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# SEED GERMINATION RESPONSES TO SOME ENVIRONMENTAL FACTORS IN THE RED FEATHER (*TRIFOLIUM RUBENS*)

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**Abstract**

A series of *experiments was conducted* to evaluate the effects of various environmental factors, such as temperature, light, planting depth, pH, osmotic and salt stress, on germination of *Trifolium rubens*. Water-*impermeability* of seed coats *prevents* germination of *T. rubens*, but the response of embryos to stratification may suggest some physiological dormancy. Thus, seed dormancy in this species is caused by a water-impermeable seed coat (physical dormancy, PY) and a (non-deep) physiologically dormant embryo (PD), i.e. combinational dormancy (PY + PD). It was also demonstrated that all concentrations of $GA_3$ increased seed germination when compared with the control treatment. Compared with distilled water, NaCl solution of 10–60 mM significantly reduced germination ($p<0.01$). The germination was <20% at an osmotic stress of −0.4 MPa, and above that, no germination was observed. Germination was affected by pH levels, with pH 8 being the optimum, whereas above or below that level, it was significantly reduced. The highest emergence (68 and 85%) was observed at 1 and 2 cm depth. In conclusion these results suggest that the seeds of *T. rubens* can germinate during favorable spring weather conditions.

**Key words:** Combinational dormancy, Dormant embryo, Planting depth, Osmotic and Salt stress.

## Introduction

*Trifolium* is one of the largest genera of the Leguminosae (= Fabaceae) family with ca. 255 species (Zohary & Heller, 1984). All species are herbaceous perennials or annuals. Their habitats are temperate and, to a lesser extent, subtropic regions of the Northern and Southern Hemispheres. Red feather *Trifolium rubens* L. (sect. *Trifolium*) belongs to the Papilionoideae (Faboideae) subfamily. It is a herbaceous perennial with erect usually simple stem that grows to 30–60 cm. Leaflets are oblong-lanceolate, 10 cm long and 2 cm broad. Fruits are usually only 1–seeded. It grows in grassland, scrub and woodland. It is found in central Europe (Coombe, 1968; Ellison *et al.*, 2006).

Leguminosae species produce seeds that are non-dormant. No previous germination studies have been reported on *T. rubens*, although the presence of physical (PY), combinational (PY + PD) or physiological dormancy (PD) has been observed in other members of the family Leguminosae (Baskin & Baskin, 2004). The seeds of many species, especially Leguminosae, have hard seed coats, which can be broken by different methods such as e.g., mechanical or acid scarification, cooling at very low temperatures under moist or dry conditions and storage regimes (Pritchard *et al*., 1988; Luan *et al*., 2017).

Van Asche & Vandelook (2010) studied seed germination of four species of *Trifolium* and came to the conclusion that the seeds of these species had combinational dormancy (PY + PD). Combinational dormancy was also reported for the seeds of *T. subterraneum* L. (Ballard, 1958). This species loses physical dormancy during dry storage (Ballard, 1958; Taylor, 1981). Thomson (1965) showed that seeds of *Trifolium* sp. germinated best at lower temperature. Additionally, Colgecen *et al.* (2008) stated that germination rate of natural *T. pratense* seeds was very low due to hard seed coat. Asci *et al.* (2011) reported that germination was affected by cold stratification.

*Trifolium rubens* is the most ornamental of *Trifolium* sp. Little information, however, is available regarding the seed germination strategies of this species in response to major environmental parameters like temperature, light, planting depth, pH, osmotic and salt stress. This is the first detailed study on seed germination of *T. rubens*.

The main aim of this study was to investigate the germination variability of *T. rubens* seeds and their ability to germinate under diverse environmental conditions. The specific objectives were: (1) to determine if freshly matured seeds are dormant, and (2) to evaluate the effects of different factors such as alternating temperature regime, light, planting depth, pH, osmotic stress, salt stress on seed germination and seedling emergence. It was hypothesized that: (1) scarification treatment can improve germination percentage of *T. rubens* seeds, (2) increased salt and water stress lower germination percentage of *T. rubens* seeds, and (3) seedling emergence decreases with increasing seed burial depth in soil.

## Materials and Methods

**Study site and seed collection:** Ripe seeds used in this study were harvested in August 2015 from wild plants grown in open woodland in the vicinity of Poddębice (51°91’72″N, 18°89’42″E), central Poland. They were collected from approximately 50 individuals plants. The seeds were dried in air for 1-wk immediately after collection. Then, the seeds were divided into sub-samples and subjected to different treatments, namely: (a) 15-min

acid scarification in concentrated (98%) $H_2SO_4$, (b) 4-month cold stratification at the constant temperature of 4°C following acid scarification, (c) dry storage – the seeds were stored in an air-conditioned laboratory at approximately 22°C for 15 months and (d) no treatment – intact or non-stratified seeds (control).

The tests were conducted in incubators with 12/12 h alternating temperature regimes of 15/5, 20/10 and 22/15°C at a 14/10 h photoperiod 40 μmol $m^{-2}$ $s^{-1}$, 400–700 nm cool white fluorescent light each day or in darkness for 20 d. These regimes simulated spring (20/10°C), summer (22/15°C) and autumn (15/5°C) temperatures (Institute of Meteorology and Water Management 1961–2000). The seeds were placed on moistened Whatman No.1 filter paper in 9-cm-diameter petri dishes with four replicates of 25 seeds for each treatment. At 24 h intervals the germinated seeds were counted and removed; deionized water was added as needed. For experiments conducted in darkness, the dishes were placed into black boxes at the beginning of the experiment and the observations were made under green safe light (wavelength of 510 nm and PPFD of 0.2 μmol $m^{-2}$ $s^{-1}$). Treatment solutions were made using deionized water and analytical grade chemicals. For the initial seed moistening 10 ml of appropriate solution was used. Any loss of moisture during the experiments was replaced with deionized water. The seeds were considered to have germinated at the first visible radicle emergence. All experiments were of a completely randomized factorial design, with four replicates of each treatment.

**Water uptake by intact and scarified seeds:** In order to find out whether *T. rubens* seeds are non-dormant or have PY (due to water-impermeable seed coat), imbibition was monitored for the seeds subjected to sulphuric acid scarification and intact seeds (control). Imbibition rates of four replicates of 25 freshly collected scarified and the intact seeds were compared. Imbibition tests were done at room temperature (21–23°C). The intact seeds were placed for 10 min in individual Petri dishes (9-cm in diameter) on a double layer of germination paper moistened with 10 ml of distilled water, then they were blotted dry and weighed. Next, the seeds were removed from the dishes, scarified in concentrated sulphuric acid (96%) for 15 minutes after which they were washed with abundant water and put back into Petri dishes onto the moistened germination paper. The scarified and intact seeds were reweighed again after 1, 12, 24, 48, 72 and 96 h of water absorption. The percentage increase in seed mass (Turner *et al.*, 2006) was calculated by the formula:

$$[(W_1 - W_d)/W_d] \times 100$$

in which $W_1$ = seed mass after imbibitions and $Wd$ = seed mass at the beginning.

**Effects of scarification and stratification:** The test was performed to determine if acid scarification, cold stratification, and combination of the two would be better for promoting seed germination than each of the two treatments carried out separately. According to a classification system for seed dormancy (Baskin & Baskin, 2004) seeds with germination enhanced following scarification and cold stratification were classified as the seeds with PY + PD. The scarified and stratified seeds were placed for 20 d in a growth chamber with 22/15°C alternating temperatures in 14/10 h photoperiod. Preliminary tests showed that this temperature range was optimum for germination.

**Effects of temperature and light on germination:** The seeds after scarification and stratification were tested for germination at daily alternating temperature regimes of 15/5°C, 20/10°C and 22/15°C for 20 d in light and darkness.

**Effects of dry storage on physical dormancy:** It has been shown that seeds of many species lose dormancy during dry storage. Therefore, to determine whether dormancy loss is the effect of storage, germination of intact seeds was tested immediately after harvesting and after 5 months of dry storage. According to the results of experiment 1, germination percentage was determined for 20 d at 22/15°C in a 14/10 h photoperiod because the highest total germination was observed under these conditions.

**Effects of gibberellic acid ($GA_3$):** The following pretreatments were tested to determine the effect of $GA_3$ on seed germination. Scarified seeds were immersed for 24 h in solutions of 0, 250, 500, 750, 1000 and 1250 ppm $GA_3$ at 22/15°C in darkness. The germination test was performed at 22/15°C alternating temperature with light for 20 d.

In another experiment the scarified seeds were immersed for 24 h in either 750 ppm $GA_3$ solution or deionized water. Then the seeds were kept at 4°C and 40% relative humidity for 16-wk stratification period. The germination test was performed as described above.

**Effects of osmotic stress:** The scarified and stratified seeds were treated with aqueous solutions of polyethylene glycol 8000 (PEG 8000) with the following osmotic potentials 0.0 (deionized water control), –0.3, –0.4, –0.6, –0.9, and –1.3 MPa by dissolving 0, 154, 191, 230, 297, or 350 g of PEG 8000 in 1 liter of deionized water, respectively (Michel, 1983; Singh *et al.*, 2012). Petri dishes with the seeds were placed in growth cabinets with 22/15°C day/night temperatures and 14/10 h photoperiod.

**Effect of salt stress:** Sodium chloride (NaCl) solutions of 0 (deionized water control), 20, 40, 60, 80, 100, and 120 mM concentrations were prepared in deionized water. Petri dishes with the scarified and stratified seeds containing 10 ml of the appropriate salt solution were placed in a growth cabinet with 22/15°C day/night temperatures and 14/10 h photoperiod.

**Effects of pH:** The seeds were placed in a buffer solutions of pH range of 4 to 9, prepared according to the method described by Lu *et al.* (2006). Distilled water (pH

5.7) was used as the control. The seeds were subjected to a combination of acid scarification and cold stratification. The germination test was performed as described above.

**Effects of depth of sowing**: The seeds were sown in 15-cm in diameter plastic pots, filled with white moist sand, at depths of 0, 1, 2, 3, 4, 6, 8 or 10 cm below the soil surface. The pots were placed in a greenhouse with 22/15°C day/night temperatures and 14/10 h photoperiod and watered as needed to maintain adequate soil moisture. Germinated seedlings were counted and removed every 7 d for 28 d. Appearance of two cotyledons called 'seed leaves' above ground level was considered as seedling emergence.

**Statistical analysis:** The germination percentage data were arcsine transformed before they were subjected to ANOVA analysis using Statistica v 10 (Statsoft. Inc., Tulsa, OK, USA). The data were tested for normality with the Kolmogorov-Smirnov's test with the Lilliefors correction and homogeneity of variance with the Brown-Forsythe test. The effect of stratification and scarification on germination was examined using two-way ANOVA followed by Tukey's post-hoc comparison tests. Two-way ANOVA was also used to analyze the effect of temperature and light on seed germination. Significant differences in seed mass increase during imbibition were estimated using all-pair Student's *t*-test comparisons. Student's was also performed to test for statistical differences in germination percentage between the seeds immersed with either 750 ppm $GA_3$ solution or deionized water.

## Results

**Water uptake by non-treated and scarified seeds:** The non-treated seeds imbibed very little water, and at the end of the 96 h imbibition period their weight increased by only 24%. On the other hand, the scarified seeds imbibed water readily, and after 96 h their mass increased by 98% (Fig. 1).

**Effects of scarification and stratification**: The fresh intact seeds germinated poorly, while the combined treatment of scarification and stratification considerably increased germination (Fig. 2). The two-way ANOVA for final germination showed significant effects of stratification ($F_{(2.72)}$ = 12.84, $p$<0.05), or scarification ($F_{(2.72)}$ = 21.45, $p$<0.05) and a highly significant effect of their interaction ($F_{(2.72)}$ = 8.23, $p$<0.05).

**Effects of temperature and light on germination: G**ermination percentages of the seeds after scarification and stratification in both light and in darkness increased significantly with the increase in temperature ($p$<0.01). The highest germination was observed at 22/15°C in light, and the lowest at 15/5°C in darkness (Fig. 3). A two-way ANOVA showed a highly significant effect of temperature on final germination ($F_{(1.54)}$ = 25.56, $p$<0.001) but not of light ($F_{(1.54)}$ = 4.23, $p$>0.05), and a highly significant ($F_{(1.54)}$ = 15.23, $p$<0.05) effect of their interaction.

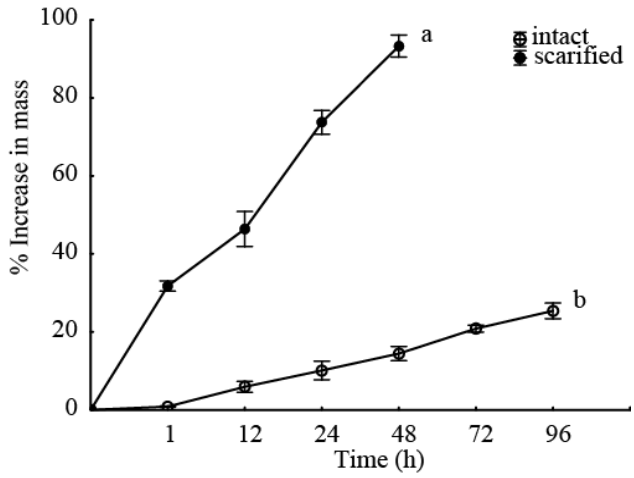


Fig. 1. Imbibition curves for non-treated intact and scarified seeds of *Trifolium rubens*. Means with the same letter do not differ (Student's *t*-test at p<0.05). Values are means ± SD.

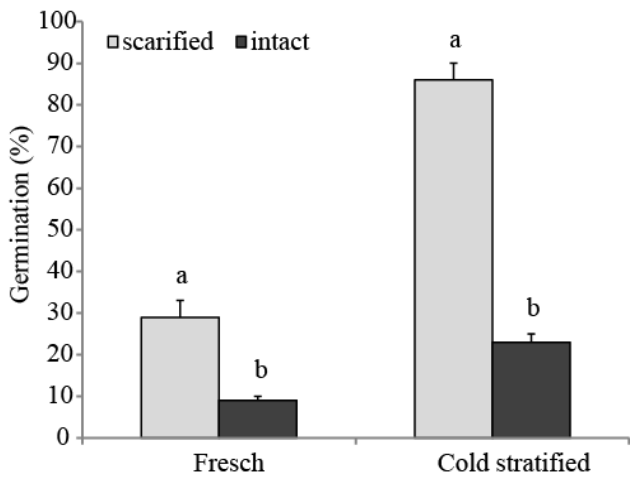


Fig. 2. Effect of scarification and cold stratification on germination of *Trifolium rubens* seeds incubated at 22/15°C in 14/10 h photoperiod for 20 d. Overall comparison between means was performed by Student's *t*-test; different letters indicate significant ($p$<0.05) differences between means. Bars indicate ± SD.

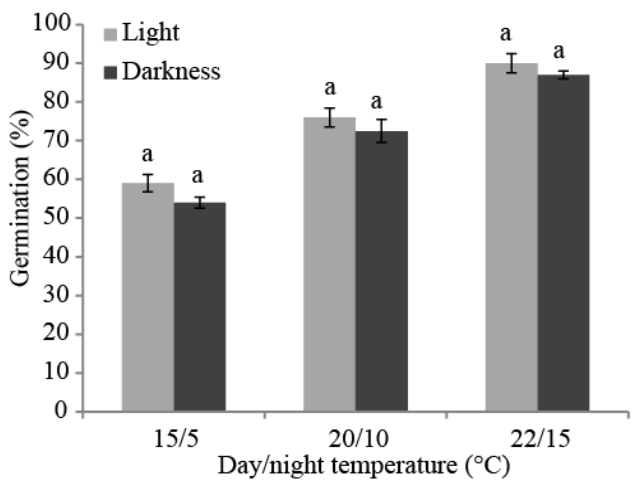


Fig. 3. Effect of temperature and light on germination of *Trifolium rubens* seeds after 20 d of incubation in deionized water. Means with the same letter are not significantly different from each other ($p$>0.05; ANOVA with Tukey's test for multiple comparisons). Values are means ± SD.

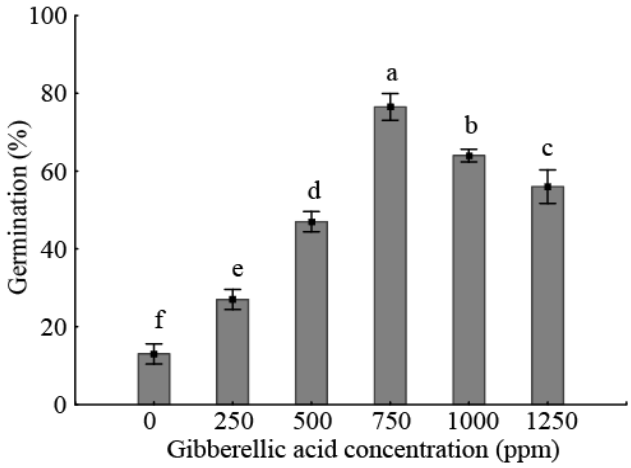


Fig. 4. Effect of the gibberellic acid concentration on the germination of *Trifolium rubens*. Freshly collected scarified seeds were immersed for 24 h in the solutions of 0, 250, 500, 750, 1000 and 1250 ppm $GA_3$ at 22/15°C in darkness. Different letters indicate significant differences by ANOVA followed by Tukey's test at $p<0.05$. Values are means ± SD.

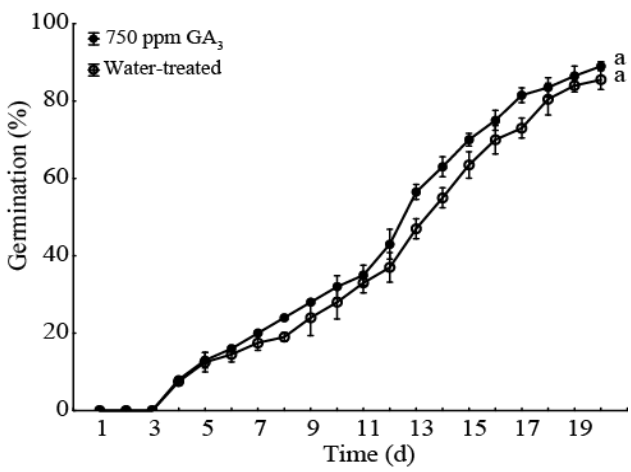


Fig. 5. Germination of scarified seeds of *Trifolium rubens* after treatment with water or 750 ppm $GA_3$ and stratification for 16 weeks. Means with the same letter are not significantly different (Student's *t*-test at $p>0.05$). Values are means ± SD.

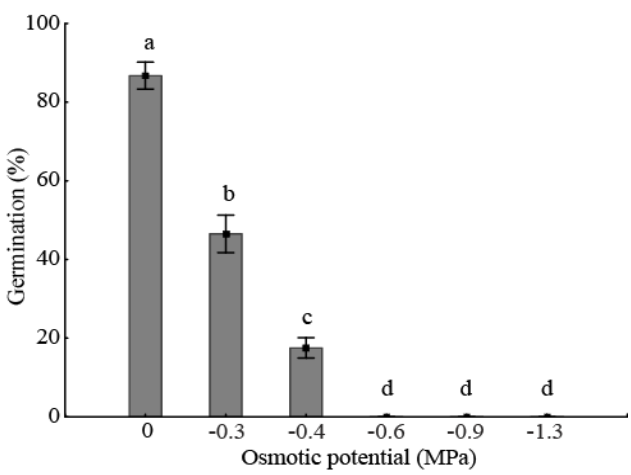


Fig. 6. Effect of moisture stress (osmotic potential MPa) on the seed germination percentage. Means followed by the same letter are not significantly different ($p>0.05$; ANOVA with Tukey's test for multiple comparisons). Values are means ± SD.

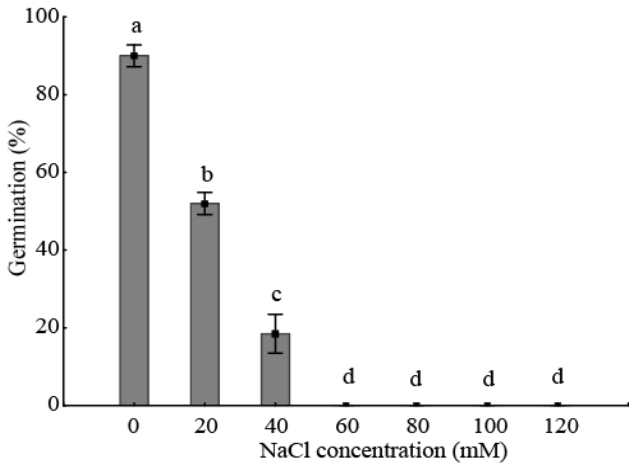


Fig. 7. Effect of NaCl concentration on germination of *Trifolium rubens* seeds. Means with the same letter are not significantly different ($p>0.05$; ANOVA with Tukey's test for multiple comparisons). Values are means ± SD.

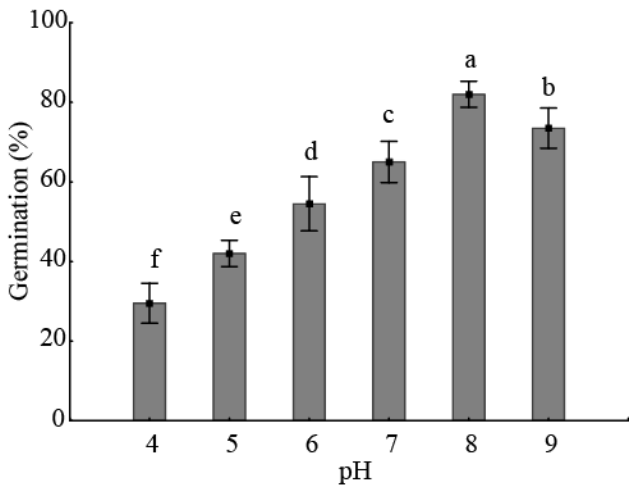


Fig. 8. Effect of pH on germination of *Trifolium rubens* at 22/15°C with a 14/10 hphotoperiod for 20 d. Means with the same letter are not significantly different ($p>0.05$; ANOVA with Tukey's test for multiple comparisons). Values are means ± SD.

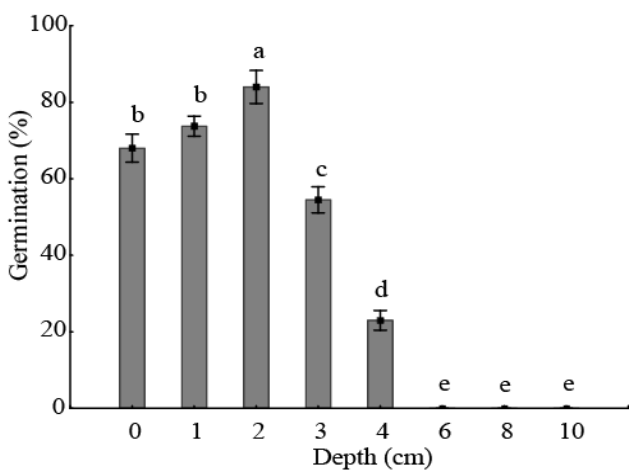


Fig. 9. Effect of depth of sowing on emergence of *Trifolium rubens* seedlings. The seedlings were counted and removed every 7 d for 28 d. Means followed by the same letter are not significantly different ($p>0.05$; ANOVA with Tukey's test for multiple comparisons). Values are means ± SD.

**Effects of dry storage on physical dormancy:** Dry storage did not improve germination significantly. Germination at 22/15°C after dry storage was lower than 6% and nearly identical to that of fresh seeds (results are not shown), indicating that the seeds remained impermeable.

**Effects of gibberellic acid ($GA_3$):** All $GA_3$ treatments increased germination of the fresh seeds which were scarified. Furthermore, the seed germination increased from 0 to 750 ppm reaching 78% and then dropped with the concentration increasing to 1250 ppm (Fig. 4). Application of 750 ppm $GA_3$ for 24 h resulted in 92% germination, whereas germination of water-treated seeds was 87% after 16 weeks of stratification; germination percentages were not significantly different ($p$>0.05) from each other (Fig. 5).

**Effect of osmotic stress**: Maximum germination (88%) was obtained under no-salt conditions. Germination decreased as osmotic potential increased from 0 MPa to –0.4 MPa. It was very low (<20%) at an osmotic potential of –0.4 MPa and above that, no germination was observed (Fig. 6).

**Effect of salt stress:** The germination of *T. rubens* was <60% at 20 mM NaCl, at 40 mM NaCl it decreased to <25%, whereas at 60 mM NaCl it was completely inhibited (Fig. 7). In general, germination of *T. rubens* seeds was inhibited by salinity.

**Effect of pH:** The optimum pH range for *T. rubens* germination (>70%) was between 8 and 9. It decreased at pH levels between 4 and 7 and it was only <30% at pH 4 (Fig. 8).

**Effects of depth of sowing:** Seedling emergence of *T. rubens* was greatly influenced by seed burial depth. 20 d after sowing the highest emergence (68% and 85%) was obtained from the seeds at 1 and 2 cm depth, respectively. It decreased at 3 cm and no seedlings emerged from the seeds placed at a depth of 6 cm or more (Fig. 9).

## Discussion

Embryos of *T. rubens* were completely developed suggesting that these seeds had no morphological or morphophysiological dormancy. On the other hand, as is the case for other Leguminosae species, it was shown in this study that the seeds of *T. rubens* had physical exogenous dormancy, as a consequence of seed coat impermeability to water and gas due to its thickness, its composition and microstructure. Physical seed dormancy prevents seeds from germinating at the time when seedlings are unlikely to survive (Baskin & Baskin, 2004). Other *Trifolium* species, for example *T. arvense* L., *T. dubium* Sibth., *T. pratense* L., *T. repens* L. (Van Asche & Vandelook, 2010) and *T. resupinatum* L. (Ates, 2011) also exhibit strong seed dormancy. In the wild, seeds are subjected to many different abiotic and biotic factors that make hard *seed* **c**oats permeable (Baskin & Baskin, 1997). These factors are more intense at the soil surface than soil depth. Thus seeds *germinate* best at or *near* the *soil surface* (Baskin & Baskin, 1997). High constant temperatures and natural fluctuating temperatures are major factors softening some hard-coated seeds (Baskin & Baskin, 1997; see also Argel & Paton, 1999 for a review). Quinlivan (1961) showed rapid increase in permeability of *T. subterraneum* seeds subjected to temperature fluctuations and recorded maximum permeability within the range of 15/60°C. The resulting data show that scarification process improved germination of *T. rubens* (average germination 29%), but not as much as the combination of scarification plus stratification (average germination 86%). This suggests that *T. rubens* may exhibit not only physical but also physiological dormancy. Therefore, according to the classification of seed dormancy (Baskin & Baskin, 2004) the seeds of *T. rubens* can be described as having a combination of physical (PY) and (non-deep) physiological (PD) dormancy [physical (PY) + physiological (PD)], Combinational dormancy is quite common in plants of other families, for example in Sapindaceae (Baskin *et al.,* 2004), Geraniaceae (Baskin & Baskin ,1974; Van Asche & Vandelook, 2006), and Malvaceae (Van Asche & Vandelook, 2006).

The seeds of many temperate plants need dry storage (after-ripening) period (Forbis, 2010). Seed dormancy of *T. rubens* is not broken by dry storage conditions. Instead, this species require a period of moist cold stratification. Seeds of *T. subterraneum* (Quinlivan, 1961) and *T. willdenovii* Spreng (Russell, 2011) also require cold-treatment to germinate.

This study also showed that germination percentages were highest at 22/15°C (> 80%) then dropped with temperature and were > 60% at 20/10°C and > 50% at 15/5°C. Overall, the seeds germinated over a temperature range of 5–22°C which could allow for germination throughout the spring and summer months after cold stratification in the temperate regions of the world.

It has long been known *that* light can stimulate the seeds of *many plant species* to *germination* (Dill & Sun, 2001). The seeds of *T. rubens* were insensitive to light; relatively high germination levels were observed in darkness. This fact denotes that *T. rubens* does not form a long-lived seed bank.

The phytohormone gibberellic acid (GA) has long been known to promote seed germination. Gibberellic acid substituted for low temperatures, long day and light in promoting germination (Dill & Sun, 2001). During germination GAs induce the synthesis of hydrolytic and proteolytic enzymes which act to mobilize food reserves in cotyledons and endosperm (Adkins *et al.,* 2002). *Trifolium rubens* germination was enhanced greatly when the scarified seeds were treated with $GA_3$. Furthermore, these results show that $GA_3$, a germination promoter, plus stratification did not increase final germination of sulphuric acid scarified seeds above the levels attained by stratification without the seed germination promoter. This result is in agreement for example with the study of Rogis *et al.* (2004) on *Tripsacum dactyloides* L.

To the best of my knowledge there are no data concerning pH in growing substrates and *T. rubens* germination. In the current study seed germination was 30% to 83% over the pH range from 4 to 8, respectively. This result indicates that *T. rubens* can germinate over a wide range of pH thus it is not an important factor limiting this process. However, its greater germination may be expected in alkaline than in acidic soil. This characteristic is common for many weed species such as *Sedum* species (Zheng & Clark, 2013).

Another major factor determining seed germination is soil moisture. In this study, germination of *T. rubens* seeds was greater than 40% at −0.3 MPa, however it was completely inhibited at −0.6 MPa (Fig. 6). These data suggest that the seeds germinate under moderate water-stress conditions and are sensitive to low water potential. These result suggest that germination may be largely restricted to well-drained, moist soils.

It was showed in this study that *T. rubens* was NaCl sensitive during the stage of seed germination and unable to germinate *even* at fairly low levels of *salt* concentration. This may be due to ionic imbalance and osmotic stress (Rehman *et al.,* 2000). Other non- halophyte species are known to tolerate salt stress during germination. For example, *Sonchus oleraceus* L. germinated at the salt concentration of 160 mM (Chauhan *et al.,* 2006). However, halophytes can grow in high salinity (over 250 mM NaCl) (Khan & Weber, 2008).

The results of this study show that seedling emergence on the soil surface was lower than that from the seeds buried at 1- and 2-cm depths; no seedlings emerged from the seeds placed below 6 cm. Reduced seedling emergence on the soil surface was probably due to limited soil-seed contact and, consequently, poor seed imbibition (Michel, 1983). Low or no emergence from deeply buried seeds could be due to death of seeds or no germination (Chauchan, 2016). Moreover, lower seedling emergence from deeper sown seeds may be associated with their limited reserves (Mennan & Ngouajio, 2006). Baskin and Baskin, (1997) stated that larger seeds often had greater reserves and could emerge from greater planting depth.

## Conclusions

Seeds of *T. rubens* were strictly dormant at dispersal. Their dormancy was characterised as a combinational dormancy. The combined treatment of scarification and stratification considerably increased germination. The seeds prefer an average alkaline and well-drained soils. The findings of this study suggest that *T. rubens* normally becomes established during spring and summer through the germination of seeds dispersed in the previous growing season.

## References


Adkins, S., D.S. Loch and S. Bellairs. 2002. Seed dormancy mechanisms in warm season grass species. *Euphytica*, 126**:** 13-20.

Asci, O.O., Z. Acar, I. Ayan, U. Basaran and H. Mut. 2011. Effect of pre-treatments on seed germination rate of red clover (*Trifolium pratense* L.) populations. *Afr. J. Agric. Res.*, 6(13): 3055-3060.

Ates, E. 2011. Influence of some hardseededness-breaking treatments on germination in persian clover (*Trifolium resupinatum* ssp. *typicum* Fiori et Paol.) seeds. *Rom. Agric. Res.*, 28: 229-236.

Ballard, L.A.T. 1958. Studies of dormancy in the seeds of subterranean clover (*Trifolium subterraneum* L.). 1. Breaking of dormancy by carbon dioxide and by activated carbon. *Aust. J. Biol. Sci.*, 11: 246-260.

Baskin, C.C. and J.M. Baskin. (2004). A classification system for seed dormancy. *Seed Sci. Res.*, 14: 1-16.

Baskin, J.M. and C.C. Baskin. 1974. Some eco-physiological aspects of seed dormancy in *Geranium carolinianum* L. from Central Tennessee. *Oecologia*, 16: 209-219.

Baskin, J.M. and C.C. Baskin. 1997. Methods of breaking seed dormancy in the endangered species *Iliamna corei* (Sherff) Sherff (Malvaceae), with special attention to heating. *Nat. Area. J.*, 17: 313-323.

Baskin, J.M., B.H. Davis, C.C. Baskin, S.M. Gleason and S. Cordell. 2004. Physical dormancy in seeds of *Dodonaea viscosa* (Sapindales, Sapindaceae) from Hawaii. *Seed Sci. Res.*, 14: 81-90.

Chauchan, B.S. 2016**.** Germination biology of *Hibiscus tridactylites* in Australia and the implications for weed management. *Sci. Rep.*, 6: 26006.

Chauhan, B.S., G. Gill and C. Preston 2006. Factors affecting seed germination of annual sowthistle (*Sonchus oleraceus*) in southern Australia. *Weed Sci.*, 54: 854-860.

Colgecen, H. H.N., H.N. Buyukkartal and M.C. Toker. 2008. *In vitro* germination and structure of hard seed testa of natural tetraploid *Trifolium pratense* L. *Afr. J. Biotechnol.*, 7(10): 1473-1478.

Coombe, D.E. 1968. *Trifolium* L. In: (Eds.): Tutin T.G. *et al., Flora Europaea* vol. 2, Cambridge University Press, UK, pp. 157-172.

Dill, A. and T. Sun. 2001. Synergistic derepression of gibberellin signaling by removing RGA and GAI function in Arabidopsis thaliana. *Genetics*, 159: 777-785.

Ellison, N.W., A. Liston, J.J. Steiner, W.M. Williams and N.L. Taylor. 2006. Molecular phylogenetics of the clover genus (*Trifolium*-Leguminosae). *Mol. Phylogenet. Evol.*, 39: 688-705.

Forbis, T.A. 2010. Germination phenology of some Great Basin native forb species. *Plant Species Biol.*, 25: 221-230.

Khan, M.A. and D.J. Weber. 2008. Ecophysiology of high salinity tolerant plants (Tasks for Vegetation Science), Springer, Amsterdam.

Lu, P., W. Sang and K. Ma. 2006. Effects of environmental factors on germination and emergence of Crofton weed (*Eupatorium adenophorum*). *Weed Sci.*, 54: 452-457.

Luan, Z., Z. Shao, J. Zhao and G. Sun. 2017. A comparison study of permeable and impermeable seed coats of legume seed crops reveals the permeability related structure difference. *Pak. J. Bot.*, 49: 1435-1441.

Mennan, H. and M. Ngouajio. 2006. Seasonal cycles in germination and seedling emergence of summer and winter populations of catchweed bedstraw (*Galium aparine*) and wild mustard (*Brassica kaber*). *Weed Sci.*, 54: 114-120.

Michel, B.E. 1983. Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant Physiol.*, 72: 66-70.

Pritchard, H.W., K.R. Manger and F.G. Prendergast. 1988. Changes in *Trifolium arvense* seed quality following alternating temperature treatment using liquid nitrogen. *Ann. Bot.*, 62: 1-11.

Quinlivan, B.J. 1961. The effect of constant and fluctuating temperatures on the permeability of the hard seeds of some legume species. *Aust. J. Agric. Res.*, 12(6): 1009-1022.

Rehman, S.P., J.C. Harris, W.F. Bourne and J. Wilkin. 2000. The relationship between ions, vigour and salinity tolerance of *Acacia* seeds. *Plant Soil*, 220: 229-233

Rogis, C., L.R. Gibson, A.D. Knapp and R. Horton. 2004. Enhancing germination of eastern gamagrass seed with stratification and Gibberellic Acid. *Crop Sci.*, 44: 549-552.

Russell, M. 2011. Dormancy and germination pre-treatments in Willamette Valley native plants. *Northwest Sci.*, 85: 389-402.

Singh, M., H.M., A.H.M. Ramirez, S.D. Sharma and A.J. Jhala. 2012. Factors affecting the germination of tall morningglory (*Ipomoea purpurea*). *Weed Sci.*, 60: 64-68.

Taylor, G.B. 1981. Effect of constant temperature treatments followed by fluctuating temperatures on the softening of hard seeds of *Trifolium subterraneum* L. *A. J. Plant Physiol.*, 8(6): 547-558.

Thomson, J.R. 1965. Breaking dormancy in germination tests of *Trifolium* spp. *Proceedings of the International Seed Testing Association*, 30: 905-909.

Turner, S.R., D.J. Merritt, J.M. Baskin, C.C. Baskin and K.W. Dixon. 2006. Combinational dormancy in seeds of the Western Australian endemic species *Diplopeltis huegelii* (Sapindaceae). *Aust. J. Bot.*, 54: 1-6.

Van Assche, J.A. and F.E.A. Vandelook. 2006. Germination ecology of eleven species of Geraniaceae and Malvaceae, with special reference to the effects of drying seeds. *Seed Sci. Res.*, 16: 283-290.

Van Assche, J.A. and F.E.A. Vandelook. 2010. Combinational dormancy in winter annual Fabaceae. *Seed Sci. Res.*, 16: 283-290.

Zheng, Y. and M.J. Clark. 2013. Optimal growing substrate pH for five *Sedum* species. *Hortscience*, 48: 448-452.

Zohary, M. and D. Heller. 1984. The genus *Trifolium*. Israel Academy of Sciences and Humanities, p. 606, Jerusalem, Israel.

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