Factors affecting seed germination of threehorn bedstraw (*Galium tricornutum*) in Australia

Author(s): Bhagirath S. Chauhan, Gurjeet Gill, and Christopher Preston
Source: Weed Science, 54(3):471-477.
Published By: Weed Science Society of America
https://doi.org/10.1614/WS-05-176R1.1
URL: http://www.bioone.org/doi/full/10.1614/WS-05-176R1.1

Weed Science, 54:471–477. 2006

Factors affecting seed germination of threehorn bedstraw (*Galium tricornutum*) in Australia

Bhagirath S. Chauhan
Corresponding author. School of Agriculture, Food and Wine, The University of Adelaide, Roseworthy Campus, South Australia, Australia 5371; bhagirath.chauhan@adelaide.edu.au

Gurjeet Gill
School of Agriculture, Food and Wine, The University of Adelaide, Roseworthy Campus, South Australia, Australia 5371

Christopher Preston
School of Agriculture, Food and Wine, The University of Adelaide, Waite Campus, South Australia, Australia 5064

Threehorn bedstraw is an important dicotyledonous weed in southern Australia that is particularly difficult to control in pulse crops. Knowledge of the germination ecology of this weed would facilitate development of effective weed-control programs. Experiments were conducted to study the germination of two populations, Roseworthy Campus (RC) and Yorke Peninsula (YP), of threehorn bedstraw from South Australia. In the absence of chilling, seeds germinated only in the darkness. Germination was considerably higher under an alternating day/night temperature range of 13/7 C compared with 20/12 or 25/15 C day/night temperature. Germination was inhibited by light; however, when seeds were subsequently transferred to complete darkness they germinated readily. Potassium nitrate (0.005 M KNO_3) and gibberellic acid (0.001 M GA_3) stimulated germination in the darkness in both populations. This concentration of KNO_3 increased germination of the RC and YP populations from 26 and 37% to 56 and 68%, respectively; however, higher concentrations of KNO_3 inhibited germination. GA_3 added in combination with KNO_3 further increased germination to 81 and 94%, respectively. Germination was also promoted by cold-stratification treatment (5 C). Complete germination (100%) was achieved within 4 wk of cold stratification, when seeds were incubated in sand. In the field, seedling recruitment of both populations was higher under minimum tillage (25 to 27%) than no-till (15 to 18%) conditions, reflecting greater exposure of seeds to light under no-till systems.

Nomenclature: Threehorn bedstraw, *Galium tricornutum* Dandy, GALTC.

Key words: Germination, tillage, light, weed seed.

Threehorn bedstraw is an important dicotyledonous weed of winter crops in southern Australia. It has recently assumed widespread importance as a declared weed in Western Australia, where it has recently been introduced inadvertently (Department of Agriculture, Western Australia 2005). This means that the movement of machinery from contaminated fields is prohibited unless that machinery is thoroughly cleaned. In addition, any plants found must be destroyed. Threehorn bedstraw is present in other states of Australia, including South Australia and the Wimmera region of Victoria, where it can be a troublesome weed in wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), field peas (*Pisum sativum* L.), chickpeas (*Cicer arietinum* L.), and faba beans (*Vicia faba* L.) (Amor and Kloot 1987; Black et al. 1994; Moerkerk 1999). There is little information on the ecology of threehorn bedstraw in the literature, making it difficult to develop strategies for the management of this weed. Such information would also be important for programs attempting to eradicate this weed species.

Two closely related species, catchweed bedstraw (*Galium aparine* L.) and false cleavers (*G. spurium* L.), have been well studied (Froud Williams 1985; Malik and Vanden Born 1988; Mennan 2003; Taylor 1999). In the absence of information about threehorn bedstraw, some insights can be gained from these closely related species. Catchweed bedstraw can cause a significant yield loss in crops. For example, Peters (1984) reported winter wheat yield losses of 30, 47, and 52% at densities of 25, 100, and 520 catchweed bedstraw plants m^{-2}. A U.K. survey of seed drills showed that 21% of cereal grain samples were contaminated with catchweed bedstraw (Tonkin and Phillipson 1973). Catchweed bedstraw and false cleavers are also common weeds in Canada (Hall et al. 1998; Malik and Vanden Born 1988), where an infestation of 100 plants m^{-2} can decrease canola (*Brassica napus* L.) yield by 18% (Malik and Vanden Born 1987a). In addition, continuous use of acetolactate synthase inhibitors in cereal crops in Alberta has led to the evolution of resistance to these herbicides in false cleavers (Hall et al. 1998). These studies suggest that threehorn bedstraw may have the potential to become an even more problematic weed, should it spread to the rest of the grain-growing areas of Australia.

Germination of catchweed bedstraw is inhibited by light (Froud Williams 1985), so few seeds germinate from the soil surface (Boyd and Van Acker 2003; Taylor 1999), with greatest germination at depths up to 5 cm (Boyd and Van Acker 2003; Froud Williams 1985). Light also inhibits germination of false cleavers, but dormancy induced by light can be overcome by potassium nitrate (KNO_3) and, to a lesser extent, by gibberellic acid (GA_3; Malik and Vanden Born 1987b). Germination of a closely related species, false cleavers and catchweed bedstraw, has been reported to be influenced by tillage (Reid and Van Acker 2005). However, such information is not available for threehorn bedstraw.

Although information on closely related species can be useful, it is possible that the germination requirements of threehorn bedstraw are different to those of false cleavers and catchweed bedstraw. At the moment, the information available is anecdotal but suggests that threehorn bedstraw germination is inhibited by light and is discontinuous

throughout the season (Bedggood 1999). A better understanding of threehorn bedstraw germination will help in developing weed-control programs for this species. Therefore, the objectives of this study were to examine the effects of environmental and chemical factors on the germination of this species.

Materials and Methods

Seed Collection

Experiments were conducted in 2005 at the Roseworthy Campus, The University of Adelaide, South Australia. In November 2004, seeds of threehorn bedstraw were collected at maturity from wheat fields at the Roseworthy Campus (RC) and in Warooka, on the Yorke Peninsula (YP) of South Australia. Seeds were considered mature when the plants of threehorn bedstraw had completely senesced. The distance between the two collection sites was 200 km. Seeds were threshed and separated from chaff manually. The 1,000-seed weight of the RC and YP populations were 5.279 g and 5.412 g, respectively. Seeds were stored in a naturally lit glasshouse until used for experiments. The glasshouse temperature was set at 25/15 ± 5 C (day/night).

General Germination Test Protocol

Threehorn bedstraw germination was evaluated by placing 25 seeds evenly in a 9-cm-diam petri dish containing two layers of Whatman No. 1 filter paper, moistened with either 5 ml of distilled water or a treatment solution. Dishes were sealed with Parafilm[1] and placed in an incubator at fluctuating day/night temperatures of 13/7 C, unless otherwise specified. The photoperiod was set at 12 h to coincide with the high-temperature period. Fluorescent lamps were used to produce a light intensity of 85 μmol m^{-2} s^{-1}. For germination in complete darkness, dishes were wrapped in two layers of aluminum foil. The number of germinated seeds was counted 14 d after the start of the experiment, with the criterion for germination being visible protrusion of the radicle. Tetrazolium chloride[2] was used to test viability of the ungerminated seeds (Steadman et al. 2003).

Effects of Temperature, Light, and Subsequent Dark Incubation on Germination

Germination of seeds of both populations at the time of maturity and 11 mo after maturity was determined in growth chambers under fluctuating day/night temperatures (25/15, 20/12, and 13/7 C). Germination was determined under both light/dark and dark regimes.

To test the effects of after-ripening on germination, further samples of both populations were placed in the light/dark regime for 14 d at 3 and 6 mo after seed maturity. The dishes were then placed in the dark for another 7 d. Germination was examined after this period, and the number of germinated seeds counted.

Effects of Physical Scarification, Germination Media, and Leaching on Germination

To test whether the inhibition of threehorn bedstraw germination was due to an impermeable seed coat, seeds of the RC population were physically scarified by cutting the seed coat with a scalpel. The scarified seeds were then incubated in the darkness. The effect of different germination media on seed germination was studied by incubating seeds in the dark. Seeds of the YP population were placed in petri dishes containing distilled water, field soil, 0.01 M of KNO_3,[3] 0.1 M KNO_3, 0.001 M GA_3,[4] 2% soil extract (10 g of soil in 500 ml of distilled water), and 4% soil extract (20 g of soil in 500 ml of distilled water). The soil used in the experiment was a clay loam soil (8 mg kg^{-1} NO_3–N, 0.15% total nitrogen, pH 7.5, 1.49% organic carbon). Soil extract was used because it has been reported to stimulate germination of false cleavers (Malik and Vanden Born 1987b). Where the soil medium was used, seeds were buried in the soil rather than being placed on the soil surface.

The effect of leaching on seed germination was examined by placing seeds of both populations in permeable nylon bags that were then placed in water for imbibing and leaching. Water was changed whenever samples were taken for germination tests. Following various periods of leaching (0, 2, 4, 6, 24, and 48 h), samples of 25 seeds were removed and placed in darkness for 14 d to test germination.

Effects of KNO_3 and GA_3 on Germination

A preliminary experiment was conducted under the light/dark and dark conditions to study the effects of KNO_3 on germination of the RC population. When no germination was found in the light/dark regime, detailed experiments were performed by incubating seeds only in the darkness. Seeds of both populations (3 mo after maturity) were treated with different concentrations of KNO_3 (from 0 to 0.1 M) and in combination with 0.001 M GA_3. Germination was analyzed separately for each population and allowing for the interaction of KNO_3 and GA_3.

Effects of Seed Size on Germination

Seed size effects on germination were studied at 0, 3, 6, and 9 mo after seed maturity in both light/dark and dark conditions. Cleaned seeds of the RC population were sieved through a 2-mm-diam aperture sieve. Seeds that passed through the sieve were categorized as small seeds and the others as large seeds. The 1,000-seed weight of small and large seeds was 3.16 and 6.19 g, respectively. In a random sample, 30% of seeds were in the small seeds category and 70% in the large. The seeds of different sizes were tested for germination by placing them on water-moistened filter paper in petri dishes. Seeds were placed at 13/7 C in the dark or in an alternating light/dark regime for 14 d.

Effects of Cold Stratification on Germination

Seeds of the RC population that had been stored on the soil surface for 5 mo after maturity in a field were subjected to cold stratification at 5 C for 5 wk. Seeds (n = 50) were placed in petri dishes containing sand in continuous darkness at 5 C. Every week, the dishes were removed from the incubator and seeds exhumed from the dishes. Seeds that had germinated at 5 C were counted and represented the germination that occurred at 5 C. The ungerminated seeds were placed on filter paper and incubated at 13/7 C in both light/dark and dark regimes. Seeds germinating at 13/7 C

were counted after 14 d of incubation at this temperature regime and calculated as a percentage of the ungerminated seeds.

In addition, the effects of temperature, time (week of incubation), and KNO_3 (0.005 M) on germination of the RC population were tested. Seeds were incubated at 5 C (cold stratification temperature) or 13/7 C (optimum temperature). Four sets of dishes were placed in each incubator. Every week, one set was removed from the incubators and germination was checked.

Effects of Tillage System on Seedling Recruitment Pattern

This experiment was conducted during the growing season of 2005 on the Roseworthy Campus Farm of the University of Adelaide, South Australia. The tillage systems included were no-till (NT) and minimum-tillage (MINT). Seeds (200 m^{-2}) of both populations were spread in fixed quadrats (size 2 m by 1 m) in mid-March of 2005. Control plots, where seeds were not spread, indicated that there was no background seed bank of threehorn bedstraw in the study area. Two presowing cultivations were given to the MINT plots to a depth of 8 cm before crop sowing. The width of the tines used for cultivation was 100 mm. In the NT plots, soil disturbance was limited to the sowing operation only. Wheat (cv. 'Krichauff') was sown with a combine fitted with knife point soil openers (16 mm wide) in the NT plots, whereas 100 mm-wide tines (to create more soil disturbance) were used for sowing in the MINT plots. The crop was sown in rows 25 cm apart on June 17, 2005. Weed seedling recruitment was measured at 7, 14, 21, 28, 35, 42, and 49 d after crop sowing (DAS) from the whole plot and expressed as a percentage of the seed bank at sowing. The census of seedlings was discontinued when no further emergence was recorded on three consecutive measurements.

Statistical Analysis

All laboratory experiments were conducted in a randomized complete-block design. Treatments of each experiment were replicated three times, and each experiment was repeated except the seed-size experiment, which was conducted at four different times after maturity. The other data represent the average of the two experiments because there was no time-by-treatment interaction. Analysis of variance was performed on the original data obtained as percentage germination (Genstat 5 Committee 1993). The tillage experiment was arranged in a split-plot design, with tillage systems as the main-plots and populations as the sub-plots. There were three replicates of each treatment. Seedling recruitment of each population under MINT and NT systems was analyzed at each sampling time using ANOVA. Seedling recruitment values for each population under MINT and NT were fitted to a functional three-parameter sigmoid model using SigmaPlot[5] 2004 (Version 9.0). The model fitted was

$$R\,(\%) = E_{max}/\{1 + \exp[-(x - t_{50}/E_{rate})]\} \qquad [1]$$

where R is the total seedling recruitment (%) at time x, E_{max} is the maximum seedling recruitment (%), t_{50} is the time to reach 50% of final seedling recruitment (d), and E_{rate} is the slope around t_{50}. Parameter E_{rate} provides an indication of the rate of seedling recruitment.

TABLE 1. Effects of subsequent dark incubation of light-inhibited seeds on germination for Roseworthy Campus (RC) and Yorke Peninsula (YP) populations of threehorn bedstraw at 3 and 6 mo after seed maturity.

	Germination			
	RC		YP	
Time	Light/dark	Dark	Light/dark	Dark
mo	%			
3	0	53	0	60
6	0	55	0	65
LSD ($P < 0.05$)	14		16	

Results and Discussion

Effects of Temperature, Light, and Subsequent Dark Incubation on Germination

Seeds from the RC and YP populations of threehorn bedstraw were subjected to three day/night temperature ranges (25/15, 20/12, and 13/7 C) and two light regimes (light/dark and dark). Germination at the time of seed maturity and 11 mo after maturity was achieved only at the alternating temperature regime of 13/7 C and in complete darkness. No germination was observed under any other temperature regime or in the light. Germination at the time of seed maturity was low for both populations (16 to 18%); however, the viability of the seeds as determined by the tetrazolium chloride test was almost 100% for both populations (data not shown). This suggests that the inhibition of germination immediately after maturity was very high in these populations. Even though germination after 11 mo of seed maturity increased for both populations (52 to 55%), a considerable proportion of seeds were still not able to germinate. In addition, light inhibited germination even at 13/7 C. This study suggests that threehorn bedstraw can germinate only at low temperatures, which would usually occur from June to August in southern Australia. The optimum temperature ranges reported for germination of related species are variable and generally lower for catchweed bedstraw than false cleavers. Similar to our study, lower optimum temperature ranges of 9 to 12 C (Froud Williams 1985) and 12 to 15 C (Sjostedt 1959) have been reported for catchweed bedstraw. Our results also demonstrate that light inhibited germination in this species, and this is similar to the response of false cleavers to light (Malik and Vanden Born 1987b). The results suggest that threehorn bedstraw may be similar to other negative photoblastic seeds in which phytochrome far-red (P_{fr}) remaining after seed ripening may trigger germination upon rehydration in the dark (Rollin 1972). In such seeds, exposure to light during germination may convert the P_{fr} to the inactive phytochrome red (P_r) form.

Reversibility of light-inhibited germination by subsequent dark incubation was studied with seeds of both populations at 3 and 6 mo after seed maturity. Germination was not observed during the initial light/dark incubation for 14 d. However, subsequent dark incubation for another 7 d enabled germination in both populations (Table 1). The ger-

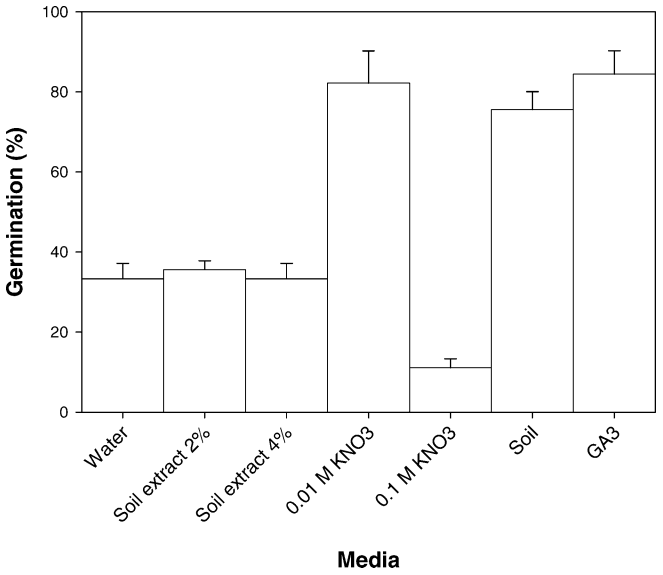

FIGURE 1. Effect of solutions and media on seed germination of the Yorke Peninsula (YP) population of threehorn bedstraw. Vertical bars represent standard errors.

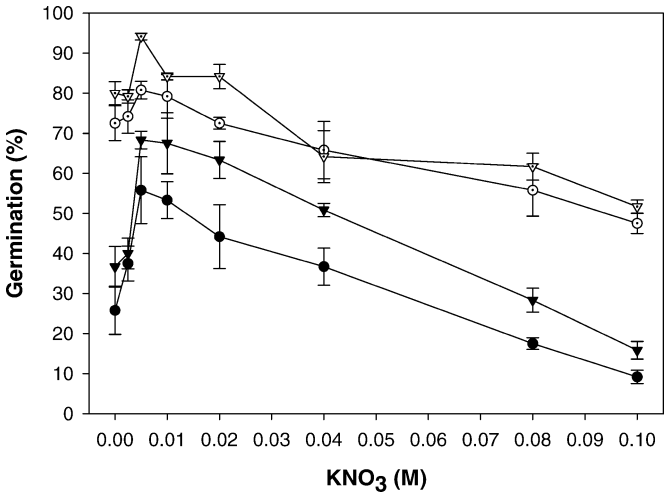

FIGURE 2. Effect of different concentrations of KNO_3, with (open symbol) and without (solid symbol) GA_3, on germination of the Roseworthy Campus (RC, circle) and Yorke Peninsula (YP, inverted triangle) populations of threehorn bedstraw. Populations were analyzed separately. Vertical bars represent standard errors.

mination percentage after this treatment was similar among populations and times after maturity at 53 and 55% and 60 and 65% for the RC and YP populations at 3 and 6 mo after seed maturity, respectively. Light-inhibited germination in seeds of threehorn bedstraw can be alleviated by a period in the dark. The requirement of darkness for germination in this species means that a high proportion of seeds will remain ungerminated on the soil surface, either until seed death (due to desiccation or predation activity) or until burial occurs through tillage or sowing operations. Therefore, greater seedling recruitment would be expected in tilled compared with untilled fields.

Effects of Physical Scarification, Germination Media, and Leaching on Germination

These experiments were conducted to understand the mechanism of inhibition of germination in this species. Germination caused by scarification (cutting) was similar to germination of intact seeds (data not shown). This suggests that inhibition of germination in this species is not due to an impermeable seed coat.

Germination was stimulated by 0.01 M KNO_3, whereas 0.1 M KNO_3 inhibited germination (Figure 1). Planting seeds in soil, or treating them with GA_3 also stimulated germination to the same level as 0.01 M KNO_3. Soil and soil extracts have been reported to be better media than distilled water for germination of catchweed bedstraw and false cleavers (Malik and Vanden Born 1987b; Sjostedt 1959). Soil extracts are likely to contain soluble nitrates, and this may be the reason for their ability to stimulate germination. However, germination of threehorn bedstraw was not stimulated by soil extracts. This could be the result of very low nitrate (8 mg kg^{-1} soil) present in the soil extracts used here. There may also be other factors involved because placing seed in the soil caused a stimulation of germination. Because the seeds tested have a relatively large size, it is possible that the "soil effect" could be due to better seed imbibition. In addition, ethylene (an important hormone) produced in the soil could have stimulated seed germination.

Inhibition of germination may be the result of germination inhibitors present in the seed or seed coat that need to be leached by rainfall before the seed will germinate. Germination of the RC and YP populations in the absence of leaching was 17 and 27%, respectively. However, leaching of seeds with water from 2 to 48 h inhibited germination (data not shown). This indicates that inhibition of germination in this species is unlikely to be the result of the presence of leachable germination inhibitors.

Effects of KNO_3 and GA_3 on Germination

KNO_3 and GA_3 are known to be agents that can overcome germination inhibitors (Fawcett and Slife 1978; Hendricks and Taylorson 1974; Karssen et al. 1989). In a preliminary experiment, stimulation of germination by KNO_3 only occurred in the darkness (data not shown). Therefore, experiments on the effects of KNO_3 and GA_3 were conducted only in the darkness. Germination of seeds of both populations was stimulated by either KNO_3 or GA_3 (Figure 2; Table 2). KNO_3 at 0.005 M stimulated germination of both populations to a maximum level of 56 and 68%, respectively (Figure 2). However, germination of both popu-

TABLE 2. Degrees of freedom (df) and level of significance for the effects of potassium nitrate (0 to 0.1 M KNO_3) and gibberellic acid (0.001 M GA_3) and their interaction on germination of seeds of the Roseworthy Campus (RC) and Yorke Peninsula (YP) populations of threehorn bedstraw.

		F probability	
Factor	df	RC[a,b]	YP[c]
KNO_3	7	$P < 0.001$	$P < 0.001$
GA_3	1	$P < 0.001$	$P < 0.001$
KNO_3 by GA_3	7	NS	$P < 0.01$

[a] Abbreviation: NS, not significant.
[b] Residual mean square of RC population = 82.72 with 30 df.
[c] Residual mean square of YP population = 39.62 with 30 df.

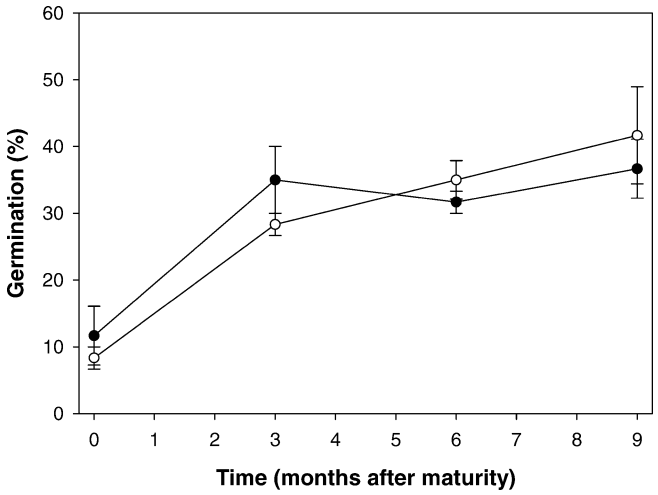

FIGURE 3. Effect of seed sizes on germination of threehorn bedstraw (Roseworthy Campus [RC] population) when tested in the darkness at different times after seed maturity. Seeds were classified as small, < 2 mm (solid circle), and large, > 2 mm (open circle). Vertical bars represent standard errors.

lations started to decline with concentrations of KNO_3 greater than 0.005 M. At the highest concentration of KNO_3 (0.1 M), germination was lower than without KNO_3. Germination of both populations was promoted by the combination of GA_3 with KNO_3. However, the interaction between KNO_3 and GA_3 was found to be significant only in the YP population (Table 2). The difference in germination between GA_3 alone and combinations of KNO_3 with GA_3 was not large. Although GA_3 can stimulate germination with KNO_3, germination was still inhibited by higher concentrations of KNO_3 even when GA_3 was present. Stimulation of germination by GA_3 has been shown in many plant species and could be due to mobilization of food reserves in the seeds (Karssen et al. 1987). Germination promotion by KNO_3 has also been reported for false cleavers (Malik and Vanden Born 1987b). The results from this experiment suggest that field application of N-containing fertilizers may be able to influence germination of threehorn bedstraw seeds.

Effects of Seed Size on Germination

Seed size had no impact on germination in the light/dark regime because seeds of this species did not germinate under these conditions. Therefore, data are shown only for the dark treatment (Figure 3). Germination was also not influenced by the size of the seeds in the dark; however, overall germination was higher at the later stages of the experiment than at the time of seed maturity. This observation indicates that germination in this species will increase with time. In addition, small and large seeds had similar seed viability over the period of this study and will contribute to plant populations in the future.

Effects of Cold Stratification on Germination

Seeds of the RC population were chilled in moist sand at 5 C for 5 wk to stimulate germination. Following this, seeds were placed in light/dark or dark conditions at 13/7 C. Cold stratification caused seeds to germinate even in the absence

TABLE 3. Effects of cold stratification on seed germination of the Roseworthy Campus (RC) population. Before cold stratification treatment, seeds were stored on the soil surface in a field. During cold stratification (5 C), seeds were placed in petri dishes containing sand, and every week, seeds were exhumed from sand and ungerminated seeds were placed on filter papers at 13/7 C day/night temperature in both light/dark and dark conditions. Germination at 13/7 C day/night temperature was examined after 14 d of incubation and represented as the percentage of the ungerminated seeds.

	Germination		
		Sequential treatment for 14 d at 13/7 C	
Time	5 C	Light/dark	Dark
wk		%	
0			35
1	15	23	35
2	60	8	15
3	88	3	3
4	100	—	—
5	100	—	—
LSD ($P < 0.05$)	4	20	

of incubation at 13/7 C. Complete (100%) germination occurred within 4 wk of cold stratification at 5 C (Table 3). The seeds that did not germinate after 1 to 3 wk of cold stratification were transferred to 13/7 C temperature cabinet in the light/dark and dark regimes. In the absence of chilling, seed germination only occurred in the dark. However, the chilling treatment enabled some seed germination to take place even in the light/dark treatment. It is known that seeds of some species require a period of chilling, while in the imbibed state, to stimulate germination. For example, germination promoted by cold stratification in catchweed bedstraw has been reported previously (Grime et al. 1981; Slade and Causton 1979). Temperatures lower than 5 C often occur in the southern Australian grain belt in winter (June to August) and this periodic cold stratification may be important for germination of threehorn bedstraw. It is also noteworthy that cold stratification was effective in the dark. In fields, seeds may be on the soil surface, so their response could be different to that observed here.

To further study the effects of cold stratification, seeds were incubated with or without KNO_3 (0.005 M) at 13/7 C and 5 C for 1 to 4 wk. Only 17% of seeds moistened with water germinated at 13/7 C, even after 4 wk of incubation (Figure 4). In contrast, treating seeds with KNO_3 increased germination to 47% after 1 wk of incubation. Cold stratification increased germination, and germination was higher with KNO_3 than without KNO_3 after 2 and 3 wk of incubation. However, after 4 wk of incubation, there were no differences between these treatments. Statistically the differences in germination rates between treatments, times, and temperature were significant ($P < 0.01$) as was the interaction between most of these factors (Table 4). Only the interaction of all three factors (temperature by time by KNO_3) was nonsignificant. This experiment clearly demonstrates that germination in this species is responsive to both cold stratification and KNO_3.

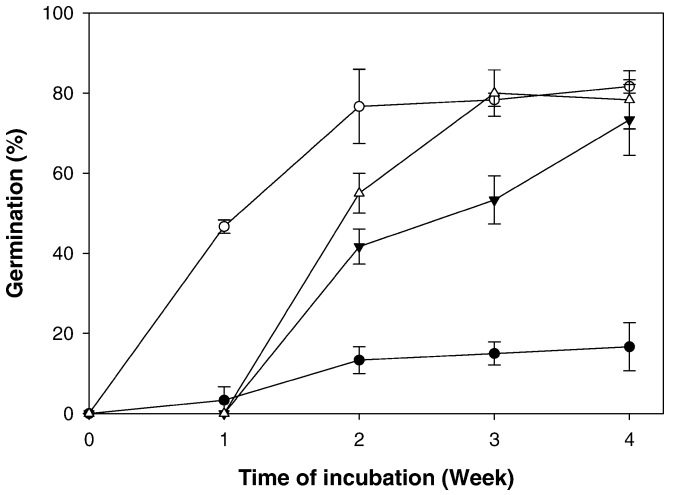

FIGURE 4. Interaction effects of temperature and KNO_3 on germination of threehorn bedstraw (Roseworthy Campus [RC] population) seed when incubated in darkness for different periods of time. Seeds were placed at 5 C (triangle) and 13/7 C (circle) day/night temperature with (open symbols) and without (solid symbols) 0.005 M KNO_3. Vertical bars represent standard errors.

TABLE 4. Degrees of freedom (df) and level of significance for temperature (13/7 C and 5 C), time (1 to 4 wk), potassium nitrate (KNO_3; 0.005 M or absence), and their interactions on germination of seeds in the Roseworthy Campus (RC) population of threehorn bedstraw. Data were analyzed by using randomized block design.[a,b]

Factor	df	*F* Probability
Temperature	1	P = 0.01
Time	3	P < 0.001
KNO_3	1	P < 0.001
Temperature by time	3	P < 0.001
Temperature by KNO_3	1	P < 0.001
Time by KNO_3	3	P = 0.01
Temperature by time by KNO_3	3	NS

[a] Abbreviation: NS, not significant.
[b] Residual mean square = 65.03 with 30 df.

Effects of Tillage System on Seedling Recruitment Pattern

Tillage treatment influenced seedling recruitment of both populations of threehorn bedstraw. In both populations, plots sown with MINT had significantly higher seedling recruitment than those sown with NT (Figure 5). The maximum seedling recruitment of the RC and YP population was 25 and 27% in MINT-sown plots compared with 15 and 18% in NT-sown plots. Not only was seedling recruitment higher under MINT, the time taken for 50% seedling emergence (t_{50}) was also shorter by 2 to 5 d under MINT. Comparisons between the two populations showed that the maximum seedling recruitment attained in the field was similar. Although this study was conducted in only one growing season, the results from two populations confirmed that seedling recruitment in threehorn bedstraw is stimulated by tillage. Within the NT plots, seedling recruitment tended to occur within or very near to the crop rows, suggesting that the seedling recruitment was promoted by the soil coverage caused by the sowing operation. This is consistent with the inhibitory effects of light on germination. It is possible that a greater proportion of seeds might be left on the soil surface under NT, as minimal soil disturbance occurs. In addition, the increased burial due to tillage could be linked to better seed–soil moisture contact (and consequently better seed imbibition) of this relatively large-seeded species. Germination of a closely related species, catchweed bedstraw, has also been reported to be influenced by cultivation. Froud Williams (1985) reported that 44% of catchweed bedstraw seedlings emerged following tine cultivation as compared with 29% following direct drilling. Similarly, the seedling recruitment of both catchweed bedstraw and false cleavers was found to be stimulated by tillage in Canada (Reid and Van Acker 2005).

There are similarities in the factors that influence germination of threehorn bedstraw and those that influence germination of the related weed species, catchweed bedstraw and false cleavers. Light inhibits germination of all three species (Boyd and Van Acker 2003; Malik and Vanden Born 1987b). Cold stratification and nitrates both stimulate germination (Grime et al. 1981; Malik and VandenBorn 1987b; Slade and Causton 1979). These similarities mean that similar responses of the three weed species to agricul-

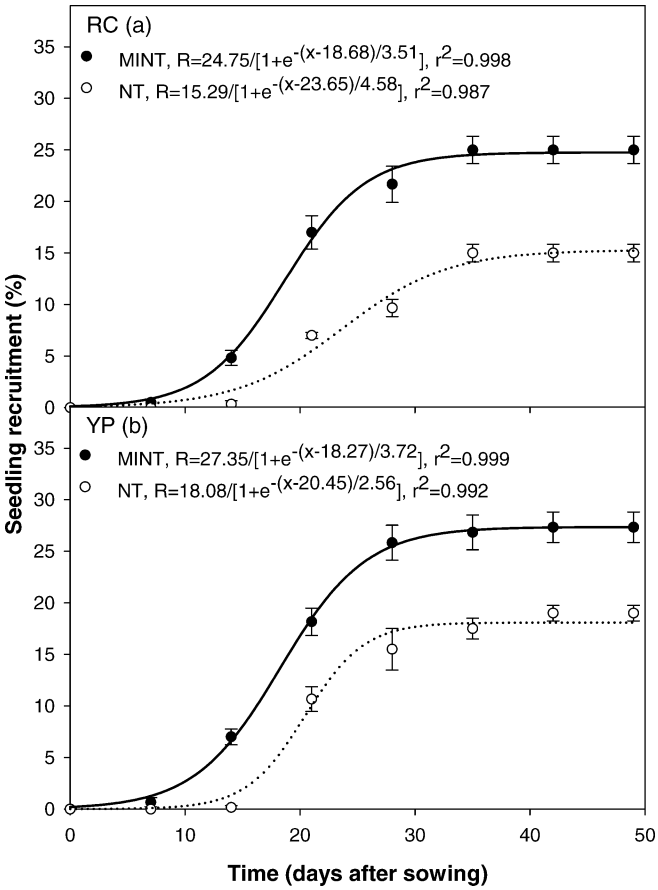

FIGURE 5. Effect of minimum tillage (MINT, solid line) and no-till (NT, dotted line) seeding systems on seedling recruitment pattern of (a) Roseworthy Campus (RC) and (b) Yorke Peninsula (YP) populations of threehorn bedstraw. Vertical bars represent standard errors. A three-parameter sigmoid model $\{R\,(\%) = E_{max}/1 + \exp[-(x - t_{50}/E_{rate})]\}$ was fitted to the data. R is the total seedling recruitment (%) at time x, E_{max} is the maximum seedling recruitment (%), t_{50} is the time to reach 50% of final seedling recruitment (d) and E_{rate} is the slope around t_{50}. Treatments were significantly different ($P < 0.05$) at all census dates except 7 d after sowing.

tural operations are likely. For example, the effect of tillage on germination of catchweed bedstraw and false cleavers reported by Reid and Van Acker (2005) are similar to the results reported for threehorn bedstraw here (Figure 5).

The practical implications for this study are (1) seeds will not germinate on the soil surface, (2) N-containing fertilizers will stimulate germination, and (3) seeds will germinate only at low temperature, which usually occurs from June to August in southern Australia. No-till systems have been shown to concentrate weed seeds on the soil surface. For this negatively photoblastic species, such vertical seed distribution is likely to result in lower and discontinuous germination under no-till systems.

Sources of Materials

[1] Parafilm, Pechiney Plastic Packaging, 289 River Street, Menasha, WI 54952.

[2] 2,3,5-triphenyltetrazolium chloride, Sigma-Aldrich Co., 3050 Spruce Street, St. Louis, MO 63103.

[3] Potassium nitrate, VWR International Ltd., Merck House Poole, Dorset BH15 1TD, England.

[4] Gibberellic acid, Sigma-Aldrich Co., 3050 Spruce Street, St. Louis, MO 63103.

[5] SigmaPlot 2004 (Version 9.0), Systat Software, Inc., 501 Canal Boulevard, Suite C, Point Richmond, CA 94804-2028.

Acknowledgment

A Ph.D. scholarship by the Australian Centre for International Agricultural Research (ACIAR) supported B.S. Chauhan during this research program at the University of Adelaide, South Australia.

Literature Cited

Amor, R. L. and P. M. Kloot. 1987. Changes in weed flora in south-eastern Australia due to cropping and control practices. Plant Prot. Q. 2:3–7.

Bedggood, W. 1999. Integrated Weed Management of Bedstraw. www.dpi.vic.gov.au.

Black, I. D., A. Mayfield, and R. Matic. 1994. Chemical control of bedstraw (*Galium tricornutum* Dandy) and bifora (*Bifora testiculata* L.) in wheat, barley and field peas. Plant Prot. Q. 9:24–27.

Boyd, N. S. and R. C. Van Acker. 2003. The effects of depth and fluctuating soil moisture on the emergence of eight annual and six perennial plant species. Weed Sci. 51:725–730.

Department of Agriculture, Western Australia. 2005. Bedstraw Confirmed in Cereal Crops. www.agric.wa.gov.au.

Fawcett, R. S. and F. W. Slife. 1978. Effects of field application of nitrate on weed seed germination and dormancy. Weed Sci. 26:594–596.

Froud Williams, R. J. 1985. The biology of cleavers (*Galium aparine*). Asp. Appl. Biol. 9:189–195.

Genstat 5 Committee. 1993. Genstat 5, Release 3 Reference Manual. Oxford, U.K.: Clarendon.

Grime, J. P., G. G. Mason, A. V. Curtis, J. Rodman, and S. R. Band. 1981. A comparative study of germination characteristics in a local flora. J. Ecol. 69:1017–1059.

Hall, L. M., K. M. Stromme, G. P. Horsman, and M. D. Devine. 1998. Resistance to acetolactase synthase inhibitors and quinclorac in a biotype of false cleavers (*Galium spurium*). Weed Sci. 46:390–396.

Hendricks, S. B. and R. B. Taylorson. 1974. Promotion of seed germination by nitrate, nitrite, hydroxylamine and ammonium salts. Plant Physiol. 54:304–309.

Karssen, C., S. Groot, and M. Koornneef. 1987. Hormone mutants and seed dormancy in *Arabidopsis* and tomato. Pages 109–113 *in* H. Thomas and D. Grierson, eds. Symposia of the Society for Experimental Biology, XXXII: Developmental Mutants in Higher Plants. Cambridge, U.K.: Cambridge University.

Karssen, C. M., S. Zagorski, J. Kepczynski, and S.P.C. Groot. 1989. Key role for endogenous gibberellins in the control of seed germination. Ann. Bot. 63:71–80.

Malik, N. and W. H. Vanden Born. 1987a. False cleavers (*Galium spurium* L.) competition and control in rapeseed. Can. J. Plant Sci. 67:839–844.

Malik, N. and W. H. Vanden Born. 1987b. Germination response of *Galium spurium* L. to light. Weed Res. 27:251–258.

Malik, N. and W. H. Vanden Born. 1988. The biology of Canadian weeds. 86. *Galium aparine* L. and *Galium spurium* L. Can. J. Plant Sci. 68: 481–499.

Mennan, H. 2003. The effects of depth and duration of burial on seasonal germination, dormancy and viability of *Galium aparine* and *Bifora radians* seeds. J. Agron. Crop Sci. 189:304–309.

Moerkerk, M. R. 1999. Chemical control of bedstraw (*Galium tricornutum* Dandy) in wheat, barley, field peas, chickpeas and faba beans in southern Australia. Plant Prot. Q. 14:24–29.

Peters, N.C.B. 1984. Competition between *Galium aparine* (cleavers) and cereals. Pages 3–4 *in* Abstract of AAB Meeting: Understanding Cleavers (*Galium aparine*) and Their Control in Cereals and Oilseed Rape. Wellesbourne, U.K.: Association of Applied Biologists.

Reid, D. J. and R. C. Van Acker. 2005. Seed burial by tillage promotes field recruitment of false cleavers (*Galium spurium*) and catchweed bedstraw (*Galium aparine*). Weed Sci. 53:578–585.

Rollin, P. 1972. Phytochrome control of seed germination. Pages 229–257 *in* K. Mitrakos and W. Shropshire, Jr., eds. Phytochrome. New York: Academic.

Sjostedt, A. S. 1959. Germination biology of cleavers, *Galium aparine* L. Vaxtodling 10:87–105.

Slade, E. A. and D. R. Causton. 1979. The germination of some woodland herbaceous species under laboratory conditions: a multifactorial study. New Phytol. 83:549–557.

Steadman, K. J., G. P. Bignell, and A. J. Ellery. 2003. Field assessment of thermal after-ripening time for dormancy release prediction in *Lolium rigidum* seeds. Weed Res. 43:458–465.

Taylor, K. 1999. *Galium aparine* L. J. Ecol. 87:713–730.

Tonkin, J.H.B. and A. Phillipson. 1973. The presence of weed seeds in cereal seed drills in England and Wales during spring 1970. J. Natl. Inst. Agric. Bot. 13:1–8.

Received December 5, 2005, and approved March 2, 2006.