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Recruitment of *Phalaris aquatica* within existing swards 1. Effects of biomass manipulation, seed level modification and site preparation

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Abstract. A successful recruitment event in perennial grasslands is infrequent and when it occurs, the rate of recruitment and survival is low, <1% in most occasions. This paper reports on two field experiments that investigated the effects of biomass manipulation, seed level modification and site preparation on the recruitment of *Phalaris aquatica* seedlings. The experiments were done through drier than average years, where *P. aquatica* achieved successful recruitment of seedlings. Recruitment rates proportional to total seed set were 1.3-13.3% in experiment 1 and 0.5-4.2% in experiment 2. The control treatment, on average, resulted in 352 seedlings/ m^2 in experiment 1 and 16/m^2 in experiment 2 compared to the best treatments which had 500/m^2 and 38/m^2 respectively, on average. There was poor seed set in experiment 2 before the recruitment event. Presence of existing biomass compared to either removing or leaving the plant material on the ground had greater success on seedling emergence, whereas seed addition had little effect suggesting that microsites may be more important than seed availability for *P. aquatica* seedling emergence. Soil scarification in general failed to have significant effects in both experiments. Seedlings survived until the following summer, but few then remained through the ensuing drought. This research showed that minimal intervention was needed to encourage emergence and early survival, and provides data on the mechanisms involved for recruitment of a perennial grass species in existing swards.

Additional keywords: seedling recruitment, seedling survival, seed set, soil scarification, insecticide, seed addition

Introduction

Across much of the continent livestock production depends upon existing species as it is not profitable or practical to sow new pastures, except in higher rainfall more fertile regions. Many of the existing pastures have low perennial grass content, the main component that determines livestock productivity. Limited research has been done to investigate low-cost means of encouraging the recruitment of new desirable perennial grasses in these existing pastures. *Phalaris aquatica* L. is a temperate (C_3) perennial grass species that has been widely sown in the Australian environment since it was introduced in 1884 from Europe via the United States (Watson *et al.* 2000). *Phalaris aquatica*, a native of southern Europe, North West Africa and the Mediterranean region, is one of the more persistent and productive temperate perennial pasture grasses. The usefulness of *P. aquatica* as a permanent pasture species has been recognised and well demonstrated in the temperate regions of Australia (Watson *et al.* 2000; Lamp *et al.* 2001). The species is drought tolerant, can withstand extended periods of heavy grazing, performs well in poorly drained and waterlogged soils, and provides good quality forage for all types of grazing livestock. It is known to grow successfully on a wide range of soil types, from heavy soils to sandy soils, although it is not very tolerant of soils with high aluminium, acidity and low fertility (Lamp *et al.* 2001). A number of cultivars have now been bred for Australian conditions and these are more tolerant of low soil pH.

Phalaris aquatica produces large quantities of seed. Kelman and Culvenor (2007) observed 2000 to 11000 seeds/m^2 dispersed, with an average of 4600/m^2 over 3 years of study at a field site near Canberra, Australia. In ungrazed *P. aquatica* grasslands in Spain, up to 25000 seeds/m^2 have been recorded (Leiva and Alés 2000). However, little recruitment within *P. aquatica* stands has been reported (Dowling *et al.* 1996; Virgona and Bowcher 2000; Lodge 2004). Kelman and Culvenor (2007) reported recruitment was around 0.1% of the total seed rain in any one year during 3 years of the experiment. The most often advanced reason for low *P. aquatica* recruitment is competition from annual grasses (Virgona and Bowcher 1998; Lodge 2000) and sown legumes (McWilliam *et al.* 1970; Lamp *et al.* 2001), which prevent the weak *P. aquatica* seedlings from establishing. High levels of seed harvesting by ants is an additional factor that contributes to low recruitment in *P. aquatica* swards (Campbell 1966; Campbell and Gilmour 1979; Kelman *et al.* 2002). *Phalaris aquatica* recruitment is further limited by the ability of adult plants to colonise new spaces (Campbell *et al.* 1981; Lodge 2004). In contrast, *P. aquatica* spreads readily by seedling recruitment along ungrazed roadsides in south-eastern Australia (Kelman *et al.* 2002; Lodge 2004). Recruitment obviously occurs at times under natural conditions, but the mechanisms are poorly understood.

To achieve recruitment of new plants several stages need to occur. Once the current seed is mature and ready to germinate, it then needs to reach the ground to establish new plants. Seeds can remain within seed heads above ground and drop gradually to the ground. Once on the ground surface seeds can be taken by predators such as seed harvesting ants. This could mean only a small quantity of seed is available for potential germination at one time. As seeds drop, the ground surface acts as a potential site for germination. The ground could be bare, covered with litter, interspersed with rocks and twigs, intersected by fissures or simply occupied by existing mature plants. Apart from the soil surface features there are likely to be underground roots of existing perennial plants already exploiting the area and competing for the below ground resources of soil, water and nutrients.

Our study investigated low-cost strategies for pasture rehabilitation by identifying practices that encouraged recruitment of *P. aquatica* within existing swards. The approach taken was to investigate management options that encouraged seed set, to prepare more suitable sites for seedling recruitment and to identify better post-emergence tactics that aided young plant survival in the short- to medium-term. Treatments were designed to create variation in the vegetation structure of the sward, viable seed levels and suitable microsites for germination. The main aim of the experiments reported in this paper was to determine whether or not manipulating biomass, modifying viable seed levels and preparing sites affected seedling emergence and survival.

Methods

Site

The experiment was located at Orange on the Central Tablelands of New South Wales (149°07' E, 33°14' S) within the Orange campus of Charles Sturt University. The site had an elevation of 826 m (a.s.l.) and an average annual rainfall of 890 mm. There was a slight easterly slope on the site. Prior to the experiment the paddock had been rotationally grazed by cattle and sheep, with longer rest than graze periods. The site had a history of fertiliser application and had been sown with introduced pasture species many years before. The soil type was a Vertosol (Isbell 1996) with a light clay surface texture (McDonald and Isbell 1990), and a pH of 5.2 ($CaCl_2$) and 5.9 (water). The experiment was established in October 2005 within an existing *P. aquatica* dominant pasture (40 plants/m^2). Annual grasses were limited to a few species (*Bromus* spp., *Lolium rigidum* Gaud., *Poa annua* L., *Vulpia* spp.). *Dactylis glomerata* L., a sown C_3 perennial grass, was present. There were limited weed problems and the main competitor for emerging *P. aquatica* seedlings was mature *P. aquatica* plants. *Phalaris aquatica* is a very competitive plant and this site was chosen to provide a moderately to highly competitive environment. The field research started when the *P. aquatica* seed heads were starting to emerge. The site was fenced off to exclude grazing.

Climate data measured at the site included: soil temperature, soil moisture (0-50 mm), relative humidity, air temperature, solar radiation, wind speed and rainfall. Weather was recorded using an automatic weather station (Tain Electronics). The monthly rainfall and average temperature (minimum and maximum) recorded for the duration of the experiment are summarised in Fig. 1, along with the long-term averages (1968-2007) measured at an official Australian Government Bureau of Meteorology site located within 15 km of the experimental site (BOM 2008). Rainfall was below the long-term average in most months during the experiment and 2006 was an extremely dry year with only 304 mm of annual rainfall. The mean annual maximum daily temperature was 17.6°C with monthly means ranging from 25.9°C in January to 9.3°C in July. The mean annual minimum daily temperature was 6.1°C with monthly means ranging from 12.1°C in January to 0.7°C in July. Average maximum daily temperature was higher and minimum daily temperature was lower than the long-term averages in most months during the experiment.

[Fig. 1]

Experimental design

The experimental design was based upon a factorial combination of 3 seed delivery mechanism × 3 seed × 3 site preparation treatments laid out in a randomised block with 4 replicates. The experiment was repeated over two years (experiments 1 and 2) on separate plots to capture the establishment in two different years; experiment 2 (second year) being a repeat of experiment 1 (first year) on an adjoining site. Plots in experiment 2 were split into 2 halves each with 2 replicates due to an area constraint. Treatments were applied respectively in January 2006 and 2007 for experiments 1 and 2.

Treatments

The seed delivery mechanism treatments were applied when it was considered seeds were reaching maturity but before seed fall. They varied the level of mechanical disturbance, litter levels, plant cover and structure and delivery mechanism of mature seed to the soil surface. The 3 seed delivery mechanism treatments were: uncut or control (UC), cut and leave (CL), and cut and remove (CR). In UC, the sward was left uncut with seed heads standing to follow the natural cycle. The CL had standing plant material cut to a height of 20-50 mm above ground but plant material and seed heads were left on the ground. This aimed to increase the litter level as a means to retain more moisture near the soil surface and also to maximise the number of seeds on the ground at one time. It was postulated that a large amount of seed reaching the ground at once could limit the ability of seed harvesting ants to completely remove them and enable some seeds to germinate. In CR, herbage mass was reduced to a level similar to grazing by cutting to a height of 20-50 mm above ground and plant matter removed. This treatment aimed to simulate the physical movement from grazing that would have caused some seeds to drop to the ground, while others could be consumed through grazing.

The seed treatments were designed to determine if current seed rain would maximise seedling numbers, if it was limiting, or if seed predation was a problem. Seed harvesting ants can be a major problem with some grass species, *P. aquatica* in particular. There were 3 seed treatments: no seed addition or control (NS), insecticide application (IS) and seed addition (SA). In IS, dead seeds (~50 kg/ha) treated with 600g a.i./L imidacloprid were added to limit predation from ants on existing viable seeds. These insecticide treated seeds were tested for germination (no germination recorded) before field application. *Phalaris aquatica* seeds were used as they are very attractive to ants (Campbell 1966; Campbell and Gilmour 1979). In SA, extra *P. aquatica* seeds were added (~50 kg/ha) to test if current seed rain had saturated the system and if recruitment was limited by seed availability. The aim was to flood the system with additional seeds. These seeds were tested for germination (85% at 15°C; 87% at 25°C; 68% at 35°C) before field application. A response to this treatment would also indicate that there was no limitation of microsites for seedling recruitment.

The site preparation treatments were designed to modify the potential sites for seedling recruitment by modifying the soil surface layer and by reducing competition for emerging seedlings through herbicide application. The 3 treatments included: no preparation or control (NP), herbicide application (HA), and scarify and rake (SR). The plot was left unmodified in NP. In HA, 212 g a.i./L FLUAZIFOP-P present as the butyl ester at a rate of 250 mL/ha was applied (25 January 2006 in experiment 1; 7 February 2007 in experiment 2) to kill any annual grasses that were germinating prior to *P. aquatica* germination events while limiting damage to the existing mature plants. This treatment was applied before any seed addition. In SR, the ground surface was scarified to remove small competitors and existing adult plants. Approximately 50% of the plot area was scarified uniformly to create more bare ground and roughen soil surfaces to create microsites that may be favourable for germination under suitable climatic conditions. This treatment changed the location and density of litter biomass.

Plot layout

Plots were 2 × 2 m. The centre of the plot was 0.9 × 0.9 m and permanently marked for routine measurements. The distance between the measurement area and the adjacent treatment was 0.55m, which allowed seeds from uncut treatments to fall into the measurement area of the adjacent plots, but this was unavoidable. However, the proportion of seeds falling into the adjacent plots would have been low compared to viable seed level present in those plots and the implication from this was assumed to be low. Outside the 0.9 × 0.9 m was a buffer between the adjoining plots used to collect additional measurements (e.g. soil seed bank and soil samples). The 0.9 × 0.9 m area was subdivided into nine 0.3 × 0.3 m contiguous quadrats and used to measure biomass and plant species composition. Each 0.3 × 0.3 m quadrat was further divided into 0.1 × 0.1 m sub-quadrats to record seedling numbers.

Measurements

Dry weight ranks of the 3 most abundant species and the total biomass of all species were estimated using BOTANAL procedures (Tothill *et al.* 1992). Ranked species were combined into plant functional groups using a subjective method defined by Gitay and Noble (1997) based on a combination of life history, physiological and abundance characteristics. The functional groups formed were *P. aquatica* (species under study), other C_3 perennial grasses (second largest group with same physiological characteristic), annual grasses (yearly life cycle length) and other species including forbs, legumes and sedges (considered weeds). Dry weights of standing and litter biomass (t DM/ha) were estimated separately and the estimates were corrected using 15-20 calibration cuts at each sample period (Sanford *et al.* 1998). Green biomass was estimated as the proportion of standing biomass and calibration cuts, after oven dried, were used to determine their dry weights. Sampling for pasture biomass was made every 3 months in late summer (February), autumn (May), winter (August) and spring (November) of each experimental year. Plant cover, litter cover and bare ground percentages were visually estimated (Sanford *et al.* 1998) and ratings were in 5% increments (e.g. 0, 5, 10 to 100). Plant cover was defined as the area on the ground covered by standing biomass when projected vertically on to the soil surface. Litter cover represented the portion of the ground surface covered by detached and dead material excluding the basal area of the standing plants. Bare ground corresponded to the area which was bare in terms of soil exposure plus the area covered by any non-plant material (e.g. cow manure, rocks and tree branches) present.

Sampling frequency for seedling monitoring varied with time from germination events. The emergence immediately following treatment application and a subsequent substantial rainfall event was the major germination event to be monitored through the year. Survival was defined as the young plants that lived through the first summer. Initial counts were done within 2-3 weeks of seeds germinating (after treatment application); then approximately after 6, 24 and 52 weeks. Each sample counted all the seedlings present and marked those newly emerged with coloured nails for the monitoring of young plant survival. In experiment 1 initial observation of seedlings were made on 7 March 2006 followed by young plant survival counts on 26 April 2006, 25 August 2006 and the final count on 19 March 2007. Experiment 2 followed a similar sequence with seedling counts on 21 March 2007, 23 April 2007, 31 August 2007 and 4 March 2008.

Soil cores were taken to determine the seed bank. The cores were taken at the start of both experiments. Samples were only taken in those treatments where natural seed fall was assumed to differ, so the treatments that had addition of extra seeds were avoided. Within the plot, 2 soil cores (0.05 m diameter × 0.05 m deep) were randomly collected. The cores for each plot were mixed, brought to the glasshouse, sifted, added to the surface of sand and placed into regularly watered pots. All seedlings that emerged were identified, counted, recorded and then combined into functional groups. The experiment continued until there were no more seedlings emerging, usually for a period of 2 weeks. An aim was to determine the readily germinable species that could compete with the emerging perennial grass seedlings.

Seed production was estimated through each flowering season, which occurred over the summer. Total number of plants and seed heads in 10 randomly selected 0.3 × 0.3 m quadrats were recorded. Seeds from 10 of these seed heads were counted to estimate the amount of seed per seed head and total seed production. Seeds were tested for germination immediately (1-2 weeks) after collection.

Analyses

Differences between treatments in total standing biomass, green and litter biomass, each functional group biomass, bare ground and plant cover at each measurement period were analysed by Restricted Maximum Likelihood (REML) spatial analysis to ensure spatial variability was accounted for. A regular grid with AR1 (first-order autoregressive model) was used for experiment 1 and an irregular grid with a power model and Euclidean distance measure was used for experiment 2 due to the arrangement of plots. Significance was determined through Wald tests.

Due to data being non-normally distributed, a Generalised Linear Mixed Model (GLMM) analysis assuming Poisson distribution with a logarithmic link function was used to determine differences in seedling numbers between treatments at each measurement period. Statistical significance was determined through Wald tests. Across sites there were often trends in seedling recruitment that were a site effect, rather than of treatments. Spatial analysis techniques (REML) were able to resolve the underlying treatment effects.

All statistical analyses were done using GenStat 9.1 (Payne *et al.* 2006).

Results

Grassland description and species composition

Experiment 1

UC had about twice ($P < 0.001$) the standing biomass of CL or CR (Table 1). SR removed the bulk of the herbage mass resulting in lower ($P < 0.001$) standing biomass than NP or HA (Table 1). CL or CR had around half ($P < 0.001$) the green biomass compared to UC (Table 1), a trend which remained until summer 2007 when green biomass was about the same (~0.4 t/ha). SR had approximately half ($P < 0.001$) green biomass compared to NP or HA (Table 1) but the difference was bridged by autumn 2006 (data not presented). More litter biomass was present in CL than UC or CR and less in SR than NP or HA ($P < 0.001$; Table 1). By autumn 2006, litter biomass was ~2.6 t/ha (data not presented). On average 62% bare ground was created in SR when there was <5% naturally occurring bare soil surface (Table 1). UC had less bare ground than CL or CR and SR had more compared to NP or HA ($P < 0.001$; Table 1). More ($P < 0.001$) space for germination was available in CR × SR (Table 1). Plant cover was greater ($P < 0.001$) in UC than CL or CR (Table 1). Less ($P < 0.001$) plant cover was observed in SR than NP or HA (Table 1) but the levels were similar from autumn 2006 to the end of the experiment (data not presented).

Phalaris aquatica, accounted for >80% of the total biomass (Table 1). UC had more than double *P*. *aquatica* biomass compared to CL or CR whereas SR had less than NP or HA ($P < 0.001$; Table 1). Presence of annual grasses (2-4%), C_3 species (3-12%) and others (3-8%) were low (Table 1) and their proportions only changed marginally as the year progressed (data not presented). On average, each functional group (other than *P. aquatica*) had <1 t DM/ha (Table 1).

Experiment 2

UC had twice as much standing biomass as CL or CR and SR had less ($P < 0.001$; Table 1). UC × NP had the most standing biomass and CR × SR the least ($P < 0.05$; Table 1). Green biomass was present more in UC and less in SR ($P < 0.001$; Table 1). CL had more litter biomass than UC or CR, and SR had less than NP or HA ($P < 0.001$; Table 1). There was a very low percentage (~5%) of natural bare ground available but SR created 60% bare ground (Table 1). CR × SR had the most bare ground whereas UC × NP/HA and CL × NP did not have any bare patches ($P < 0.001$; Table 1). UC had more ($P < 0.001$) plant cover than CL or CR (Table 1). Less ($P < 0.001$) plant cover was present in SR than NP or HA (Table 1).

Phalaris aquatica and other members of the C_3 perennial grass functional group (mainly *D. glomerata*) dominated the species mix. The total biomass consisted of ~60% *P. aquatica*, 30% other C_3 species, 8% other species including broadleaf weeds and <2% annual grasses (Table 1).

[Table 1]

Flowering and seed set

Flowering in both experiments started in the second week of December continuing until mid January, ~4 weeks. Anthesis commenced at the tip of the seed head, progressing towards the base over a relatively short period of 2-3 days. Seed retention in the head was greatest soon after anthesis, but then over 3-4 weeks seeds were released. Seed production was maximum in January, declining over time but some seeds were still present in standing seed heads until May.

Seed production was lowest (57 kg/ha; Table 2) at the start of experiment 2, which coincided with the end of experiment 1. Seed weight (~1 mg/seed) remained relatively constant for all seeds recovered from seed heads throughout summer and autumn. Germination of collected seeds on average was 47 and 42% at the optimal temperature of 25°C (Table 2).

Viable seed level

Low quantities of *P. aquatica* seeds were present in the 'readily germinable' soil seed bank in both experiments accounting for only 2 and 3% respectively of the total germinating seed bank (Table 2). Annual grasses were low in numbers compared to seasonal broadleaf species or legumes, which dominated the seed bank (Table 2).

The germination rate of freshly fallen seeds was <50% (Table 2) but the amount of viable seed in control treatments was still large, 59.2 kg/ha in experiment 1 and 23.9 kg/ha in experiment 2 (Table 3). In experiment 1, in UC × SR, proportional gain from SA was 9.9% of total viable seed whereas it was 13.3% from NS or IS, though higher recruitment occurred in UC × SA × SR (Table 3). The trend was similar in CL × SR and CR × SR, where SA had higher recruitment numbers but did not improve recruitment percentage (Table 3). Viable seed level and recruitment were poorer in experiment 2 but the trends in different treatments were similar to experiment 1 (Table 3). In UC, CL and CR, recruitment increased in SA × SR but the proportional gain was lower (Table 3). Highest recruitment without SA had higher emergence than SA in both experiments, indicating extra seed only added to what happened naturally (Table 3).

[Table 2]

[Table 3]

Emergence and overall young plant survival

Experiment 1

Emergence of *P. aquatica* seedlings occurred in early March 2006. As many as 1332 seedlings/m^2 were recorded in UC × IS × NP (Table 3). UC had more seedlings than CL, which had marginally higher seedling numbers than CR ($P < 0.001$; Fig. 2*a*). Fewer ($P < 0.001$) seedlings germinated in NS than IS or SA (Fig 2*a*). Seedling recruitment was marginally more ($P < 0.05$) in HA than NP or SR (Fig 2*a*). More ($P < 0.05$) seedlings were observed in IS than SA when herbicide was applied (Fig 2*c*). In CL, less ($P < 0.05$) seedlings were observed in SR than NP (Fig 2*c*). In UC, both IS and SA increased seedling numbers in SR; in CR, IS had more seedlings germinated in HA whereas there were more in NP for SA; and in CL, IS enhanced seedlings in NP but SA had more in HA or SR ($P < 0.001$; Fig. 2*c*). Almost half the plots (46%) had >100 seedlings/m^2, but 3 plots (CL × IS × HA, CR × NS × NP and CR × IS × HA) did not record a seedling. Seedlings of species other than *P. aquatica* were present but in low numbers. The most prominent was legumes (~20 seedlings/m^2), broadleaf seedlings were minimal (~1/m^2), and perennial grass seedlings (mainly *D. glomerata*) were around ~10/m^2 (data not presented). Legumes occurred more ($P < 0.05$) in UC than CL or CR (data not presented).

Survival rates of young *P. aquatica* plants were very low. On average, 222 seedlings/m^2 germinated but there was a massive mortality through the year. The really important period of mortality was from early March to mid autumn. Over this 6 week period >90% of emerged seedlings died (Fig. 2*a*). UC had higher ($P < 0.001$) seedling numbers surviving than CL or CR (Fig. 2*a*). IS or SA had more ($P < 0.001$) seedlings surviving than NS (Fig 2*a*). In UC, more ($P < 0.01$) seedlings were surviving in SR × IS / SA; in CL, there were more ($P < 0.01$) surviving seedlings in IS × NP and SA × HA / SR; in CR, IS × HA and SA × NP had more ($P < 0.01$) seedlings surviving (Fig. 2*c*). At 24 weeks after emergence, there were 18 young plants/m^2 (on average) present. The decline was greatest in UC where the highest initial recruitment was recorded. UC had more ($P < 0.001$) seedlings surviving than CL or CR (Fig. 2*a*). HA or SR had marginally less ($P < 0.05$) seedlings surviving than NP (Fig 2*a*), except in CR there were more ($P < 0.01$) seedlings surviving in HA than SR (Fig. 2*c*). The exponential rate of decline from emergence to week 24 was higher ($P < 0.05$; $R^2 = 0.54$) in UC than CL or CR (data not presented).

Experiment 2

This experiment did not result in as many seedlings as experiment 1 but there were seedlings across all treatments and not only in UC, the more successful treatment in most of experiment 1 plots. The highest seedling number recorded was 198 seedlings/m^2 in UC × IS × HA (Table 3). More (P < 0.001) seedlings were recorded in UC and CR than in CL (Fig. 2*b*). Recruitment of seedlings occurred more (P < 0.001) in IS and SA than in NS (Fig 2*b*). In UC, more seedlings occurred in IS than SA, and in CL, more seedlings emerged in SA than IS (P < 0.001; Fig. 2*d*). In UC, fewer (P < 0.05) seedlings germinated in HA or SR than NP (Fig 2*d*). Forty two percent of the plots recorded 11-100 seedlings/m^2, but nearly a third of all plots (26 out of 108) failed to record a seedling. Low seedling numbers (1-5/m^2) were observed for legumes, broadleaves and *D. glomerata* (data not presented).

Experiment 2 had lower mortality rate in terms of young plant survival than experiment 1. Though there was far less recruitment (27 seedlings/m^2 on average), the decline through the year was only gradual. At 24 weeks after emergence, there were an average of 6 young plants/m^2 remaining. Some young plants died but there were 3 young plants/m^2 on average at 52 weeks after emergence. UC × IS × HA had the highest number of young plants (20/m^2) surviving (Fig. 2*d*). In UC, fewer seedlings were present in HA or SR than NP whereas in CL, more young plants were surviving in HA (P < 0.05; Fig. 2*d*). IS or SA had more (P < 0.001) young plants surviving than NS (Fig. 2*d*).

[Fig. 2]

Discussion

In these field experiments there was successful recruitment of *P. aquatica* with limited recruitment of the less-desirable species. Our study was based upon previous observations (Lodge 1981; Dowling *et al.* 1996) that the better time for recruitment of perennial grasses would be in late summer to early autumn, following seed set, as previous research (Winkworth 1971; Silcock *et al.* 1990; Lodge 2004) suggested the seed bank for perennial grasses was minimal and that later in autumn the dominance of annual grasses and forbs could out-compete the weaker perennial grass seedlings. In our study, measurements were taken in late summer or early autumn, late winter and through to the next summer, where the only seedlings of perennial grasses found were those that had emerged late in the previous summer. The general hypothesis regarding the better time for recruitment can therefore be considered as substantiated by these observations.

A single recruitment event of *P. aquatica* seedlings was observed in early autumn (March) in both experiments after significant rainfall events in the second half of February. The rainstorms in late February were the first major downfall after seed maturation and treatment application, and the seedling recruitment was the result of that event. The earlier rain in January possibly helped seed maturation and may have primed the seed for later germination (Bradford 1994; Villela 1998; Gutterman 2000). Similar observations on germination after the first major rainfall event have been reported for other grass species - *Themeda australis* (R.Br.) Stapf (Mott 1978), *Austrodanthonia* spp. (Hovenden *et al.* 2008; Thapa *et al.* 2011), *Stipagrostis uniplumis* (Licht. ex Roem. & Schul.) De Winter (Zimmermann *et al.* 2008), *Bothriochloa macra* (Steud.) S.T. Blake (Thapa *et al.* 2011). Grass seeds germinate and emerge only in the presence of adequate soil moisture (Wilson and Briske 1979; Maze *et al.* 1993; Hamilton *et al.* 1999; Zimmermann *et al.* 2008). However, seedlings did not emerge at other times of the year even though sufficient rainfall was received for a germination event to occur, a phenomenon also reported by Zimmerman *et al*. (2008). This could be because most of the other rain occurred in winter months (June-July) when mean maximum (~10°C) and minimum (<0°C) temperatures were at their lowest for the year, temperatures considered to be less than optimal for emergence. This highlights the fact that availability of moisture and optimal temperature are the factors that predominantly have an effect on seedling recruitment. Seedlings of species other than *P. aquatica* were present but in low numbers. The most prominent was legumes which occurred more in uncut than cut treatments. This observation seems to be inconsistent with the findings of Leigh *et al*. (1995) concerning the effects of allelopathic chemicals from *P. aquatica* on legume establishment.

Low perennial grass seed banks were observed in our study even though in this case, soil cores were monitored for only two weeks after collection. Several studies (Winkworth 1971; Mott and Andrew 1985; Silcock *et al.* 1990; Bertiller and Coronato 1994; O'Connor 1997; Lodge 2004; King *et al.* 2006) have also shown that seeds of perennial grasses were usually scarce in the soil. This is despite the fact that seed set in our study was relatively high especially in experiment 1 (>10000 seeds/m^2) where soil moisture during flowering and seed set in summer prior to the start of the experiment was considerably wetter than conditions in experiment 2 at the same period. Lodge (2004) observed the same phenomenon of high seed production but a much lower seed bank for the perennial grasses studied. Several authors (Johns and Greenup 1976; Campbell and Gilmour 1979; Kelman *et al.* 2002) report that ant harvesting can markedly affect the availability of *P. aquatica* seeds in the soil seed bank. Seedlings did not emerge at 6, 12 or 24 weeks after the initial main emergence which supports the view that the seed bank was depleted after seed fall. The minimal gain in seedling numbers from adding seed to the pasture suggests that the amount of seed set was able to saturate the number of microsites available for seedling recruitment.

The presence of established vegetation had a large positive effect on recruitment particularly in experiment 1. More seedlings were observed where mature plants remained uncut and the better treatments were those where *P. aquatica* plants were allowed to flower, set seed and to remain standing. Cutting tall grass and either removing the cut material or leaving it on the soil surface failed to achieve improvement in seedling numbers. There was a clear difference in seedling numbers between the treatments where swards were left untouched and where they were cut. This is substantiated by observations along ungrazed and undisturbed road sides where recruitment of *P. aquatica* naturally occurs. In both cases, the mechanisms involved could be that the tall uncut swards probably provide more moisture retention due to shading and a greater boundary layer resistance to air movement, which help the seedlings to germinate in comparison to open (i.e. cut or grazed) swards. Further research may show that *P. aquatica* has characteristics that allow it to recruit under these conditions. However, this was in contrast to other studies on perennial grasses (Moloney 1990; Aguilera and Lauenroth 1993; Milton and Dean 2000; Zimmermann *et al.* 2008), which reported that the presence of established competitors severely suppressed seedling emergence.

Scarifying the soil to create more potential microsites and reduce competition from established plants, did not lead to a significant increase in seedling emergence. This was in contrast to other studies (Kim *et al.* 1990; Hofmann and Isselstein 2004; Liu *et al.* 2008) which have shown that soil surface disturbance enhanced emergence and recruitment but these were different species and variations occur in seed characteristics and plant physiological function. One possible explanation for this difference in our study is that the soil at the experimental site is naturally self mulching and so no benefit was gained from disturbing the soil. Other studies, such as Hofmann and Isselstein (2005), have suggested that disturbance has little effect on seedling emergence in high productivity swards. Our study was conducted in a dense and productive *P. aquatica* sward. Soil scarification might also apply with *Phalaris* pastures growing on different soil types and in poorer condition compared to those studied in these experiments. However, soil scarification in experiment 1 improved seedling emergence where seed was added in uncut, and cut and leave treatments, which indicate scarify and rake treatments received less natural seed set.

Insecticide treatment increased seedling numbers in our study, which supports previous studies (Anslow 1958; Champ and Sillar 1961; Campbell 1966) where the use of insecticide reduced theft of seeds by ants and increased the emergence of seedlings of sown species. Though there was a slight gain at emergence from the use of insecticide, the difference between seedling numbers was overcome through the year and the numbers of surviving seedlings were similar across treatments in experiment 2 at 52 weeks after emergence. Therefore the benefit gained from using insecticide was fairly small. Also, the herbicide treatment did not benefit seedling emergence when seed was not added.

No young plant survived beyond the recruitment phase in experiment 1 supporting Lodge’s (2004) results that survival of young plants is rare. However, there was a very low survival rate in experiment 2, more in line with the data of Hume and Barker (1991) that some natural regeneration may normally occur for *P. aquatica*. The difficulty for survival may be attributed to the prevailing drought. Mortality of seedlings and young *P. aquatica* plants have previously been found to be exacerbated in summers especially when drought was prolonged (Leiva and Alés 2000). Cook (1980) identified the severity and length of the dry periods as important factors affecting the growth and survival of seedlings of oversown tropical pasture species. There is a reasonable chance that young *P. aquatica* plants may fail to survive dry summer conditions. During December-February, there were slightly better conditions in experiment 2 (304 mm rainfall) compared to experiment 1 (160 mm rainfall), hence few seedlings in experiment 2 may have survived the summer months.

The general conclusion from our study is that minimal intervention was needed to achieve maximum recruitment and seedling survival from early autumn to spring. Our study showed *P. aquatica* recruitment was not limited by seed availability. However, under common pasture management practices, *P. aquatica* is normally not able to produce high seed yields due to grazing at critical times of the year. When seed was added, seedling recruitment was higher in cut and remove, treatments closer to common practices. *Phalaris aquatica* recruitment might therefore be limited by seed availability under common agricultural practices. Swards, therefore, need to be encouraged to flower and set seed which can be achieved by implementing a summer rest from grazing. Soil scarification, insecticide and herbicide use had small effects on recruitment. Overall, the results showed that manipulating biomass, modifying viable seed levels and preparing sites for potential emergence encouraged seedling recruitment.

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Fig. 1. Monthly rainfall (mm), and maximum (Tmax) and minimum (Tmin) temperature (°C) for the experimental period plotted with the 40-year average, 1968-2007 (BOM 2008).

Fig. 2. *Phalaris aquatica* seedlings/m^2 (logarithmic scale, n + 1). Treatments include UC (uncut), CL (cut and leave), CR (cut and remove), NS (no seed), IS (insecticide application), SA (seed addition), NP (no preparation), HA (herbicide application) and SR (scarify and rake). Back-transformed means from GLMM used. For (*a*) and (*b*), within each subset of treatments at each measurement period, and for (*c*) and (*d*), within each measurement period, columns with the same letter are not significantly different, $P < 0.05$.

Table 1. Standing biomass (t DM/ha), green biomass, litter biomass, bare ground %, plant cover %, and biomass of each functional group in each treatment at the start of experiments 1 and 2

Predicted means from REML analysis presented. Least significant differences (l.s.d.) are presented for significant interactions ($P < 0.05$). Non-significant interactions are not presented. UC, uncut. CL, cut and leave. CR, cut and remove. NP, no preparation. HA, herbicide application. SR, scarify and rake. n.s., not significant.

Table 2. Production of seeds at the *Phalaris aquatica* site and seedlings/m^2 germinated in the glass house from soil cores (50mm deep) collected from the field site

Temperature range used was 30/15°C; total numbers (± standard error) in two weeks after sampling.

Table 3. Viable seed level, average and highest recruitment at the start of experiments 1 and 2

Treatment interactions, where viable seed level differed, are presented. UC, uncut. CL, cut and leave. CR, cut and remove. NS, no seed. IS, insecticide application. SA, seed addition. NP, no preparation. HA, herbicide application. SR, scarify and rake.

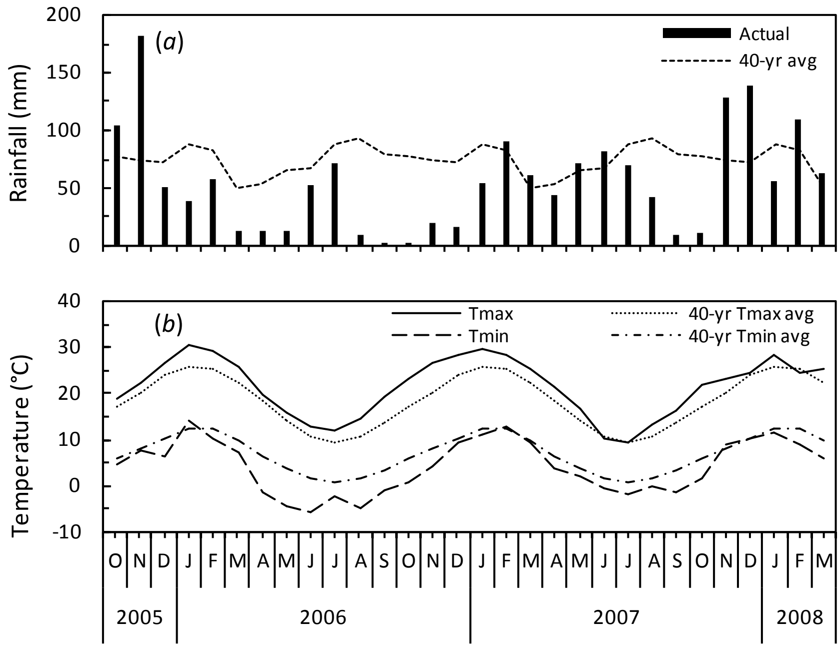
(a)
Actual
40-yr avg
Rainfall (mm)
200
150
100
50
0
(b)
Tmax
Tmin
40-yr Tmax avg
40-yr Tmin avg
Temperature (°C)
40
30
20
10
0
-10
O N D J F M A M J J A S O N D J F M A M J J A S O N D J F M
2005
2006
2007
2008

Fig. 1.

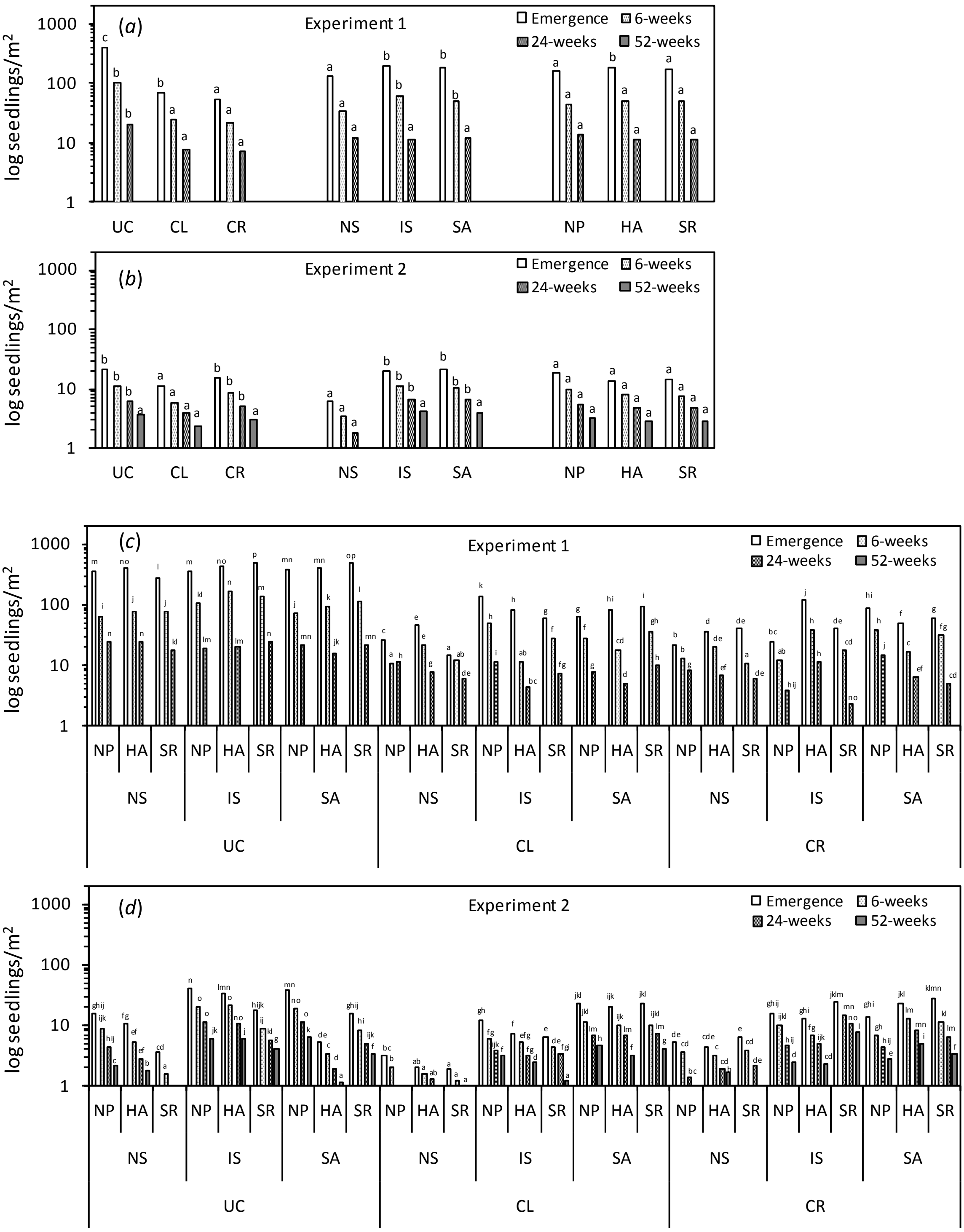
(a)
Experiment 1
Emergence
6-weeks
24-weeks
52-weeks
log seedlings/m2
1000
100
10
1
UC
CL
CR
NS
IS
SA
NP
HA
SR
(b)
Experiment 2
(c)
(d)

Fig. 2.

Table 1.

Treatments		Standing	Green	Litter	Bare	Cover	*P.aquatica*	Annual grasses	C_3 perennials	Others
Summer 2006 - experiment 1										
UC		5.35	1.06	2.81	20	30	4.48	0.14	0.10	0.63
CL		2.51	0.54	3.69	22	18	2.16	0.09	0.08	0.18
CR		2.41	0.50	3.19	25	16	2.03	0.08	0.09	0.20
l.s.d.		0.188	0.041	0.079	0.9	1.6	0.169	0.036	n.s.	0.100
	NP	3.82	0.82	3.68	3	27	3.19	0.14	0.12	0.37
	HA	3.95	0.88	3.74	1	27	3.37	0.11	0.09	0.40
	SR	2.49	0.40	2.27	62	10	2.11	0.05	0.07	0.24
l.s.d.		0.200	0.085	0.082	0.9	1.7	0.178	0.039	n.s.	0.108
UC	NP	6.07	1.21	3.07	3	37	4.98	0.20	0.14	0.76
	HA	6.76	1.53	3.03	1	39	5.58	0.13	0.15	0.91
	SR	3.22	0.44	2.34	56	12	2.89	0.08	0.02	0.21
CL	NP	2.72	0.63	4.31	1	23	2.36	0.14	0.10	0.12
	HA	2.61	0.58	4.27	1	22	2.37	0.08	0.05	0.14
	SR	2.18	0.40	2.49	62	9	1.75	0.03	0.08	0.29
CR	NP	2.67	0.62	3.67	5	21	2.23	0.09	0.12	0.24
	HA	2.47	0.52	3.91	3	19	2.17	0.10	0.06	0.15
	SR	2.09	0.36	1.98	67	7	1.70	0.05	0.10	0.22
l.s.d.		0.340	0.145	0.140	1.6	3.0	0.303	n.s.	0.085	0.182
Summer 2007 - experiment 2										
UC		1.74	0.40	1.50	19	22	1.22	0.02	0.43	0.08
CL		0.84	0.33	1.67	19	13	0.47	0.02	0.28	0.05
CR		0.78	0.29	1.57	23	12	0.49	0.01	0.23	0.06
l.s.d.		0.130	0.028	0.068	0.7	1.7	0.187	n.s.	0.163	n.s.
	NP	1.28	0.39	1.71	0	18	0.85	0.02	0.30	0.11
	HA	1.15	0.33	1.72	1	17	0.72	0.02	0.36	0.05
	SR	0.94	0.29	1.31	60	12	0.60	0.01	0.29	0.03
l.s.d.		0.135	0.028	0.070	0.7	1.8	0.197	n.s.	n.s.	0.061
UC	NP	2.06	0.48	1.57	0	29	1.51	0.03	0.37	0.19
	HA	1.77	0.38	1.68	0	25	1.16	0.02	0.57	0.05
	SR	1.39	0.35	1.24	59	13	0.98	0.01	0.35	0.01
CL	NP	0.93	0.38	1.84	0	13	0.49	0.02	0.34	0.05
	HA	0.86	0.32	1.82	0	14	0.52	0.02	0.25	0.05
	SR	0.73	0.28	1.36	57	12	0.39	0.01	0.25	0.05
CR	NP	0.84	0.33	1.72	1	13	0.56	0.01	0.18	0.09
	HA	0.82	0.28	1.67	3	13	0.48	0.01	0.26	0.05
	SR	0.69	0.25	1.33	64	11	0.42	0.01	0.26	0.02
l.s.d.		0.230	n.s.	n.s.	1.2	3.0	n.s.	n.s.	n.s.	n.s.

Table 2.

	Experiment 1	Experiment 2
Seeds/m^2	12000	5400
Seed yield kg/ha	126	57
Germination (% at 25°C)	47	42
	Seedlings no/m^2	
Phalaris aquatica	244 ± 101	308 ± 87
Annual grasses	138 ± 37	605 ± 117
Broadleaves	5602 ± 1147	4785 ± 830
Legumes	7958 ± 1548	5921 ± 901

Table 3.

Treatments			Viable seed level, no/m^2 (kg/ha)			Emergence		Highest emergence
			Natural	added	total	no/m^2	%	no/m^2
				Experiment 1				
UC	NS / IS	NP / HA	5640 (59.2)	0 (0)	5640 (59.2)	490	8.7	1332
		SR	2908 (30.5)	0 (0)	2908 (30.5)	387	13.3	664
	SA	NP / HA	4814 (50.5)	3986 (40)	8800 (90.5)	552	6.3	1032
		SR	2999 (31.5)	3986 (40)	6985 (71.5)	695	9.9	1017
CL	NS / IS	NP / HA	3567 (37.4)	0 (0)	3567 (37.4)	92	2.6	653
		SR	1811 (19.0)	0 (0)	1811 (19.0)	58	3.2	181
	SA	NP / HA	3856 (40.5)	3986 (40)	7842 (80.5)	108	1.4	362
		SR	2164 (22.7)	3986 (40)	6150 (62.7)	124	2.0	311
CR	NS / IS	NP / HA	2311 (24.3)	0 (0)	2311 (24.3)	72	3.1	551
		SR	1759 (18.5)	0 (0)	1759 (18.5)	59	3.4	172
	SA	NP / HA	2094 (22.0)	3986 (40)	6080 (62.0)	84	1.4	221
		SR	1667 (17.5)	3986 (40)	5653 (57.5)	74	1.3	237
				Experiment 2				
UC	NS / IS	NP / HA	2268 (23.9)	0 (0)	2268 (23.9)	42	1.9	198
		SR	1601 (16.9)	0 (0)	1601 (16.9)	22	1.4	117
	SA	NP / HA	2062 (21.7)	3986 (40)	6048 (61.7)	29	0.5	137
		SR	1647 (17.4)	3986 (40)	5633 (57.4)	42	0.7	90
CL	NS / IS	NP / HA	1163 (12.3)	0 (0)	1163 (12.3)	12	1.0	56
		SR	875 (9.2)	0 (0)	875 (9.2)	13	1.5	69
	SA	NP / HA	1186 (12.5)	3986 (40)	5172 (52.5)	33	0.6	115
		SR	781 (8.2)	3986 (40)	4767 (48.2)	47	1.0	59
CR	NS / IS	NP / HA	918 (9.7)	0 (0)	918 (9.7)	17	1.9	81
		SR	730 (7.7)	0 (0)	730 (7.7)	31	4.2	109
	SA	NP / HA	731 (7.7)	3986 (40)	4717 (47.7)	33	0.7	122
		SR	604 (6.4)	3986 (40)	4590 (46.4)	52	1.1	151