Journal of Applied Botany and Food Quality 83, 163 - 169 (2010)

[1]Soil Research Group, The University of Sydney, Orange, NSW, Australia
[2]Soil Research Group, Charles Sturt University, Orange, NSW, Australia
[3]EH Graham Centre for Agricultural Innovation, Wagga Wagga, New South Wales, Australia

Assessment of allelopathic effects of *Phalaris aquatica* on *Chloris truncata*, *Trifolium subterraneum*, *Medicago trunculata*, and *P. aquatica*

A.A. Adams[1], A. Raman[2,3*], H.I. Nicol[2]

(Received November 13, 2009)

Summary

We examined the effect of *Phalaris aquatica* L. cv. Sardinia on populations of *Chloris truncata* R. Br. (Poaceae) and *Trifolium subterraneum* L. (Fabaceae) under laboratory conditions to assess interspecific allelopathy and, on *P. aquatica,* to assess intraspecific allelopathy (autotoxicity). A population-ecological pilot study measured the abundance and morphometrics of *Medicago trunculata* that occurred in association with populations of *P. aquatica*. We found the abundance of *M. trunculata* was reduced in the presence of *P. aquatica* litter; lengths of shoots of *M. trunculata* were enhanced, but those of roots remained unaffected. Previous laboratory-based studies on *P. aquatica* allelopathy utilized >10% extracts in bioassays on different leguminous taxa; therefore in the present study we attempted to assess the allelopathic potential of *P. aquatica* with <10% extracts (0-2.5% in 0.5% increments), to determine the optimal rate of concentration at which the allelochemicals became either inhibitory or stimulatory. Six concentrations (0.25, 0.5, 1, 1.5, 2, 2.5%) of aqueous extracts of *P. aquatica* were applied to seeds of *C. truncata*, *T. subterraneum*, and *P. aquatica*. Germination of *C. truncata*, *T. subterraneum*, and *P. aquatica* was observed for eight days and lengths of radicles were measured as an indicator of growth. Assessment of the allelopathic potential tested with aqueous extracts of *P. aquatica* on seeds of *C. truncata* and *T. subterraneum* neither inhibited germination nor impacted on radicle lengths. However, *P. aquatica* exhibited autotoxicity by inhibiting radicle growth, although not that of germination. *Phalaris aquatica* water extracts were subjected to high-performance-liquid-chromatographic analysis revealing gramine. Gramine, known to be allelopathic in other grasses, could be a potential allelopathic agent in this taxon.

Introduction

Phalaris aquatica L. is a temperate perennial grass, grown extensively in pastures in New South Wales (NSW), Australia, which were introduced from the 'southern Europe-northwestern Africa-Mediterranean' bioregion into Australia in the 1900s (PUTIEVSKY et al., 1980). *Phalaris aquatica* is a popular pasture species in NSW, because it is drought-tolerant and provides forage almost throughout the year (WATSON et al., 2000). As a C_3 grass, it persists well under heavy grazing and is considered invaluable in Australia, particularly in those land segments that are unsuitable for cultivating *Lolium perenne* L. (Poaceae), another preferred pasture taxon in NSW (AN et al., 2007). Moreover, *P. aquatica* is adapted to a wide range of soils in the high-rainfall areas, and it can withstand reasonably long dry spells and low-medium rainfall, as well as extended periods of water-logging (RAHPC, 1972).

In spite of being a favoured pasture grass, many secondary metabolic compounds, such as monomethylated and dimethylated tryptamines, tyramines, and cyanogenic glycosides, are known to occur in *P. aquatica*. These compounds have been implicated in inducing disorders in livestock (ZHOU et al., 2006). Aqueous extracts of *P. aquatica* have been found to reduce rates of germination and productivity, inhibit seed germination, induce stunted growth of the radicle and, reduce root growth and nodulation in leguminous pasture taxa, which are usually co-cropped in *P. aquatica* pastures in temperate Australia (HALSALL et al., 1995). Although many of the livestock-affecting secondary compounds of *P. aquatica* have been characterized (R. CULVENOR, pers. oral comm., 4 August 2007), the allelochemicals responsible for the inhibition of growth of leguminous-pasture species and other grasses have not been established.

An indole alkaloid, gramine, occurs in the mesophyll and epidermal cells of *Hordeum vulgare* L. (Poaceae) (CORCUERA, 1989). Gramine in 'small' quantities in root exudates of *H. vulgare*, along with hordenine, inhibits root growth in *Sinapis alba* L. (Brassicaceae) (HOULT and LOVETT, 1993). A reduction in crop yield and quality often results when either the same crop or a related species is cultivated on the same soil successively, due to autotoxicity; several taxa of Poaceae, such as *Oryza sativa* L., *Avena sativa* L., *Triticum aestivum* L. (SÁNCHEZ-MOREIRAS et al., 2004), *Pennisetum glaucum* L. (SEXENA et al., 1996), and *L. perenne* have been shown to be autotoxic (YU, 2001).

A pilot-scale field assessment evaluating the role of *Phalaris aquatica* on populations of *Medicago trunculata* led to laboratory assessments using specific dilutions of the *P. aquatica* extract on seedlings of *Trifolium subterraneum* and *Chloris truncata*. Previous laboratory assessments of *P. aquatica* allelopathy have utilized concentrated extracts (>10%) in Petri-dish bioassays tested on different leguminous species, which highlighted impacts of high significance (e.g. HALSALL et al., 1995). To determine the optimal rate of concentration at which the allelochemicals became either inhibitory or stimulatory, dilute extracts (0-2.5% in 0.5% increments) were used.

We sought answers to the following questions: What are the effects of dilute aqueous extracts of *P. aquatica* on the germination of and the elongation of radicles in *T. subterraneum* and *C. truncata* under *in-vitro* conditions? Is *P. aquatica* autotoxic? Does *P. aquatica* contain gramine, the indole alkaloid with an allelopathic potential?

Materials and methods

Field-study site

The study was conducted at *Mendhams*, a 7 ha perennial pasture, grazed by sheep and cattle, located within the Orange Campus of CHARLES STURT UNIVERSITY [CSU-O] (33°14' S; 149°07 E). Within *Mendhams* two oppositely located paddocks (named 'A' and 'B') were used. The paddocks were visually assessed and found to include a 'similar' plant-species composition: *P. aquatica, Dactylis glomerata* L., *Medicago trunculata*, and *Echium plantagineum* L., although paddock A had not been grazed since 2004. Two 15 m transects 'P' and 'Q' were constructed east-westerly direction in

* Corresponding author

September 2007. Transect P included ±2370 kg/ha of leaf litter; transect Q included ±750kg/ha leaf litter. Along transects P and Q, ten quadrats (90 cm x 90 cm each) were constructed at 1.5 m intervals. Incidence of *M. trunculata* was recorded and removed for morphometric data. Shoots and roots of each *M. trunculata* material were measured; fresh mass of each measured plant was obtained by weighing in an electronic scale (ADAM Equipment, AFP-3100LA, Milton Keynes, United Kingdom).

Germination and radicle elongation

Chemical residue-free seeds of *C. truncata* were obtained from NATIVE SEEDS PTY LTD (Melbourne, Australia). Chemical residue-free seeds of *P. aquatica* and *T. subterraneum* were obtained from AUSWEST SEEDS PTY LTD (Forbes, NSW, Australia). Freshly collected two-year old leaves of *P. aquatica* cv. Sardinia (raised in sterile soil) were obtained from CSIRO PLANT INDUSTRY LABORATORY (Canberra, Australia) in August 2007. These materials were air dried in CSU-O laboratory at room temperature (20±2°C) for two weeks. The dried leaves were ground in a micro hammer-cutting mill (# 14/680, GLEN CRESTON LTD, Stanmore, Middlesex HA7 1BU; mesh screen size: 2 mm). Six aqueous extracts of concentrations 0.25, 0.5, 1.0, 1.5, 2.0, and 2.5% of ground *P. aquatica* leaf materials were prepared for *in-vitro* bioassays, by placing 3 g of ground material in 100 mL of double-distilled water and shaken continuously for 4 h on a flask shaker (DYNAMAX, Sydney, Australia). The extracts were stored at 4°C for 24 h before further extraction allowing sedimentation. The sediment was removed by placing each solution in a bench centrifuge (MSE MISTRAL 2000, Leicester, United Kingdom) at 4000 G for 10 min. The supernatant was filtered through WHATMAN® filter paper, sterilized through filtration with sterile-syringe driven filter units. Each solution was stored separately at 4°C for 24 h before use.

Seeds were surface sterilized with 1% NaOCl solution for 5 min, washed thoroughly in distilled water repeatedly, and allowed to stand for 10 min in the sterile-water rinse before repeating the rinses. Twenty seeds were set to germinate on WHATMAN® filter paper moistened with 5 mL of each extract of determined concentration in sterile plastic Petri dishes (9 cm Ø) with their edges sealed with PARAFILM®. The dishes were placed in an incubator (MIR-253, SANYO, Osaka, Japan) for 10 days with a light-dark cycle of 14:10 h and temperature cycle at 25°:12°C. Petri dishes were observed under a stereo-binocular microscope (SMZ-2, NIKON®, Tokyo, Japan) at 24 h intervals during incubation. Six replicates of each species/concentration were used, i.e., 120 (20 seeds/dish, 6 dishes) seeds treated with extract concentration. Due to limited incubator space, two batches were run, i.e., 60 (20 seeds/dish, 3 dishes) seeds of each species in the first batch and 60 of each in the second batch. A seed was considered to have 'germinated' when the radicle tip was visible in a stereo-binocular microscope. Radicle length was measured and recorded on day 8. Radicle length was recorded as 'zero' when seeds did not germinate. Seeds with some sign of germination response were extracted from the Petri dishes only on day 8.

High-performance-liquid chromatography (HPLC)

A SHIMADZU™ system (LC-10AT VP, Kyoto, Japan) was used. Gramine was determined on a WATERS SUNFIRE C18 (5 μm) column equipped with a guard column. The solvent system, consisting of two solvents (A, B) was made up following MUIR et al. (1992): Solvent A – sodium acetate trihydrate diethylamide and Na_2EDTA dissolved in HPLC grade water; Solvent B – acetonitrile and Na_2EDTA with HPLC grade water. The column-flow rate was 1 mL/min. Detection was made at 270 nm. UV spectra measured at 240-300 nm were recorded for each peak.

Preparation of *Phalaris aquatica* extract solution: The extract solution for HPLC analysis was prepared following the procedure described under 'Germination and radicle elongation'.

Solvent preparation: Solvent A. Sodium acetate trihydrate (19 g) (SIGMA 5760-250 g CAS 6131-90-4 St Louis USA), diethylamide (0.5 mol) (FLUKA 121-44-8), and 1 mL of 0.4 mg/mL Na_2EDTA (SIGMA E4884-100 g CAS 6381-92-6) were dissolved in 1 L of HPLC grade water and adjusted to pH 6.2 with glacial acetic acid. Solvent B. Acetonitrile (600 mL) and HPLC grade water (400 mL) containing 1 mL of 0.4 mg/mL Na_2EDTA (SIGMA E4884-100 g CAS 6381-92-6) were measured separately, mixed and filtered with MILLEX sterile syringe driven filter units (0.22 μm). Solvent B was prepared before each run due to possible contamination between runs. Methanol and acetonitrile were of spectroscopic grade. Gramine standard (SIGMA G10806-25g CAS 87-52-5) used to spike plant sample extracts were prepared in MeOH-NH_3OH (99:1).

Determination of gramine: Standard gramine® (SIGMA G10806-25 g CAS 87-52-5, St. Louis, USA) was run through the HPLC at the aqueous extracts 10, 50, and 100 ppm, prepared in MeOH-NH_3OH (99:1), with five replicates of each to confirm the mobile-phase effectiveness and established the retention time of gramine. One concentration of *P. aquatica* aqueous extract (3% solution) was analyzed with HPLC. *Phalaris aquatica* aqueous extract (3% solution) was also augmented with gramine (100 ppm). The gramine peak was determined by comparing results from each tested concentration. Once the retention time of gramine was determined, the aqueous extract of *P. aquatica* was further analyzed to compare retention times. A second analysis was done on *P. aquatica* extract with an added sample containing 100 ppm of standard gramine. No quantitative analysis was carried out on *P. aquatica* extract.

Statistical analyses

In measurements pertaining to germination and radicle elongation, the data were subjected to a two-way ANOVA using GENSTAT Release 10.2 (PAYNE et al., 2007). The two treatment factors being concentration and host, with means separated using lsd. No transformations were carried out on the data, residuals were considered to be normally distributed. Germination percentages were determined by multiplying seeds sown by proportion of seeds germinated. In measurements pertaining to the field study, a two-tail 't' test was used to measure the difference between the two treatments using GENSTAT Release 10.2 (PAYNE et al., 2007).

Results

Abundance and morphometrics of *Medicago trunculata* in field conditions

Abundance of seedlings of *M. trunculata* in transect P differed significantly ($p>0.05$) from that in transect Q ($p>0.05$). The mean of 10 quadrats of transect P was 7 ($n=69$), whereas the mean for transect Q was 17 ($n=166$) (Fig. 2). The mean shoot length of *M. trunculata* in transects P and Q differed significantly ($p>0.05$): in transect P it was 16 mm (n=161), whereas transect Q was 11 mm (n=109) (Fig. 3). The mean root length of *M. trunculata* in transects P and Q did not differ significantly ($p>0.05$): in transect P it was 3.9 mm (n=69), in transect Q it was 3.8 mm (n=166) (Fig. 4).

Laboratory analysis

Germination of *T. subterraneum* treated with *P. aquatica* extract: Concentrations of *P. aquatica* extract (0.25, 0.5, 1.0, 1.5, 2.0, 2.5% extracts) did not inhibit germination of *T. subterraneum* significantly

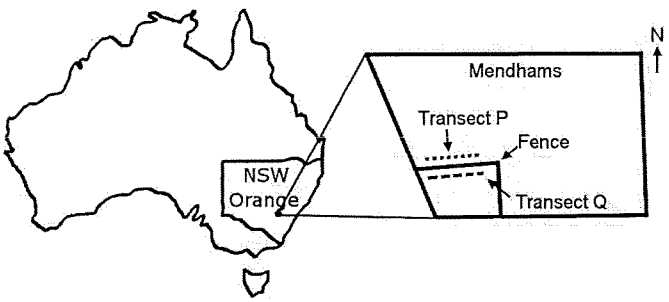

Fig. 1: Transects in *Mendhams* in the context of the state of New South Wales (NSW); transects P and Q run parallel to the fence line (not to scale).

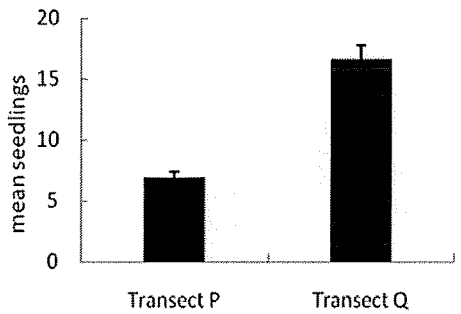

Fig. 2: Mean seedling number of *Medicago trunculata* in transects P and Q (Bars: standard error).

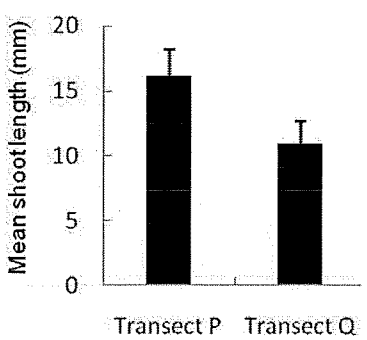

Fig. 3: Mean shoot lengths of *Medicago trunculata* plants in transects P and Q (Bars: standard error).

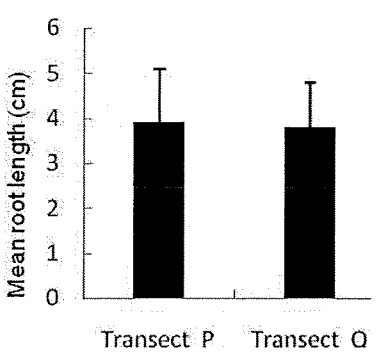

Fig. 4: Mean root lengths of *Medicago trunculata* plants in transects P and Q (Bars: standard error).

(p>0.05). However, the 0.5, 1.0, 1.5, 2.0, 2.5% extracts delayed germination, compared with the control and 0.25% extract on day 2. The rate of germination of seeds delayed on day 2 by 0.5% extract was 40%, 1.0% extract was 38%, 1.5 % extract was 50%, 2.0 % solution was 64%, 2.5% extract was 76%. Extracts 0.25, 0.50 and 1.00 had enhanced germination by 10% on day 8 (Fig. 5).

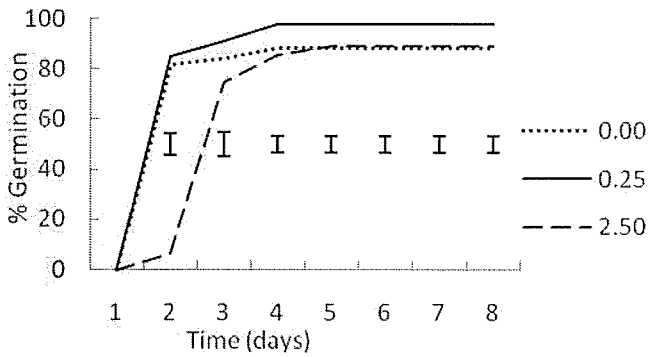

Fig. 5: *P. aquatica* extract on germination of *T. subterraneum* (Bars: standard error).

Germination of *P. aquatica* treated with *P. aquatica* extract: Extracts 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5 of *P. aquatica* did not inhibit germination of *P. aquatica* significantly (p>0.05). However, 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5% extracts had delayed the germination 12% by day 3. *Phalaris aquatica* seeds treated with 0.25% extract enhanced germination by 7% by day 8 (Fig. 6).

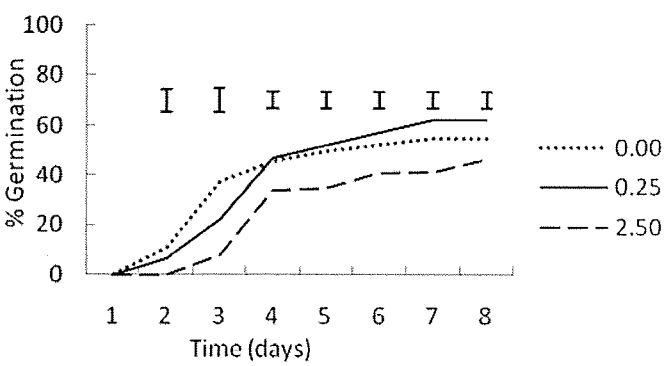

Fig. 6: *P. aquatica* extract on germination of *P. aquatica* (error bars: standard error).

Germination of *C. truncata* treated with *P. aquatica* extract: Extracts of *P. aquatica* (0.25, 0.5, 1.0, 1.5, 2.0, 2.5%) did not inhibit germination of *C. truncata* significantly (p>0.05). However, 1.0, 1.5, 2.0, and 2.5% extracts delayed germination by 5-7% by day 3. Germination of *C. truncata* seeds treated with 0.25 and 0.5% extracts was enhanced by 7% by day 8 (Fig. 7).

Radicle length of *T. subterraneum, P. aquatica* and *C. truncata* treated with *P. aquatica* extract: Radicle lengths of *C. truncata* and *T. subterraneum* were not inhibited by *P. aquatica* extract 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5% extracts significantly (p>0.05). But the radicle length of *P. aquatica* was inhibited by *P. aquatica* 0.25, 0.5, 1.0, 1.5, 2.0 and 2.5% extracts significantly (p>0.05) (Fig. 8).

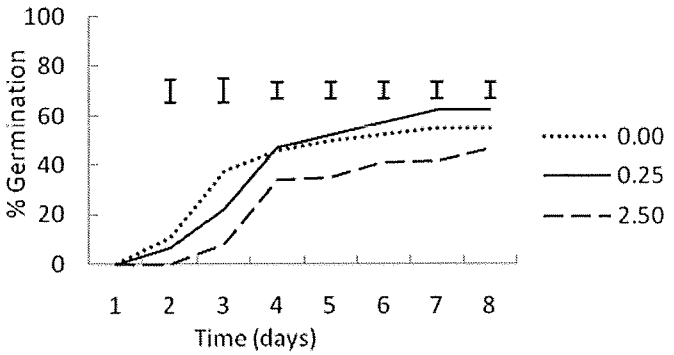

Fig. 7: *P. aquatica* extract on germination of *C. truncata* (error bars: standard error).

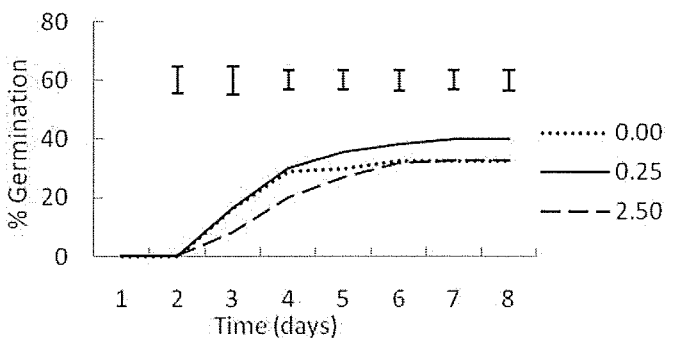

Fig. 8: Effect of *P. aquatica* extract on radicle length of *P. aquatica, T. subterraneum* and *C. truncata*

HPLC analysis of *P. aquatica* aqueous extracts

The HPLC of gramine standard at 10 ppm yielded a retention time at 5.35 min, with 0.2 mAu (Fig. 9). HPLC of *P. aquatica* extract (3% extract) peaked at 5.35 min (Fig. 10), and this was confirmed as gramine after comparing with several runs of standard gramine. Spiked *P. aquatica* extract (3% solution) correlated with the existing gramine retention time and increased from 1.0 mAu to 35 mAu, within the same retention time (5.35 min) (Fig. 11). The unidentified peaks before and after gramine include solvents used in the mobile phase i.e., acetonitrile and sodium acetate trihydrate, along with unidentified compounds from *P. aquatica*.

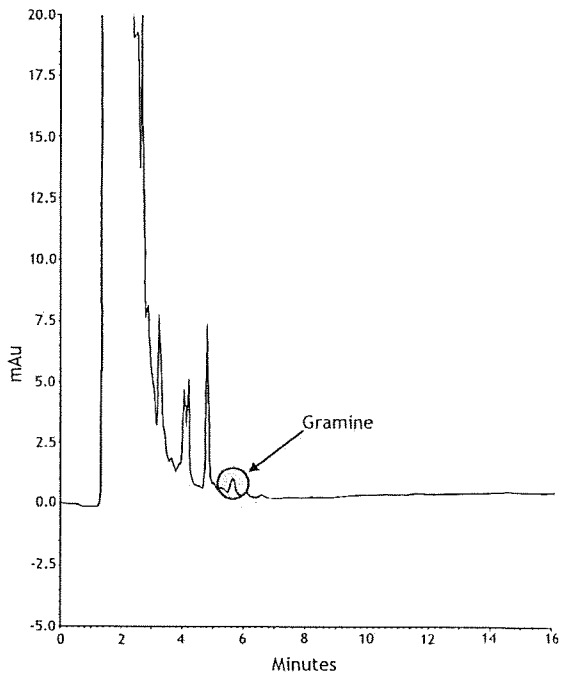

Fig. 10: HPLC of *P. aquatica* aqueous extract (3% solution).

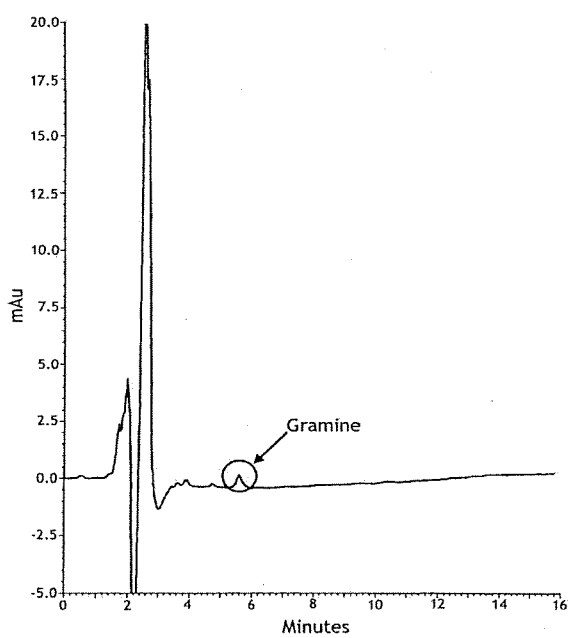

Fig. 9: HPLC of standard gramine (10 ppm).

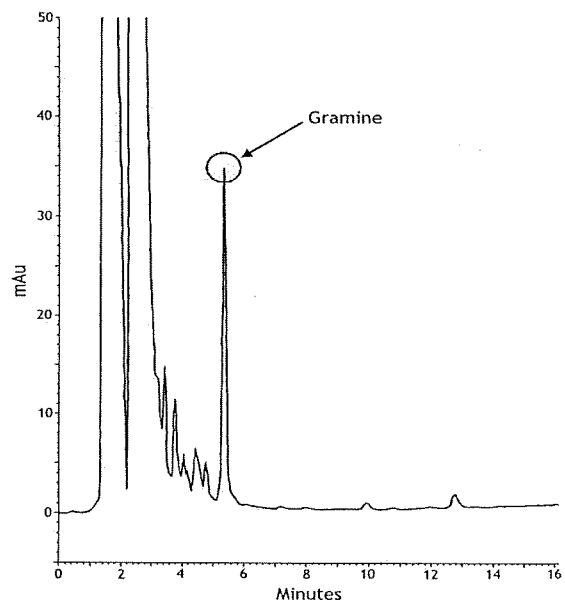

Fig. 11: HPLC of *P. aquatica* aqueous extract (3% solution) spiked with gramine standard (100 ppm).

Discussion

Abundance and growth of *Medicago trunculata* within *Phalaris aquatica* populations

Medicago trunculata seedlings were consistently lower in abundance in transect P (with 2370 kg/ha of *P. aquatica* litter) than that in transect Q (with 700 kg/ha of *P. aquatica* litter) indicating that *P. aquatica* litter impeded the growth of *M. trunculata*. Shoot growth of *M. trunculata* was significantly greater ($p>0.05$) in transect P than in transect Q, although the root growth of *M. trunculata* was not ($p>0.05$) between transects P and Q. These results match those of LEIGH et al. (1995), who found that mulched residues of *P. aquatica* affected rates of germination and life-history performance of legumes, including *M. trunculata*. Nonetheless, drop in abundance of *M. trunculata* in *P. aquatica* pasture need not only be due to the allelopathic effect of *P. aquatica*, because factors such as low seed reserves (DEAR and LOVELAND, 1985), effects of grazing, treading and ingestion of seed pods by domesticated herbivorous animals (CARTER, 1987), soil-structure decline resulting in reduced seed burial (TAYLOR, 1985) and the presence of weeds, diseases and plant residue (PRATLEY, 2001) have a bearing.

Several Poaceae species have been shown to be allelopathic and therefore this attribute is not exclusive to *P. aquatica*. Weedy *Vulpia* (Poaceae) that occurs in high numbers in pasture- and cereal cropland throughout temperate Australia (MCINTYRE and WHALLEY, 1990) impacts on the establishment of a grass-legume pastureland, because its residues are allelopathic (AN et al., 2007); residues of *Vulpia* applied to pot-raised *T. aestivum* reduced the establishment of *Triticum aestivum* L. (Poaceae) seedlings (PRATLEY, 1989) suggesting that the removal of *Vulpia* residues was critical to mitigate allelopathic effects. *Danthonia richardsonii* (Poaceae) has also been linked to legume population decline both in the field and laboratory conditions, because leachates from the residues of *D. richardsonii* have been found to reduce germination and seedling growth of different leguminous taxa (SLATER and CREGAN, 1996).

Bioassay of *Phalaris aquatica* extract on *Chloris truncata*, *Truncata subterraneum* and *Phalaris aquatica*

Extracts of *P. aquatica* (0.25, 0.5, 1.0, 1.5, 2.0, 2.5% extracts) neither inhibited seed germination nor radicle lengths of *C. truncata* and *T. subterraneum* tested *in-vitro* ($p>0.05$). These results differ from those reported by HALSALL et al. (1995) using *T. subterraneum*, who found germination rates in several legumes, including *T. subterraneum*, were reduced by *P. aquatica* extracts. Techniques of extraction used in this study were different to those used by HALSALL et al. (1995), who utilized a rotary evaporator to concentrate the extract; we extracted the *P. aquatica* in distilled water to avoid any heating of the extract in a rotary evaporator which might denature compounds and alter allelopathic potential (QASEM and FOY, 2001).

The 0.25% solution of *P. aquatica* enhanced germination of *T. subterraneum* by 10% by day 8. Although statistically non-significant, the stimulation is worth noting. Several crop species synthesize diverse allelochemicals that are capable of enhancing growth of other plant taxa (MACÍAS et al., 2003). Allelochemicals of *T. aestivum* enhance germination of *Avena sterilis* L. (KIL and LOVETT, 1999). Similarly, leaf extracts of *Helianthus annuus* L., stimulate early stages of growth in *Lactuca sativa* L. seedlings (MACÍAS et al., 2003). The grass *Vulpia* yielded characteristic allelopathic responses in tested species, i.e., at low concentrations allelochemicals stimulated growth of receiver species, but at higher concentrations were inhibitory, similar in action to that of plant-growth promoters (ÖPIK and ROLFE, 2005).

Extracts of *P. aquatica* (0.25, 0.5, 1.0, 1.5, 2.0, 2.5% extracts) did not inhibit *in-vitro* germination of *P. aquatica* at any significant level ($p>0.05$); while radicle length of *P. aquatica* was inhibited by extracts of *P. aquatica* (0.25, 0.5, 1.0, 1.5, 2.0, 2.5%) significantly ($p>0.05$) suggesting that *P. aquatica* is autotoxic. Aqueous extracts of *T. aestivum* inhibited the germination of its seeds by 21%, radicle growth by 30%, and coleoptile growth by 20%, when tested against *T. aestivum* (WU et al., 2007). In the present study, the *P. aquatica* extracts utilized were extracts either at or below 2.5% concentrations, whereas WU et al. (2007) applied aqueous extracts of *T. aestivum* as 10% extracts on to *T. aestivum* seeds and observed significant ($p>0.05$) inhibition of germination and radicle and coleoptile growth. Had *P. aquatica* leaf extract at 10% solution been applied to *P. aquatica* seeds in the present study, then that rate may have inhibited germination. Aqueous extracts of *P. aquatica* were applied to *P. aquatica* seeds raised in Petri dishes: the seed and emerging radicle were in direct contact with the extract. However, in a 'natural' pasture setting, *P. aquatica* seedlings may germinate at up to 30 cm from the parent plant and from the leachate of *P. aquatica* litter. Distance from the allelopathic compounds emanating directly from the parent plant is an important factor, as shown in the *Kalanchoë* (Crassulaceae) study (BÄR et al., 2000). *Kalanchoë* is a demonstrated autotoxic species; similar-aged daughter plants placed close to the parent plant, but separated by regular distances, suffered inhibitory effects showing reduced growth, whereas those growing farther away from the mother plant showed few signs of growth inhibition.

Gramine, an indole alkaloid in *Phalaris arundinacea* L. and *P. aquatica* (ZHOU et al., 2006), is amenable to masking by cyanogenic glycosides, which usually accumulate in mature *P. aquatica* leaves, especially during re-growth (JANZEN, 1978). This presupposes that *P. aquatica* could either manifest or suppress autotoxicity at different annual seasons because of the unmasking and masking effects of cyanogenic glycosides. *Phalaris aquatica* has a better long-term persistence capability than several other temperate perennial grasses (e.g. *Dactylis glomerata* L., *Festuca arundinacea* (SCHREB.), *Lolium perenne*) (WATSON et al., 2000). However a decline, in terms of both tiller density and clump density, occurred in *P. aquatica* pastures in a 3-year trial made under four different grazing practices (CULLEN et al., 2005), which indicates that mature *P. aquatica* plants may succumb to the effects of either autotoxicity or nutrient depletion; nonetheless, further *in-vitro* and *in-vivo* studies are necessary to confirm that *P. aquatica* is autotoxic.

HPLC analysis of *Phalaris aquatica* aqueous extracts

HPLC analyses of *P. aquatica* aqueous extract separated into distinct peaks, with retention times matching precisely with standard gramine; in the trials, aqueous extract of *P. aquatica* was spiked with the standard gramine to confirm gramine's occurrence in the extract. Although no measurement of the volume of gramine in the extract could be made, an approximate 10 ppm value could be arrived at from the 3% *P. aquatica* aqueous solution. The retention time for gramine found in the present study compares favorably with values of gramine in *Hordeum vulgare* (Poaceae) (HOULT and LOVETT, 1993). Investigating aqueous extracts of *P. aquatica*, HALSALL et al. (1995) indicated that *P. aquatica* is allelopathic, but no compound was isolated in that study. In the present study, gramine – a compound confirmed as the source for allelopathy in *Hordeum vulgare* (LIU and LOVETT, 1993) – has been isolated from leaves of *P. aquatica*. Although the isolation of gramine from *P. aquatica* agrees with the earlier indication of the occurrence of the same compound from *Phalaris aquatica* (HALSALL et al., 1995) and from *Hordeum vulgare* (LIU and LOVETT, 1993), a likely combined role of several other allelocompounds (e.g., cyanogenic glycosides, hordenine, bufotenine and monomethylated tryptamines, dimethylated tryptamines, and tyramines) cannot be overlooked. Gramine concentration, acting

singly, should be below its inhibitory threshold, because allelopathic interferences often result from the combined effect of several different compounds (AN et al., 1998).

Conclusion

In-vitro bioassays of *P. aquatica* extracts at and below 2.5% concentrations did not inhibit the seed germination in *C. truncata*, *T. subterraneum*, and *P. aquatica*. *In-vitro* bioassays of *P. aquatica* extracts at and below 2.5% concentrations did not inhibit the lengthening of the radicles in *C. truncata* and *T. subterraneum*, but those of *P. aquatica* were reduced significantly. The reduction in radicle lengths ($p<0.05$) provides evidence that *P. aquatica* is autotoxic. Because autotoxicity of *P. aquatica* has not been reported so far, further *in-vitro* and *in-vivo* investigations are warranted to confirm these results.

To date, very few studies have attempted to determine the allelochemicals produced in *P. aquatica*, thus endowing a capability for allelopathy. The presence of gramine, a known allelopathic compound of *Hordeum vulgare* was characterized from aqueous extract of *P. aquatica*, using HPLC. Extracts of *P. aquatica* in low concentrations did not exhibit allelopathy, when trialed against the nominated plant taxa, although autotoxicity was evident.

Acknowledgements

We thank BRUCE AULD and IGOR NOVAK (CSU-O), JIM PRATLEY (CSU, Wagga Wagga, NSW, Australia) for reading the draft manuscript and offering useful comments, RICHARD CULVENOR, REX ORAM, JOHN LEIGH (CSIRO, Canberra, Australia) for advising on the taxonomy of *Phalaris* and its distribution in Australia. One of us (A.A.A.) thanks the University of Sydney for financial support. This study was partly supported with financial assistance from the LITTLE RIVER LANDCARE GROUP (Molong sub-catchment, NSW, Australia), for which we thank the manager FERGUS JOB.

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Address of the authors:
Allan A. Adams, University of Sydney, PO Box 883, Orange, NSW 2800, Australia
Dr. Anantanarayanan Raman, Charles Sturt University, PO Box 883, Orange, NSW 2800, Australia. E-mail: araman@csu.edu.au.
Helen Nicol, Charles Sturt University, PO Box 883, Orange, NSW 2800, Australia.