 μM ABA, 333 mM Vanillin, 100% *Eucalyptus camadulensis* and *Juglans regia* leaf extracts was delayed for a longer period than other considered compounds concentrations. Evidences have presented that exogenous ABA controls seed germination by limiting water uptake to embryos (Schopfer and Plachy, 1984) and probably affects cell wall extensibility (Schopfer and Plachy, 1985) or membrane rigidity by biophysical interaction with phospholipids (Leshem *et al*., 1990). Also reports show that transition from dormant to the non-dormant state is marked by changes in the composition of membrane-associated proteins (Hilhorst, 1998). Three dehydrins (17, 21 and 26 Kda) and two groups 3 LEA (late embryogenesis-abundant) polypeptides (17 and 23 KDa) appear during dormancy induction (Garello *et al*., 2000). Thus changes in dormancy may be related to changes at the level of membrane proteins and ABA possibly induces expression of specific genes involved in the blocking of embryo germination (Garello *et al*., 2000). Some allelopathic compounds such as ferulic acid activate the synthesis of ABA (Olofsdotter, 1998) and the antiauxin and antigibberellin activity increased by some terpenes (Kruse *et al*., 2000). Also allelopathic agents act upon pathways that are involved in the synthesis and control of plant hormone levels. These could represent a relevant factor to regulate many metabolic processes that govern plant growth (Olofsdotter, 1998). Also the effect of the allelochemicals on the Coefficient of Velocity (CV) of *Trifolium repens* (not shown) indicate that among considered compounds ABA has the least effect on CV in respect of other allelopathic compounds. Generally, CV decreases as less seeds germinate and with longer germination time (Scott *et al*., 1984). Thus ABA delays germination start for a longer time than other allelopathic compounds. Also all employed concentrations of allelopathic compounds show significant difference at the 0.01 level between CV in treated seeds and control. Among considered concentrations of compounds 400, 700 and 1000 μM concentrations of ABA, 100% *Juglans regia* and *Eucalyptus camadulensis* leaf extracts and 333 mM concentration of vanillin have the least CV in respect with other concentrations. The results obtained (not shown)

Table 1: Effect of pelleted allelopathic compounds on percentage germination of *Trifolium repens*

	Amount of allelochemicas according to gram					
Treatment	5.07 g in 1000 g pellet material[a]			10.14 g in 1000 g pellet material[b]		
	Pellet level according to thousand-grains weight					
Vanillin[b]	Control	2†	3‡	Control	2†	3‡
	90[a]	100a (88[ab])	63.3[b] (87.5[ab])	90[a]	70[b] (88[ab])	50[b] (87.5[ab])
	40 g in 100 g pellet material[a]			50 g in 100 g pellet material[a]		
Eucalyptus[a] camadulens is leaf	Pellet level according to thousand-grains weight					
	Control	2†	3‡	Control	2†	3‡
	90[a]	83.3[a] (88[a])	80[a] (87.5[a])	90[a]	90[a] (88[a])	85[a] (87.5[a])
	0.528 g in 1000 g pellet material[a]			0.264 g in 1000 g pellet material[a]		
	Pellet level according to thousand-grains weight					
Abscisic acid[a]	Control	2†	3‡	Control	2†	3‡
	90[a]	90[a] (88[a])	93.3[a] (87.5[a])	90[a]	70[a] (88[a])	70[a] (87.5[a])

Average comparison performed using of Duncan's test at level 0.01. Different letters indicate significant differences. Values in parentheses show percentage germination in seeds pelleted by coating materials with no allelopathic compounds. †, ‡: Two-fold and three-fold of thousand-grain weight respectively

from the effect of allelopathic compounds on Coefficient of Allometry (CA) indicate that differences were not significant at the 0.01 level between concentrations of 333, 250 and 200 mM of Vanillin, 400, 700 and 1000 μM of ABA, 80 and 100% *Eucalyptus camadulensis* and *Juglans regia* leaf extracts and 80%, 100% *Onobrychis sativa* seed extract and the control. Caffeine and Ephedrine compounds had significant effect on shoot and root lengths of *Trifolium repens*. Probably these compounds affect on division, elongation and ultrastructure of the cell (Rice, 1984). In fact, slowing mitosis of the cells inhibits cell elongation. In treated seeds with leaf extract, seedlings were abnormal in form. In general data from first step of experiments shows that among considered allelopathic compounds ABA, *Eucalyptus camadulensis* leaf extract and Vanillin were selected as suitable compounds as these compounds delayed germination start for a longer time than other allelochemicals. On the other hand, these compounds do not cause any negative effect on seedlings growth. Therefore, for seed pelleting in the second stage of experiment as described in materials and methods these selected compounds were coated around *Trifolium repens* seeds. The effect of pelleted allelopathic compounds (Vanillin, *Eucalyptus camadulensis* leaf and ABA) on PG (Table 1) indicate that among considered compounds seeds which pelleted with *Eucalyptus camadulensis* leaf powder and ABA show rather PG than other considered

Table 2: Effect of pelleted allelochemicas on germination start of *Trifolium repens* seeds

	Amount of allelochemicas according to gram					
Treatment	5.07 g in 1000 g pellet material[a]			10.14 g in 1000 g pellet material[a]		
	Pellet level according to thousand-grains weight					
Vanillin[b]	Control	2†	3‡	Control	2†	3‡
	5[a]	17[a] (5[b])	21.3[a] (8[b])	5[b]	13.3[ab] (5[b])	22.3[a] (8[b])
	40 g in 100 g pellet material[b]			50 g in 100 g pellet material[a]		
	Pellet level according to thousand-grains weight					
Eucalyptus[a] *camadulensis* leaf	Control	2†	3‡	Control	2†	3‡
	5[c]	20[b] (5[c])	26.3[a] (8[c])	5[c]	25[b] (5[c])	28.3[a] (8[c])
	0.528 g in 1000 g pelletmaterial[a]			0.264 g in 1000 g pellet material[a]		
	Pellet level according to thousand-grains weight					
Abscisic acid[ab]	Control	2†	3‡	Control	2†	3‡
	5[b]	16.6[a] (5[b])	15[a] (8[b])	5[b]	18.3[a] (5[b])	18.3[a] (8[b])

Average comparison performed using of Duncan s test at the 0.01 level. Different letters indicate significant differences. Values in parentheses show percentage germination in seeds pelleted by coating materials with no allelopathic compounds. †, ‡: Two- fold and three-fold of thousand-grain weight respectively

Table 3: Effect of pelleted allelopathic compounds on coefficient of velocity of *Trifolium repens*

	Amount allelochemicas according to gram					
Treatment	5.07 g in 1000 g pellet material[a]			10.14 g in 1000 g pellet material[a]		
Vanillinb	Pellet level according to thousand-grains weight					
	Control	2†	3‡	Control	2†	3‡
	0.186[a]	0.058[b] (0.147[a])	0.046[b] (0.111[a])	0.14[a]	0.041[b] (0.147[a])	0.035[b] (0.111[a])
	40 g in 100 g pellet material[a]			50 g in 100 g pellet material[a]		
	Pellet level according to thousand-grains weight					
Eucalyptus[a] *camadulensis* leaf	Control	2†	3‡	Control	2†	3‡
	0.186[a]	0.046[b] (0.147[a])	0.036[b] (0.111[a])	0.186[a]	0.038[b] (0.147[a])	0.034[b] (0.111[a])
	0.528 g in 1000 g pellet material[a]		0.264 g in 1000 g pellet material[a]			
	Pellet level according to thousand-grains weight					
Abscisic acid[ab]	Control	2†	3‡	Control	2†	3‡
	0. 186[a]	0.064[b] (0.147[a])	0.055[c] (0.111[a])	0. 186[a]	0.062[b] (0.147[a])	0.053[c] (0.111[a])

Average comparison performed using of Duncan s test at the 0.01 level. Different letter(s) indicate significant differences. Values in parentheses show percentage germination in seeds pelleted by coating materials with no allelopathic compounds. †, ‡: Two- fold and three-fold of thousand-grain weight respectively

compounds. Also there was no significant difference between pelleted seeds with Eucalyptus leaf and ABA. Different amount of Eucalyptus leaf powder (40 and 50 g) and ABA (0.264 and 0.528 g) and also different levels of pellet with Eucalyptus leaf and ABA (two-fold and three-fold of thousand-grain weight) had no significant effect on percentage germination with respect to the control. In *Trifolium repens* seeds which pelleted with 10.4 g of Vanillin and two-fold thousands grains weight, PG find difference reduction. Also as indicated in Table 1, there is no any significant differences between bare seeds and seeds pelleted by coating materials with no allelopathic compounds. The data in Table 2 shows that Eucalyptus leaf delays Germination Start (GS) for a longer period than other tested compounds. Different amount of *Eucalyptus* leaf (40 and 50 g) and also different levels of pellet weight have significant effect on germination start. Pelleted seeds with 50 g *Eucalyptus* leaf powder and three-fold of thousand grains weight exhibited delayed germination start for a longer time than the control. Of course other considered compounds also show significant effect on germination start but this effect is more in pelleted seeds contain Eucalyptus leaf. Studies indicate that dormancy blocking is associated with higher rates of oxygen uptake. Also it is hypothesised that a stimulation of the Krebs cycle is involved in the dormancy-breaking action by metabolic compounds (Hilhorst, 1998). Thus it is possible that allelochemicals with effect on rate of oxygen uptake and also reduction of the Krebs cycle activity induce dormancy in treated seeds. As mentioned for PC, also for GS there is no any significant differences between bare seeds and seeds pelleted by coating materials with no allelopathic compounds. The effect of pelleted allelochemicals around *Trifolium repens* seeds is shown in Table 3, CV find significant reduction in pelleted seeds containing Vanillin, Eucalyptus leaf and ABA as compared with the control. Reduction in CV is an indication of slowness and delay in

germination process. As shown in Table 3, when the seeds pelleted by coating materials with no allelopathic compounds, the CV does not show significant decrease. In general, data from second step of experiments indicate that among pelleted allelochemicals around seeds, Eucalyptus leaf delayed germination start for a longer time than other allelochemicals and also had no negative effect on PG. Thus for some crops seeds, such as *Trifolium repens* seeds, the germination could be delayed from autumn to spring by pelleting with chemicals and compounds.

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