Research and development for integrated control of *Acetosa vesicaria* (ruby dock) in the Pilbara region of Western Australia.

Janet M. Anthony and I. R. (Bob) Dixon

In partnership with

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Executive summary

This report summarises and presents the major findings from the *Acetosa vesicaria* (ruby dock) integrated control project. The Department of Environment and Conservation (DEC; formerly CALM) initiated the project in 2003 in conjunction with Robe River Mining, BHP Billiton Iron Ore and Pilbara Iron. The principal sponsor for the project was Robe River Mining.

Research conducted by BGPA focused on the following core areas to assist the establishment of a management strategy for control of the environmental weed *Acetosa vesicaria* otherwise known as ruby dock.

1. Seed studies to understand key areas associated with germination, dormancy, longevity and soil seed banks in an effort to diminish and manage this weed.
2. Herbicide trials to determine the most effective herbicide/s, surfactant and application rates required to control *A. vesicaria*.

The major findings are:

- *A. vesicaria* can flower and set seed at the two leaf stage when drought stressed.
- The seed have two colour morphs, a larger pale seed and a smaller darker seed; ultimately there is no difference in germination percentage between the two types of seed.
- There is no physical dormancy to prevent germination, however there is a short period of 12 weeks after ripening following seed fall until the seed reach full germination capacity.
- There is a chemical inhibitor in the tissues of the perianth preventing precocious germination until adequate rainfall leaches the inhibitor from the perianth.
- Seeds germinate in higher numbers with alternating temperatures of 5^{o}C and 18^{o}C relating to winter conditions which are more conducive to growth. However, these seeds will germinate and grow into large fruiting plants during the summer months after cyclonic rains.
- The seeds germinate in higher numbers with a period of light.
- Relative ageing has shown that *A. vesicaria* seed viability is average for geosporous species.

- Seeds remain in the perianth and form dense mats on the ground that slowly breaks down over time to fibrous husks.
- Seeds respond with increased germination to the stimulant gibberellic acid.
- Butenolide is more effective as a germination stimulant when sprayed on seed contained within the perianth on the soil surface.
- The seed contained within the perianth are principally dispersed by wind, water and human activity.
- After testing six herbicides only glyphosate was found to be effective for controlling *A. vesicaria*.
- The efficacy of glyphosate is increased with the addition of the surfactant Pulse®.
- Glyphosate can be used when the plant appears water stressed and be equally effective.
- Glyphosate was very effective in reducing seed set when applied to flowering plants.
- Glyphosate has a detrimental effect on many native species.

These findings suggest that:

- *Acetosa vesicaria* is a disturbance opportunist and can spread quite rapidly, so control of this weed using a unified approach with all environmental and land management departments is vital.
- It is possible to manage this weed using glyphosate and the surfactant Pulse® particularly if applied before flowering and applications are repeated after each germination event (after rain).
- There is the potential to further reduce the seed bank using the germination stimulant Butenolide but further research is required after the development phase of this novel chemical is finalised.

Recommendations include:

- Where possible apply glyphosate while plants are small and before they begin to flower.
- When isolated plants are found remove them by hand and destroy any seed.
- Follow good phyto-sanitary practices by washing vehicles and equipment particularly before entering a new site.

- Make sure that everyone is aware of this weed and where to report new infestations.
- Rates for glyphosate applications are:
    - For small plants less than 15 cm high 3 ℓ/ha glyphosate 360 plus 2 ml/ℓ Pulse®. Alternatively; for the dry formulation with a concentration of 875 g/kg, 1 g/ℓ glyphosate plus 2 ml/ℓ Pulse®; or 0.04 ml/ℓ Roundup 360® plus 2 ml/ℓ Pulse®. One litre of these mixtures will cover 8m^2.
    - For larger flowering plants 6 ℓ/ha glyphosate 360 plus 2 ml/ℓ Pulse®. Alternatively; for the dry formulation with a concentration of 875 g/kg, 2 g/ℓ glyphosate plus 2 ml/ℓ Pulse®; or 0.08 ml/ℓ Roundup 360® plus 2 ml/ℓ Pulse®. One litre of these mixtures will cover 8m^2.

- Further research be undertaken to understand the impact of fire on *A. vesicaria*.
- And when available, carry out further research on the germination stimulant Butenolide.

Acknowledgements

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1.0 Introduction

Acetosa vesicaria L. formerly known as *Rumex vesicarius* is an environmental weed invading large areas of arid Australia and may prove to me the most invasive species of introduced *Rumex* (Moore and Scott 1987). Generally, weeds have the capacity to alter the balance of the native ecosystem, as they are very adaptable, produce copious quantities of seed and out-compete the native flora resulting in high environmental and social costs. The Botanic Gardens and Parks Authority (BGPA) was commissioned by The Department of Environment and Conservation (DEC) to undertake research into the control and management of *A. vesicaria*, one of the most serious environmental weeds in the Pilbara region of Western Australia. This research was conducted over a three year period.

Initially Robe River Mining generously supported the project through the auspices of the Coondewanna West, West Angelas Rail Project environmental offsets package. Supplementary funding was also obtained from BHP Billiton Iron Ore and Pilbara Iron.

Acetosa vesicaria also known as native hops, ruby dock, rosy dock, bladder dock or ambat chuka is a saharo-sindian species that is indigenous and widespread throughout desert and semidesert areas of North Africa, southwest Asia, southern Iran, Afghanistan and Pakistan. *Acetosa vesicaria* is a member of Polygonaceae (dock family) that comprise of some 1000 herbs, shrubs, climbers and a few trees. Typically for a member of Polygonaceae the base of each leaf forms a distinctive sheath (ochrea) round the stem. Flowers are small, radially symmetric and white, greenish or yellowish, but when crowded together in racemes may be showy and are most commonly bisexual. The fruit is most often a tetrahedral achene or nut with abundant endosperm and are usually enclosed in the persistent perianth (McAlister 1983). World wide there are *ca* 800 species in 45 genera and in Western Australia there are 22 native and 19 naturalised species (McAlister 1983; Watson and Dallwitz 1992 onwards; Hussey *et al.* 1997). Most genera inhabit the temperate northern hemisphere regions with a few tropical and subtropical species. Many species are mentioned as ornamentals in European garden catalogues, and unfortunately *A. vesicaria* has been cultivated as a garden ornamental in Western Australia.

There are two introduced *Acetosa* sp., *A. sagittata* (also known as *R. sagittatus* and *A. vesicaria* in Western Australia. The genus *Acetosa* is not accepted by the Integrated Taxonomic Information System (ITIS, 2005) and currently accepts the genus *Rumex,* but *Acetosa vesicaria* is the accepted form used by the Western Australian herbarium. However, it is generally accepted that *Acetosa* = *Rumex.* The genus *Rumex* is a worldwide genus of about 160 species. In Australia the genus consists of eight indigenous and nine introduced species (Rechinger 1984). Nine species of *Rumex* have been recorded as introduced into Western Australia and all introduced species except *A. vesicaria* are restricted to the south west of the state (Moore and Scott 1987), with most of the indigenous Australian species centred in the southeast of the continent (Rechinger 1984). *Rumex* species are annuals or perennials with stout taproots, opposite, entire leaves, small green or reddish flowers in racemes or panicles. The fruit is the most distinguishing feature; with a triangular nut enclosed in 3 bracts (valves) that may have wart like swellings called tubercles (Hussey *et al.* 1997).

Figure 1. Two colour forms of *Acetosa vesicaria* identified in the Pilbara region of Western Australia.

Acetosa vesicaria is a 0.1-0.8 m tall, much branched stout fleshy annual herb, with broad to very broadly triangular leaves (Fig. 1) and large showy light brown, pale

pink, purplish or red perianths (fruiting valves), that are thin, inflated and finely reticulate (Fig. 2A). The fruiting valves enclose the inconspicuous wind pollinated flowers (Fig. 2B) (Rechinger 1984). It has been suggested that there are 2 seed types (light usually 1 and dark usually 1-5 per perianth). The dark seeds vary in colouration: brown, greyish brown and black usually the smaller of the two types and are in the periphery of the fruiting valves, with the light seeds in the centre of the fruiting valves (Schatral and Osborne 2002). When grown in its native habitat it is a winter annual with a short growth, flowering and fruiting period (Sayed 1998). However, in the Pilbara region of Western Australia *A. vesicaria* can germinate with the summer rains, and providing the temperatures are not too high, can grow and set seed during this period (pers. com. van Leeuwen).

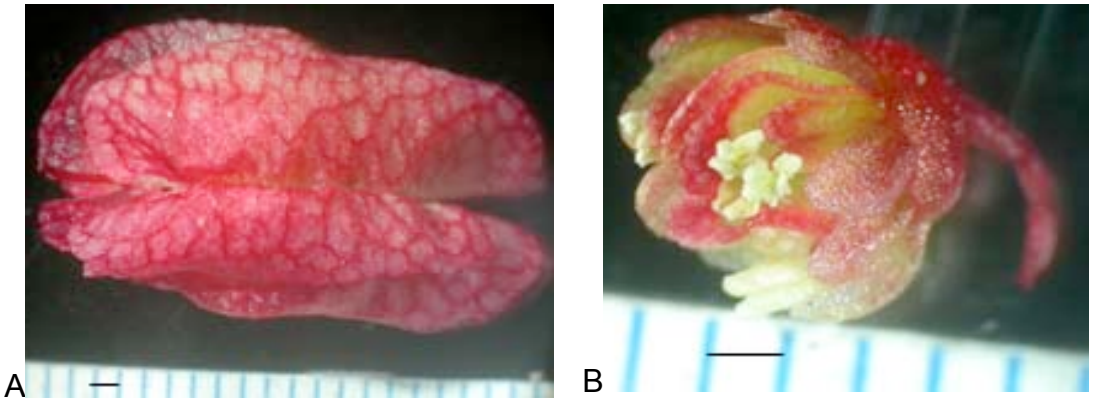

Figure 2. *Acetosa vesicaria* A. Fruiting perianth and B. Flowers enclosed within the perianth with the external valves removed. Bar represents 1 mm.

Because of its striking nature *A. vesicaria* attracts public attention by its large, often bright red purplish tinged valves and pale green, somewhat succulent leaves. The history of this species introduction to Australia is poor and it is speculated that Afghan camel drivers introduced it to Australia during the latter half of 1800's (Rechinger 1984) conversely according to Wilson (1996) there is no evidence of this. The Australian National list of naturalised and potentially invasive garden plants (2004) lists *A. vesicaria* as a significant environment weed but is only recorded as being naturalised in the Northern Territory. There are records of this weed being present in most States of Australia and it certainly covers large areas of Western Australia (Fig. 3). Western Australian Herbarium, Flora Base (1998 onward) has 57 records within the State, with the earliest recorded in the Perth region in 1892.

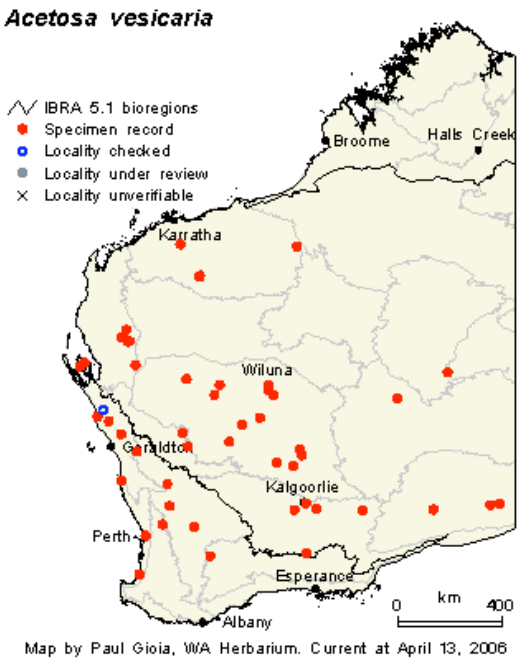

Figure 3. Distribution of *Acetosa vesicaria* in Western Australia (Western Australian Herbarium, Flora Base 1998 onward).

Acetosa vesicaria is common along roadsides and disturbed areas in the arid zone from the Pilbara to the Nullabor and is often mistaken for a native (Moore and Scott 1987). In the Pilbara this species flourishes in response to anthropomorphic ground disturbance and therefore is abundant at highly disturbed sites associated with mining operations (Fig. 4), mineral exploration activities and road maintenance. However, this environmental weed is not confined to areas of anthropomorphic disturbance and often invades native bushland in areas subject to natural disturbance regimes such as along creek lines and on rock scree slopes (Fig. 5).

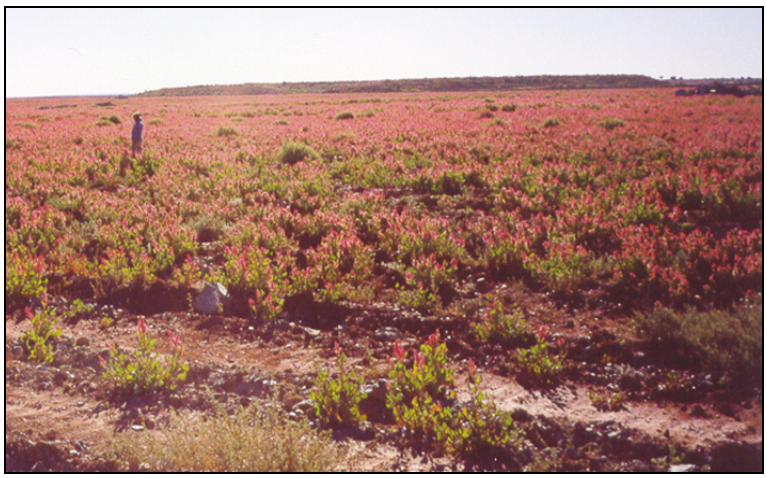

Figure 4. *Acetosa vesicaria* invading a mining revegetation site at Plutonic Mine. Photo: M. Hansen, July 2004.

Figure 5. *Acetosa vesicaria* seedlings growing on rocky scree.

A. vesicaria plants have been reported to be high in oxalate, nitrate and tannins (Auld and Medd 1987; Salama 1996), making this plant largely unpalatable to herbivores.

Various methods of weed control have been reported and generally the most common method of control is the application of herbicides. Biological control, the introduction of a natural predator or a disease that will destroy the weed without affecting non-target plants, has been used for control of *Emex australis* and *E. spinosa* (Polygonaceae) using a weevil (*Apion minatum*) from Europe and West Asia (Scott and Yeoh 2005) though biological control is costly and time consuming. These researchers found that the host range of this weevil was restricted to *Emex* species and some *Rumex* species, but the eggs laid on *A. vesicaria* did not hatch and when larvae were placed on the plant they did not prevent the plants producing seed prior to senescence.

Another weed control method is competition or suppression, creating a situation where required plants either native or cultivated are encouraged to grow and out compete the weeds. Keary and Hatcher (2003) used a native grass, *Lolium perenne,* to control *R. obtusifolius*. The impact of *E. australis* in crop and pasture rotations could be lessened by sowing mixtures of grass and legume seed into first year pastures as *E. australis* is a weak competitor and seed production is reduced in the presence of other pasture species (Panetta and Randall 1990). Schatral and Osbourne (1999 and 2002) found that chenopods do not compete or inhibit the

growth of *A. vesicaria* in pot trials. They suggested that when native vegetation is established *A. vesicaria* seedlings may have difficulties surviving.

Fire can also be used as a management tool and is generally dependent on the intensity and duration of the burn. Downey and Smith (2000) reported on the management of *Cytisus scoparius* with fire acting as a germination stimulant and as a result massively depleting the seed bank.

2.0 Research Aims

Knowledge of weed biology is essential for implementing weed management systems and relates to attributes such as morphology, seed dormancy and germination, physiology of growth, competitive ability and reproductive biology (Bhowmilk 1997). Cacho *et al.* (2006) produced a mathematical model to evaluate the feasibility of eradicating a hypothetical weed, and established that the greatest influence on the duration of the eradication effort are search speed, kill efficiency, germination rate and seed longevity.

The principal aim of our research was to produce a management plan for the control of *Acetosa vesicaria.* To undertake research to produce a decisive management plan this research was divided into two sections:

1. Seed studies with the emphasis placed on the depletion of *A. vesicaria* seed bank.
2. Herbicide control primarily to discover the most effective herbicide with the least “off-target” damage.

3.0 Seed Studies

Understanding the relationship between seeds, soil seed bank dynamics and establishment requires an understanding of factors that control germination, therefore linking field and laboratory studies is crucial. Estimates of seed bank depletion rates are essential for modelling and management of plant populations. The depletion of seed reserves in the soil is dependent upon the rates of germination, longevity and predation under any particular set of environmental conditions.

Environmental factors during seed imbibition and germination, including abiotic factors such as (soil water, temperature, light), stress factors such as (soil salinity), as well as seed factors such as germination inhibitors and presence of dormancy mechanisms, regulate seed germination behaviour (final percentage and rate). Understanding the interaction with environmental cues of *A. vesicaria* seeds will assist in understanding seed bank dynamics and identify potential control points allowing for more rapid depletion of the soil seed bank.

Seed viability and imbibition

The viability of a seed may be measured as the proportion of a given sample that has the potential to germinate (Bewley and Black 1994) and determination of seed viability is often achieved by the 'cut test'. Seed containing 'healthy' (turgid, intact, non-discoloured) endosperm and embryo are considered viable (Dixon and Meney 1994; Roche *et al.* 1997a). Measures of viability provide information with respect to persistence and survival of weed seeds (Kumar and Dhiman 2005).

The initial phase of germination is imbibition and is a purely physical process where by water moves from a high to a low potential. Water uptake is limited by the permeability of the seed coat; therefore a viable seed with an impermeable testa will not imbibe water (Bewley and Black 1978; Hartman *et al.* 1997).

Seed germination

Standard methods of assessing germinability of seed batches include seed germination on filter papers and sowing of seeds in soil (Bellairs and Bell 1993) and can be undertaken using various temperature and light regimes.

Seed colour and size polymorphism

Baskin and Baskin (2003) have reported that colour polymorphy may indicate varying levels of physical dormancy. Seed morphs displaying different dormancy levels and germination periods increase a species survival in harsh conditions (Ungar 1995). As *A. vesicaria* has previously been identified as having distinct colour polymorphism (Short 1999) it is thought that this may result in different dormancy states.

Seed size is a key determinant of the success of plant propagules (Murray *et al.* 2003) and variation in size has been documented among and within species (Geritz 1998; Jones and Nielson 1999; Murray *et al.* 2003). Larger seeds with greater reserves tend to produce larger and more vigorous seedlings than smaller seeds however larger seeds require greater plant resources that may be restricted in stressful environments. It has been shown that within species seed size variance is associated with a variety of fitness related traits such as the probability and timing of germination (Simons and Johnston 2000). Seed size has no effect on percentage germination or subsequent seedling growth of *R. obtusifolius,* however, germination of the smaller seeds of *R. crispus* is reduced (Cideciyan and Malloch 1982).

Seed dormancy

Seed germination constitutes one of the most important events in the life cycle of plants. In order to ensure survival seeds have dormancy mechanisms. A seed is said to be dormant if it fails to germinate even though it is viable and the conditions are favourable. Knowledge on dormancy provides information on the phases of bioactivity in the seed and facilitates decisions on the most suitable time, method and duration of weed control methods. Dormancy levels follow a seasonal pattern for *R. obtusifolius* (Van Assche and Van Lerberghe 1989). Conversely, mature seeds of *Rumex crispus* are not dormant during any season (Baskin and Baskin 1985) despite previous assertions by Cavers and Harper (1964) who reported that the seed lay dormant in the soil for long periods of time.

A number of studies have investigated germination stimulants responsible for alleviating dormancy such as; smoke (Roche *et al.* 1997b; Enright and Kintrup 2001; Pérez-Fernández and Rodríguez-Echieverría 2003); gibberellic acid (Koornneef *et al.* 1990; Seiler 1998; Yamaguchi and Kamiya 2002; Merritt *et al.* 2006); heat (Velempini

et al. 2003; Mwang'Ingo *et al.* 2004); stratification (Rogis *et al.* 2003; Mwang'Ingo *et al.* 2004) scarification (Albert *et al.* 2002; Gebre and Karam 2004); and more recently 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, a butenolide, the active ingredient present in plant-derived smoke (Flematti *et al.* 2004 and 2005; Merritt *et al.* 2006).

Seed longevity

Seeds deteriorate and lose their ability to germinate after prolonged *ex situ* or *in situ* storage (Walters 1998). The ageing processes (loss of viability) are generally controlled by interactions of temperature, moisture and chemical reactions (Pukacka and Ratajczak 2005). *Rumex* species have previously been reported to have long lived seed (such as *R. crispus*, Lewis 1973; Weaver and Cavers 1979 and *R. obtusifolius*, Van Assche and Van Lerberghe 1989), however this is a species specific trait.

One method of determining seed longevity is to carry out seed burial at different depths, over a long period of time, removing seed at regular intervals to assess the germination rate (Lewis 1973; Egley and Chandler 1978; Hill and Vander Kloet 2005).

A collaborative project between the Seed Conservation Department of Royal Botanic Gardens Kew and Nottingham Arabidopsis Stock Centre (NASC) initiated in 1997 aimed to fit an established model used for predicting seed longevity under any constant storage environment to two ecotypes of *Arabidopsis thaliana*. Currently this model is being applied and compared to other species of plants and this will enable assessment of seed longevity to be undertaken over a considerably shorter period than the seed burial technique (Millennium Seed Bank Project 2006).

Inhibition of germination and seedling growth

Plant phenolics have been implicated in the regulation of seed germination and/or seedling growth (Djurdjevic *et al.* 2004; Garnett *et al.* 2004; Kim *et al.* 2005a and 2005b; Rachid *et al.* 2005). *Acetosa vesicaria* plants have been reported to be high in phenolic compounds (Auld and Medd 1987) with leachate from bracts has been implicated in the inhibition of germination (such as for *Atriplex griffithii*, Ungar and Khan 2001; *Triticum taushii,* Gatford *et al.* 2002).

Soil seed bank studies

Soil seed banks play an important role in plant populations and their dynamics have been studied and modelled to predict emergence and population development especially for weeds (Grundy *et al.* 1999 and 2003; Buckley *et al.* 2004). Soil seed bank refers to all viable seeds and fruits present in or on the soil (Wassie and Teketay 2006). This will provide a predictive science and allow an understanding of the conditions promoting seed bank storage and the likelihood of regeneration of plant populations via seed after major disturbances (Hill and Vander Kloet 2005). The use of soil sampling to estimate the numbers weed seed in the soil is labour intensive regardless of the techniques used and the limiting factor is ultimately the total volume of soil that can be sampled and processed (Benoit *et al.* 1989).

3.1 Materials and Methods

All germination trials were carried out using 8 ml of treatment solution on 84 mm industrial filter paper in 90 mm plastic petri dishes and placed in a 5^0C 12 h dark: 18^0C 12 h light incubator for germination unless otherwise stated. All germination trials were replicated five times with twenty seed per replicate and germination was recorded every 2-3 days for a period of 14 days unless otherwise stated.

3.1.1 Seed numbers and size

Fruiting perianths were collected from the soil surface at two field sites; Newman Light Industrial Area (LIA) and Plutonic Mine waste dump in March 2003. Seed were extracted from within the fruiting perianth, using 100 randomly selected perianths from each site and the seed number/perianth was recorded. Seed were also extracted from the perianths of pot grown plants and number of light and dark seed recorded from each fruit. A random sample of 200 seed was divided into two colour cohorts (light and dark) and seed length was recorded.

3.1.2 Seed viability and imbibition

Seed was collected from various field sites: Newman LIA (seed age unknown); West Angelas Mine Turee B Borefields (seed age unknown) in March 2003; Plutonic mine waste dump (seed age unknown and fresh seed) in May 2003; Main roads quarry 90 km from Newman (fresh seed) August 2003. Fresh seed was also collected from pot grown plants (grown at BGPA under irrigation during October-December 2003). Viability for each collection sample was estimated using a cut test on a random sample of 100 seeds; viability was determined by the presence of a healthy white embryo (Fig. 6) and endosperm.

Seed was divided into five lots of 100 seed, weighed and placed into labelled petri dishes containing filter paper moistened with 8 ml distilled water. Seed weight was measured at 0, 0.5, 30, 60, 120, 240, 480 and 1440 minutes to determine imbibition rates with each seed lot being removed and dried with tissue paper prior to weighing (method pers. com. S. Turner; BGPA).

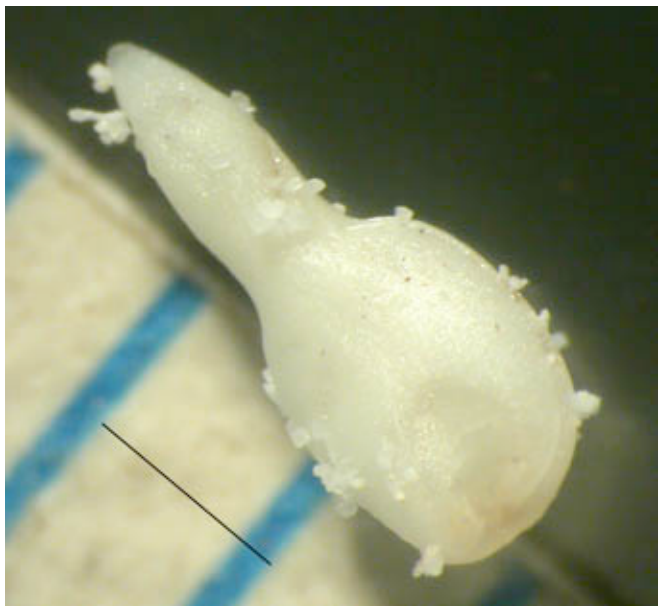

Figure 6. *Acetosa vesicaria* spatulate embryo. Bar =1 mm. (Classification based on Baskin and Baskin 2003).

3.1.3 Temperature and light

Mature *A. vesicaria* fruit were collected from two field sites (Karijini National Park and Newman LIA), labelled with collection site and date and stored at room temperature (*ca.* 20^{o}C) in calico bags for three months. Seed was removed from the fruiting valves and exposed to different germination treatments (Table 1) using only mature, undamaged, firm seed. Seed were considered germinated with emergence of a radicle and germination numbers were scored every 2-3 days for three weeks.

Table 1. Experimental treatments for temperature and light germination trials.

Treatment name	Treatment
5:18 light	5^{0}C 12 h dark: 18^{0}C 12 h light
5:18 dark	5^{0}C 12 h dark: 18^{0}C 12 h dark
13:26 light	13^{0}C 12 h dark: 26^{0}C 12 h light
13:26 dark	13^{0}C 12 h dark: 26^{0}C 12 h dark
18:33 light	18^{0}C 12 h dark: 33^{0}C 12 h light
18:33 dark	18^{0}C 12 h dark: 33^{0}C 12 h dark

Using results of the initial temperature and light experiment (Fig. 13) as a guide, a further experiment was designed to test alternating temperature against constant temperature (Table 2).

Table 2. Experimental treatments for alternating temperature trial

Treatment name	Treatment
5 light	5°C, 12 h dark, 12 h light
5 dark	5°C, 24 h dark
18 light	18°C, 12 h dark, 12 h light
18 dark	18°C, 24 h dark
5:18 light	5°C 12 h dark: 18°C 12 h light
5:18 dark	5°C 12 h dark: 18°C 12 h dark

3.1.4 Seed dormancy

Mature fruit were collected and stored at room temperature (*ca.* 20°C) in paper bags from pot grown *A. vesicaria* plants, grown outside in the BGPA nursery during October-December 2003 and irrigated twice daily. These seed were used to examine after ripening dormancy and seed polymorphy. Seed was extracted from the fruiting valves 0, 2, 4, 8, 12 and 16 weeks after collection. Only mature, undamaged, firm seed were used in germination trials. Seed were exposed to different germination treatments (Table 3) and within each treatment seed were divided into two colour morphs (light and dark).

Table 3: Experimental treatments used in seed dormancy trials

Treatment name	Treatment
Control	Sterile distilled water (SDW)
SW	1:10 smoke water*:SDW
Butenolide	100ppb butenolide**
GA_3	1000ppm gibberellin

*Smoke water was derived by combustion of straw in a metal drum and the smoke generated by the combustion process was bubbled through distilled water for one hour to produce a smoke saturated solution.

**Butenolide is under a development licence held by the University of Western Australia (UWA), Murdoch University and BGPA and is unavailable for commercial use at this time.

Mature fruit collected from pot grown plants were used to determine the effectiveness of the germination stimulants (Table 3) on seed remaining in the perianth. Each treatment consisted of a petri dish containing 50 fruit on industrial filter paper moistened with 15 ml of treatment solution, with five replications. After 14 days seed were extracted from the fruit and the number of germinants, non-viable seed and viable seed recorded.

Mature fruit collected from pot grown plants were used to determine the effectiveness of the germination stimulants (Table 4) on seed remaining in the perianth and sown on the soil surface of seedling trays (340 mm x 280 mm). Each treatment was

replicated three times with 100 fruit per tray. Treatment dilutions (Table 4) were adjusted appropriately to apply 50 ml of solution to each tray using a hand spray to ensure an even application. After 14 days seed were extracted from the fruit and the number of germinants, non-viable seed and viable seed recorded to determine the percentage of seed germinating.

Table 4. Experimental treatments used in seed dormancy trials

Treatment name	Treatment
Control	Distilled water (DW)
Butenolide 1 g/ha	1 g/ha butenolide
Butenolide 10 g/ha	10 g/ha butenolide
Gibberellin	1 g/ha gibberellin
SW 10	10 ml/ m^2 smoke water
SW 50	50 ml/ m^2 smoke water
SW100	100 ml/ m^2 smoke water

3.1.5 Seed relative ageing

Seeds were divided into 11 x 50 seeds for germination testing, seeds were rehydrated at 47% relative humidity (RH) at 20^{o}C until equilibrium was reached. The seed lots were then transferred to the ageing environment of 60% RH at 45^{o}C (Millennium Seed Bank Project 2006). Samples (1 x 50 seeds) were removed 0, 1, 2, 5, 10, 20, 30, 50, 75, 100 and 125 days after being placed in the ageing environment. Seed were sown as previously described, placed in the incubator and germination percentage was recorded after ten days.

3.1.6 Germination inhibitor

Mature fruiting perianths collected from pot grown plants were used to establish the possibility of a chemical inhibitor within the valves. All seed were removed and the perianth valves were soaked in distilled water. The difference between wet and dry weights of 10 individual perianths was averaged to determine the amount of water required to soak the valves at field capacity. Two hundred fruit were soaked for one hour and the fruit pressed to remove all the fluid (leachate solution). The experiment was repeated using inhibitor solution from fruit soaked for 24 hours. The leachate was applied to seed in petri dishes at 0, 25%, 50% and 100% concentrations and placed in the germination incubator. Both germination numbers and seedling length were recorded.

3.1.7 Seed bank studies

Thirty replicates of random soil samples were collected from one site (Karijini National Park) for soil seed bank analysis. At each sampling point, a 25x25 cm soil core was removed at two depths, 0-5 cm (including leaf litter) and 5-10 cm to determine the depth in which the majority of seed occur. Soil was transported to the laboratory at Kings Park and sieved to remove all large debris such as rocks and sticks. All perianths from the current season and previous seasons were placed in paper bags. The remaining soil was sieved through decreasing mesh sizes and any loose *A. vesicaria* seed were removed with the aid of a magnifying glass. Data recorded from soil samples at each depth included:

- Number of current season perianths (fresh), number of seed both viable and nonviable from these perianths.
- Number of past season perianths (old), number of seed both viable and nonviable from these perianths.
- Number of loose seed, including viability.

Seed from each data set were placed in individual petri dishes and positioned in the germination incubator to determine the percentage germinable.

3.1.8 Statistical Analysis

Final germination percentage and seedling length data were statistically analysed by analysis of variance (ANOVA), using Minitab® statistical software package. Germination percentages were arcsine-transformed prior to analysis (untransformed data appears in all tables and figures). Fisher's least significant difference ($P<0.05$) was used to determine significant differences between treatments.

3.2 Results

3.2.1 Seed number and size

The number of seed per fruiting perianth was significantly greater (P<0.001) from the pot-grown plants than the two field samples that do not vary significantly from each other (Fig. 7). The light seed with a size range of 2.8-5.4 mm is significantly larger than the darker seed with a range of 2.7-4.5 mm (Figs 8 & 9). There was always one light seed per fruit and the number of dark seed ranged from 1 to 4 per fruit with a mean of 2 dark seed per fruit.

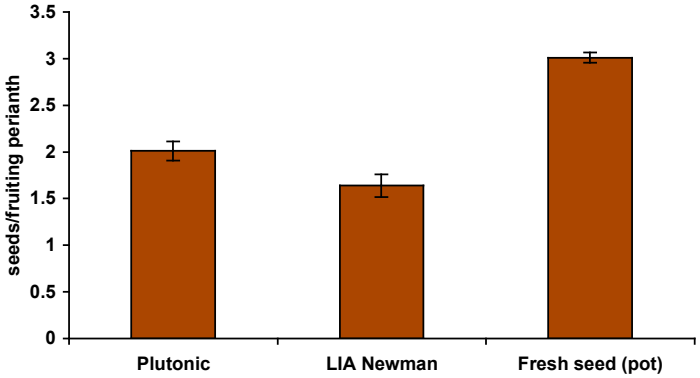

Figure 7. Mean seed number per fruiting perianth of *Acetosa vesicaria* collected from two field sites and pot grown plants. Vertical bars represent ± standard error of the mean (n=100).

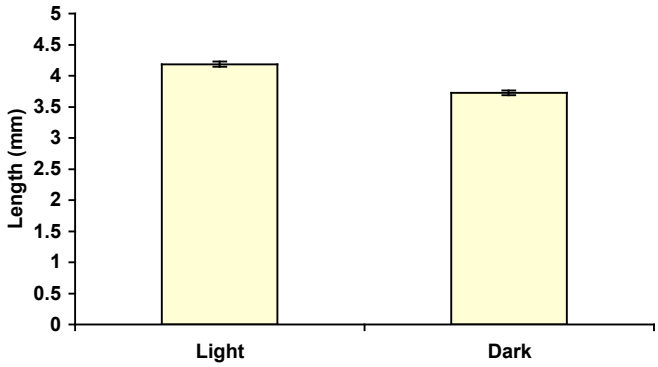

Figure 8: Mean length of *Acetosa vesicaria* seed colour morphs (light and dark). Vertical bars represent ± standard error of the mean (n=200).

Figure 9. Colour polymorphism of *Acetosa vesicaria* seed. Bar=5 mm.

3.2.2 Viability

The viability of fresh seed was considerably higher than seed of unknown age with 100% viable from Newman and less than 10% viable from West Angelas, there was also considerable variability in viability from the different collection sites (Fig. 10). It must be noted that many seed from collection sites with seed of unknown age were empty and may have already germinated.

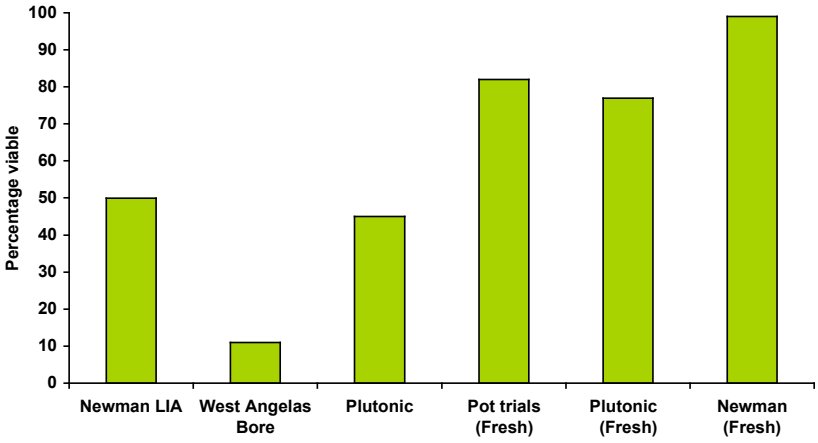

Figure 10. Viability of seed collected at various field sites and pot grown plants. (n=100).

Water uptake for *A. vesicaria* was initially very rapid for the first 240 minutes (4 hours) after which there no significant increase water uptake (weight) (Fig. 11), indicating that they are not physically dormant.

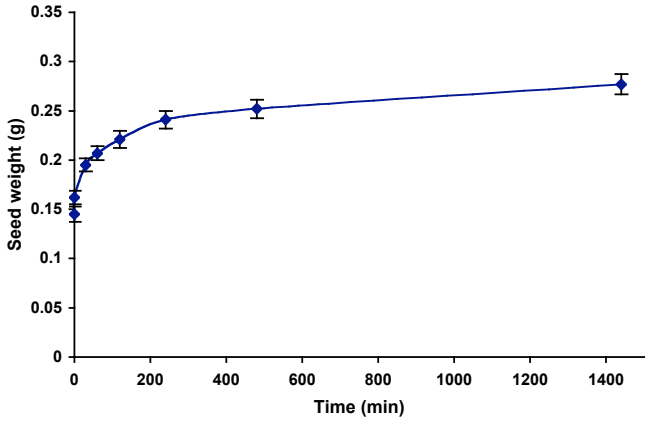

Figure 11. Imbibition of *Acetosa vesicaria* seed. Vertical bars represent ± standard error of the mean (n=5).

3.2.3 Temperature and Light

Germination (Fig. 12) was significantly higher (P<0.001) in the lowest temperature regime compared to the higher two temperature regimes (Fig. 13). Generally light significantly improved germination compared to dark, particularly for seed collected at the Karijini National Park site. There are differences in the response between the two collection sites but overall the preferred germination conditions are the same.

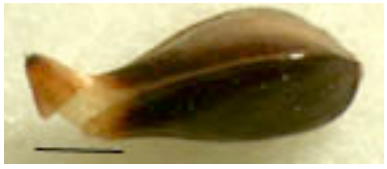

Fig. 12. *Acetosa vesicaria* germination with the appearance of the radicle. Bar = 1 mm.

Alternating temperature did not significantly affect germination percentage however; alternating temperature combined with light produced a twofold increase in germination (Fig. 14). Constant darkness resulted in significantly less germination regardless of the temperature regime.

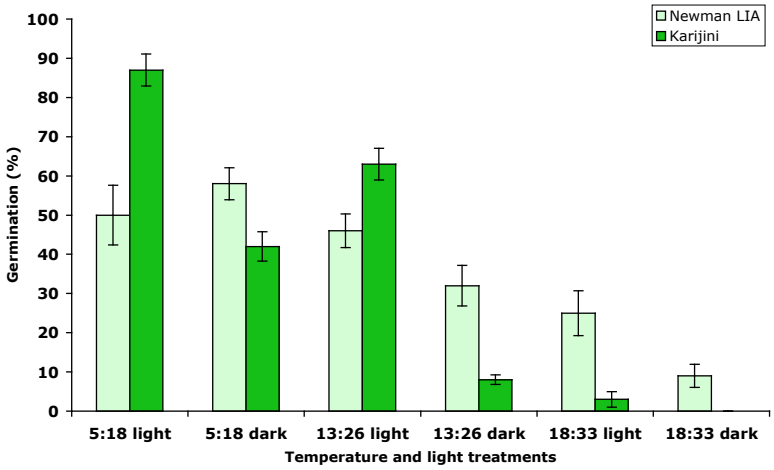

Figure 13. Mean percentage of *Acetosa vesicaria* from two collection sites with various light and temperature regimes.
Description of treatments presented in Table1. Vertical bars represent ± standard error of the mean (n=5).

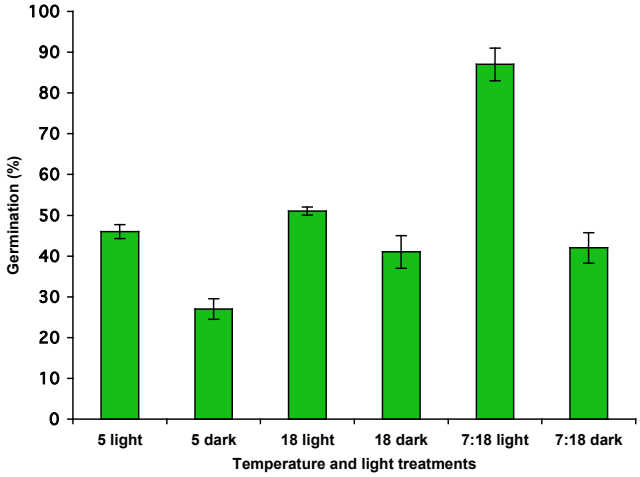

Figure 14. Mean percentage of *Acetosa vesicaria* seed germination collected from Karijini; comparing alternation of temperatures and light/dark with constant temperatures and darkness.
Description of treatments presented in Table 2. Vertical bars represent ± standard error of the mean (n=5).

3.2.4 Seed dormancy

Seed reached maximum germination 12 weeks after seed collection (Fig. 15). There was a significantly greater germination of light coloured seeds (P<0.001) during the first eight weeks after seed collection, after this time there was no difference between the colour morphs (Fig. 16).

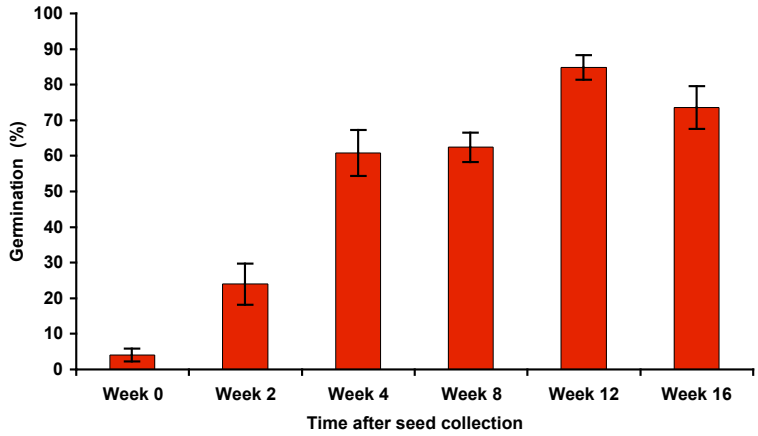

Figure 15: Comparison of germination percentage of *Acetosa vesicaria* seed with time since collection. Vertical bars represent ± standard error of the mean (n=5).

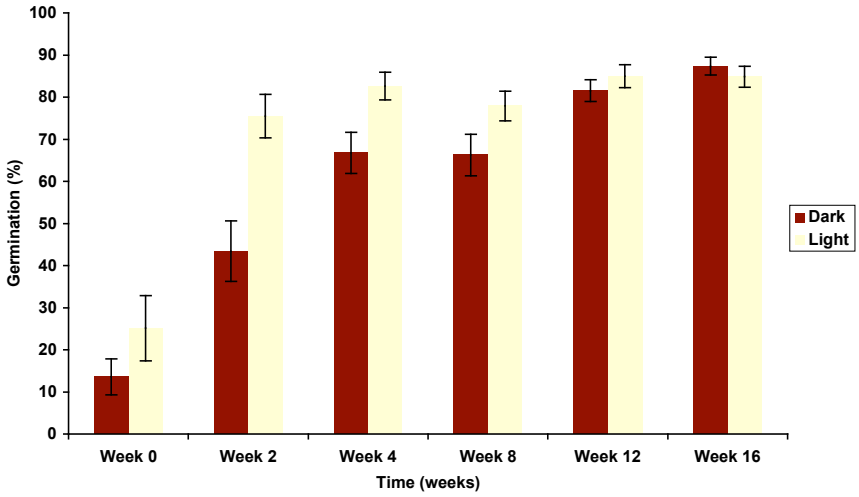

Figure 16. Germination of *Acetosa vesicaria* light and dark seed over time since collection. Vertical bars represent ± standard error of the mean (n=5).

Fresh seed commence germinating after six days even when treated with the germination stimulant GA_3 (Fig. 17A), compared to 12 week old seed that commence germination on day 1 (Fig. 17D). Gibberellin and Butenolide increase germination considerably for eight weeks after collection, but these two germination stimulants have no significant effect on germination of 12 week old seed (Fig. 17). Smoke water appears to inhibit germination to some extent (Fig. 17C & D).

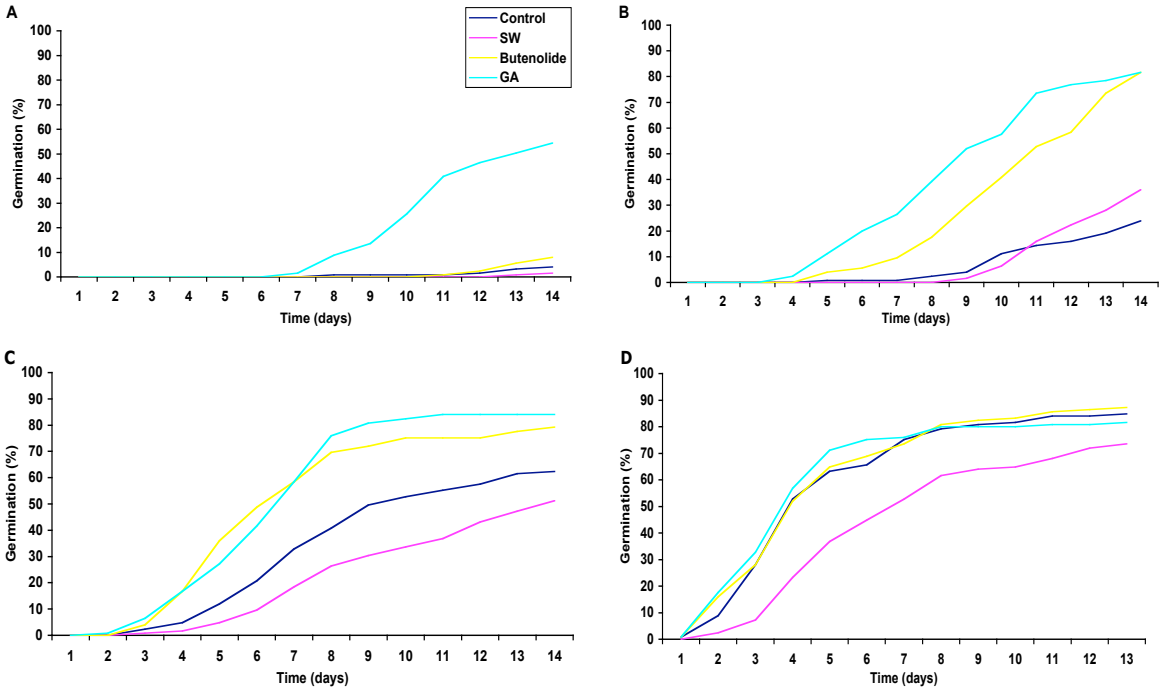

Figure 17: Cumulative germination/day of *A. vesicaria* seed following treatment with various known germination stimulants (Table 2).
A. Seed sown on day of collection; B. Two weeks after collection; C. Eight weeks after collection and; D. Twelve weeks after collection. Description of experimental treatments presented in Table 3. Vertical bars represent ± standard error of the mean (n=5).

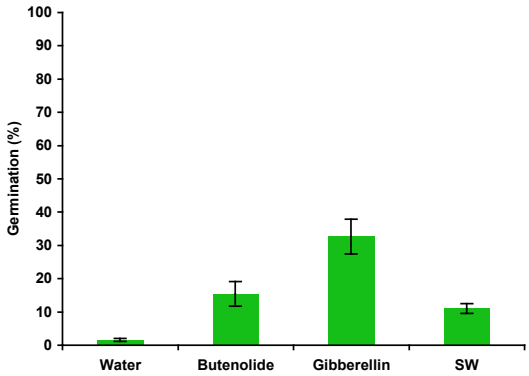

Figure 18. Percentage of seed germinated from within fruiting perianths of *Acetosa vesicaria* following treatment with germination stimulants and sown on petri dishes. Description of experimental treatments presented in table 3. Vertical bars represent ± standard error of the mean (n=5).

Gibberellin significantly (P<0.01) increases the germination of seed twofold whilst enclosed within the fruiting perianths of *A. vesicaria*. Butenolide and smoke water also stimulate germination but to a lesser extent (Fig. 18).

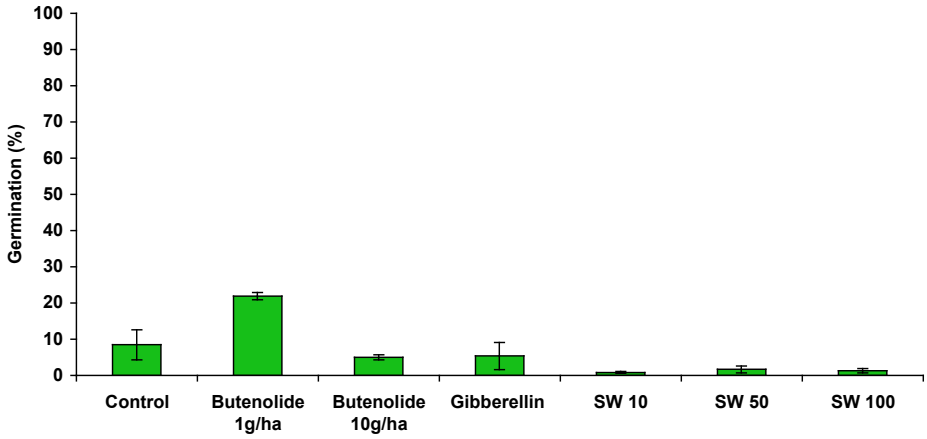

Figure 19. Percentage of seed germinated from within the fruiting perianths of *Acetosa vesicaria* sown on the surface of seedling trays and treated with chemical stimulants.

Vertical bars represent ± standard error of the mean (n=5). SW 10= 10ml/m^2 smoke water; SW 50= 50 ml/m^2; SW 100= 100 ml/m^2.

Acetosa vesicaria perianths sprayed with 1 g/ha butenolide resulted in a significantly greater number (P<0.001; Fig. 19) of seed germinating compared to all other treatments including gibberellin that was more effective in petri dishes (Fig. 18). All concentrations of smoke water appeared to inhibit germination of seed from the fruit compared to the distilled water control (Fig. 19).

3.2.5 Relative seed ageing

Acetosa vesicaria seeds reached 50% of maximum germination by 92 ageing days (Fig. 20). Maximum germination percentage was 82% and there was a gradual decline in germination until 75 ageing days after which germination sharply fell.

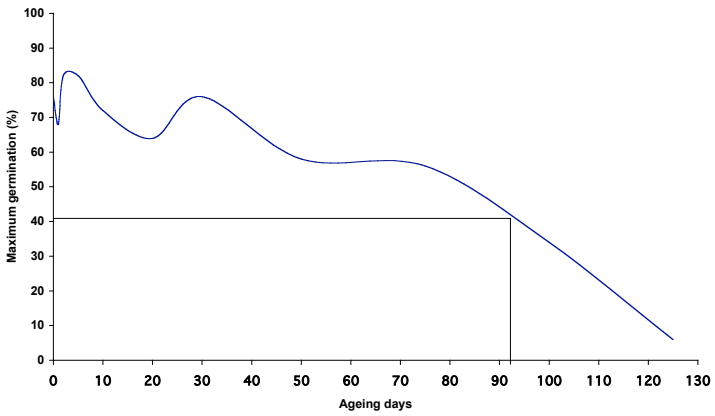

Figure 20. Relative ageing curve for *Acetosa vesicaria* seeds.

3.2.6 Germination inhibitor

Leachate derived from soaking valves of *A. vesicaria* fruiting perianths significantly reduced (P<0.001) germination when applied at greater than 50% concentration (Fig. 21). Leachate solution applied at 100% significantly reduced the average length of the germinant by greater than three times (Fig. 22). There is no significant difference in germination between soaking the fruit for one hour or 24 hours.

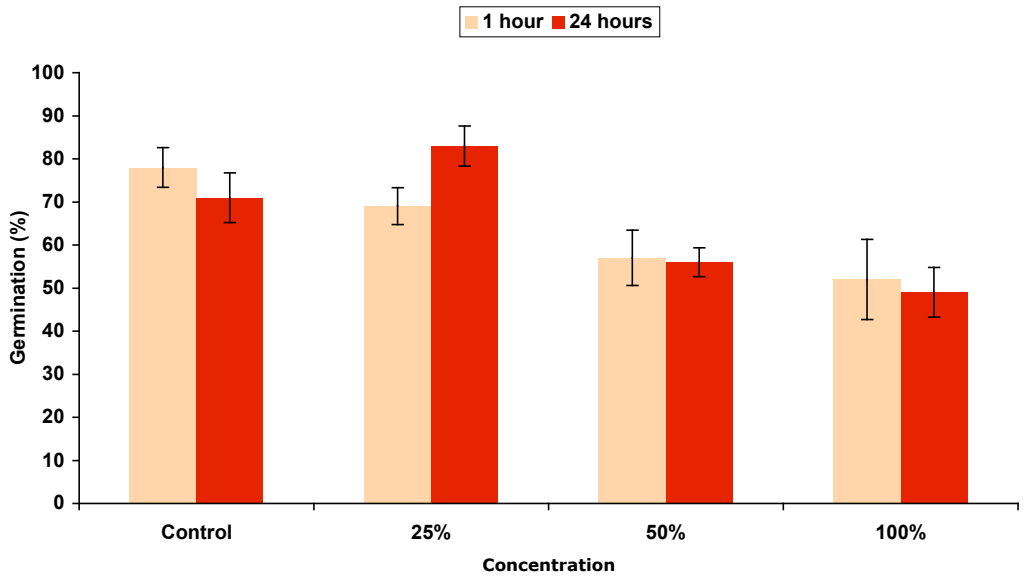

Figure 21. Percentage of *Acetosa vesicaria* seed germinating following application of various concentrations of leachate derived by soaking the fruiting valves for one and twenty four hours. Vertical bars represent ± standard error of the mean (n=5).

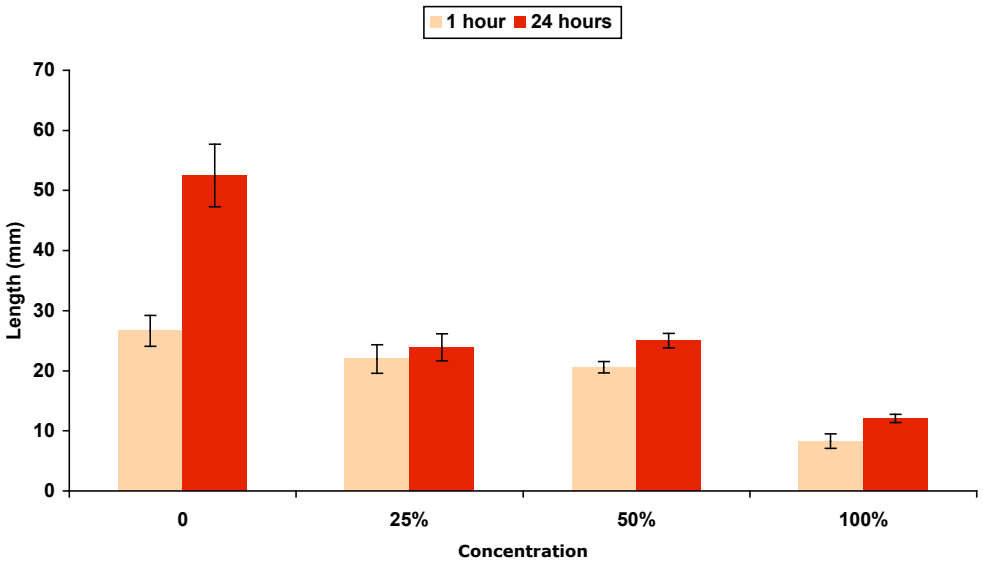

Figure 22. Length of *Acetosa vesicaria* germinants after 10 days following application of various concentrations of leachate derived by soaking the fruiting valves for one and twenty four hours. Vertical bars represent ± standard error of the mean (n=5).

3.2.7 *Seed bank studies*

The seed of *A. vesicaria* are present in the top 5 cm of soil in significantly greater numbers (> 100 times more) than the 5-10 cm depth (Fig. 23). There was a greater number of old fruit than fresh fruit (most new season fruit was still on the plant), hence a comparatively larger number of old seed. A vast majority of the seed remains within the fruit (Figs. 23 & 24) compared to the loose seed recovered from the soil samples. Less than 10% of the loose seed was viable and germinated, compared to 75% of fresh seed and 15% of old seed (Fig. 25).

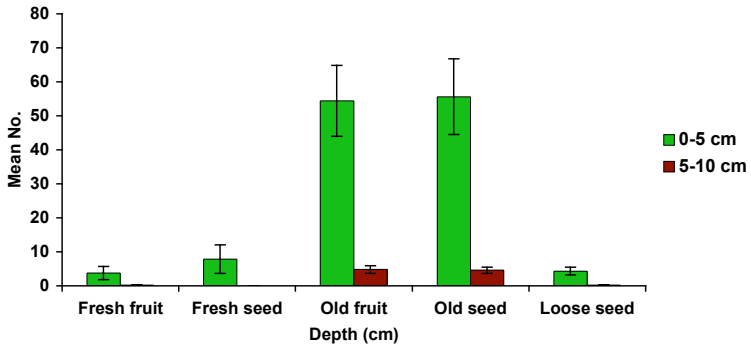

Figure 23. Mean number of *Acetosa vesicaria* fruit and seed recovered from soil cores at two depths. Vertical bars represent ± standard error of the mean.

Figure 24. Seed of *Acetosa vesicaria* within the fruiting perianth valves. Bar = 2 mm.

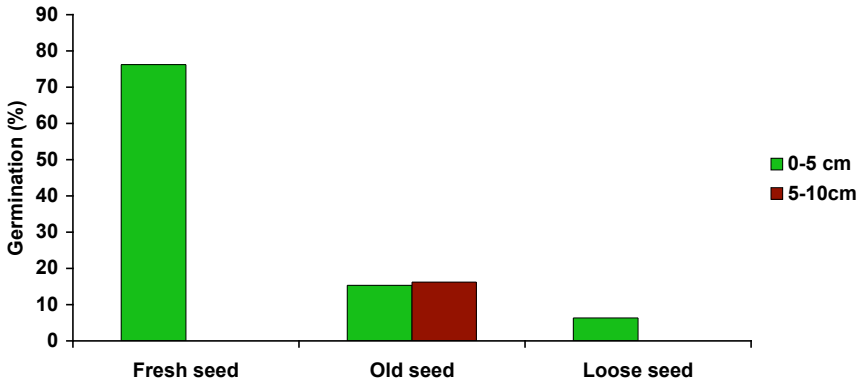

Figure 25. Percentage of germinated seed recovered from soil samples at two depths (Fig. 23).

3.3 **Discussion**

One of the features of *A. vesicaria* is the ability to flower and set viable seed after minimal vegetative growth (at the two leaf stage) (Fig. 26), and can continue to flower until senescence. This adaptive feature has been reported previously in other flowering plants (Houle 2002), as a weedy species the unfortunate result of this ability is copious amounts of seed can be produced under poor growing conditions for example drought.

Figure 26. *Acetosa vesicaria* flowering at the seedling stage.

Seed polymorphy

Within species variation in seed size and colour has been associated with dormancy and timing of germination, longevity, dispersal, competition and geographic position (Jones and Nielson 1999; Espinosa-García *et al.* 2003; Murray *et al.* 2003). This research found that *A. vesicaria* has two colour morphs, one light significantly larger seed and one to four dark smaller seeds per perianth, confirming previous research (Schatral and Osborne, 2002). However, contrary to Short (1999), we found that although the larger pale seeds initially germinated in significantly greater numbers than the darker morphs they do not germinate “readily” once released but require at least two weeks of after-ripening and, after 12 weeks there is no difference in germination rates between the colour morphs (Fig. 16). Seed size did not affect germination in other docks (*R. obtusifolius* and *R. crispus*) (Cideciyan and Malloch 1982). *Acetosa vesicaria* produce small numbers of the larger pale seeds than the smaller darker seed, given the harsh environment that these plants grow in it is more

likely that the production of the larger seed increases the likelihood that some plants will survive during adverse growing conditions. The production of a larger seed, positioned in the centre of the perianth, may simply be because it is older with more resources available during development and slightly more after-ripened, this has been suggested by Tieu *et al.* (2001) for *Anigozanthos manglesii* seed.

Seed germination and dormancy

Rapid water uptake by *A. vesicaria* seeds in the initial phase of germination (Fig. 11) indicates there is no physical barrier to germination confirming previous research undertaken (Hansen 2000). This further supports our assertion that the short period of dormancy and low germination of seeds remaining in the fruiting perianth of *A. vesicaria* seeds is potentially physiological rather than physical.

Mature seeds of many species germinate immediately after release from the parent plant, whilst others require a period of after-ripening (Baskin and Baskin 1998). Seed dormancy is an inherent block of germination and can be released during after-ripening that corresponds to a low-hydrated state achieved by air-dry storage (Leubner-Metzger 2005). Germination of *A. vesicaria* was very low in freshly collected seed and increased after harvest peaking in 12 week old seed, however 50% of viable seed were able to germinate after four weeks. It has been suggested that desert annuals maintain dormant seeds to avoid extinction (Tielbörger and Valleriani 2005) but results also show that germination rates are variable.

Seed germination and temperature

Varying the germination temperature to relieve seed dormancy has been described for other *Rumex* sp. Van Assche and Van Lerberghe (1989) found that sudden fluctuations in temperature would result in significantly greater germination for *R. obtusifolius*. Asrar (2000) thought that low temperature (15^{o}C) alleviated the dormancy in fresh seed of *A. vesicaria* however, our study shows that low temperature (5^{o}C and 18^{o}C; Fig. 14) did not improve germination of fresh seed but gibberellin did (Fig. 17). Instead, alternating diurnal temperatures (5/18^{o}C with 12 h dark/ 12 h light) is ideal for *A. vesicaria* seed germination after the period of after ripening. *Rumex crispus* and *R. obtusifolius* also respond to alternating diurnal temperatures (Roberts and Totterdell 1981). Our research confirmed investigations

previously undertaken by Gardiner (1997) and Asrar (2000) that high temperatures are not conducive to germination of *A. vesicaria* (Fig. 13). This is somewhat surprising considering that it is a desert annual but it is most likely a survival strategy allowing germination to occur during the wet cooler winter months. *Acetosa vesicaria* is a C_3 winter annual herb with succulent leaves and vegetative growth and is restricted to the short growing period of December to February (in the northern hemisphere). This species also appears to exert little control over its water-use efficiency attributed to the C_3 mode of photosynthesis (Sayed 1998). This lower water use efficiency may contribute to rapid water loss thereby prompting precocious flowering for this species.

Germination and light

Light stimulates germination in *A. vesicaria* seeds (Figs. 13 & 14). This research has demonstrated that the seed bank is largely stored on the surface of the soil and is retained within the fibrous perianth perhaps explaining the light requirement. *Rumex crispus* and *R. obtusifolius* also germinate more readily on the soil surface than when buried (Weaver and Cavers 1979).

Germination stimulation/ dormancy alleviation

Application of smoke, either aqueous or aerosol, is an established method of stimulating germination for several species (Roche *et al.* 1997b; Enright and Kintrup 2001; Pérez-Fernández and Rodríguez-Echieverría 2003) and increased seedling vigour (Sparg *et al.* 2005), but there have been some reports of smoke suppressing germination (Razanmandranto *et al.* 2005). In the current study the germination response of *A. vesicaria* to smoke was variable (Figs. 17, 18 and 19) and it seems likely that dosage and length of exposure of smoke application is an important factor. This was further supported by the significantly higher germination response to a low concentration of Butenolide (1g/ha) the active chemical in smoke, whereas the higher dosage supress germination of *A. vesicaria* (Fig. 19). Butenolide has been isolated and synthesised and is present in plant- and cellulose-derived smoke (Flematti *et al.* 2004, 2005). Recently butenolide has been reported to stimulate germination of five Asteraceae species (Merritt *et al.* 2006) and this study has shown that butenolide will stimulate germination of *A. vesicaria* (Fig. 19). Currently research is being

undertaken at KPBG into the use of butenolide for the stimulation of weed seed from the soil seed bank.

There are numerous reports of gibberellic acid (GA_3) enhancing germination and removing physiological dormancy (Bewley and Black 1994; Baskin and Baskin 1998). In this research we found that GA_3 enhanced the germination of *A. vesicaria* seed during the after ripening period. After this period no germination stimulants were required (Fig. 17).

Seed bank dynamics

Seed banks are highly variable in composition, lifetime and functional significance (Adams *et al.* 2005). The formation of a persistent seed bank is a feature of many weedy species (Martínez-Ghersa *et al.* 2000; Espinosa-García *et al.* 2003) and Cohen (1966 and 1967) suggested that development of a persistent seed bank is advantageous to the long-term survival of plant species in highly variable environments. Sester *et al.* (2006) studied the persistence of the weed beet (*Beta vulgaris*) and reported that the seed bank followed cyclic periods of seed decay and was highly dependent on the seasons. Short (1999) suggested that the dark seed morphs of *A. vesicaria* formed the persistent seed bank. Our studies revealed that both seed morphs were present in the seed bank and, not surprisingly because they are produced in larger numbers, there were many more of the dark seed morphs. This confirms previous research undertaken by Andreoli (2002) who reported the presence of both seed morphs in soil cores collected from Plutonic Mine at the revegetation sites.

Benoit *et al* (1989) estimated that no fewer than 60 soil cores should be used to quantify the weed seed bank abundance and that it was the number of sample points and not the quantity of soil sampled that reduced the sample variance. In our research we sampled 30 cores at two different depths and attribute the sample variance to the patchy distribution of the plants and consequently the seed. *Acetosa vesicaria* seed tends to be shed close to the parent plant and forms large mats on the soil surface (Fig. 27). These mats slowly break down over time and the fibrous husks containing seed become less detectable. Increasing the number of soil samples would have reduced the variation, however the results would remain the same, with

the majority of the seed contained within the fruiting perianths present on the soil surface (Fig. 23). When the soil sampling was undertaken the majority of inflorescences were still on the plants resulting in significantly larger numbers of old fruit than new fruit in the soil cores.

Figure 27. Dense mats of dead *Acetosa vesicaria* plants and fruit on the soil surface.

Seed dispersal is an important aspect of understanding weed abundance as the mechanism allows offspring to colonise sites near or far from the parent plant simultaneously ensuring the chance that those seeds will survive and become established adults (Nogales *et al.* 2005). During the course of this study we have recorded that the principal form of seed dispersal for *A. vesicaria* is wind, water and anthropogenic; particularly noticeable along road verges. It is possible, but highly unlikely given the unpalatable nature of this plant, that animals may disperse the seed during times when there is little feed around. Our observations indicate rabbits and kangaroos may browse the softer growth but not the mature seeding inflorescences. Paradoxically, according to Reddy and Bhatt (2001), *A. vesicaria* and *Spinacea oleracea* (spinach) are the commonly consumed green leafy vegetables in different parts of India. The seed contained within the fruiting valves tends to settle within the rocky crevices, for example along rivers and creeks, road verges, quarries and mining rehabilitation sites. It is important to note that this weed is a disturbance opportunist and relishes moving into areas where grading and ripping or other earthmoving activities have occurred.

Relative ageing

Acetosa vesicaria seeds reached 50% of maximum germination in 92 ageing days, which is average for geosporous seeds (pers. com. Merritt (BGPA)). These results are used to compare longevity between taxa and currently BGPA is testing many species both native and exotic. *Rumex* species have previously been reported to have long lived seed (such as *R. crispus*, Lewis 1973; Weaver and Cavers 1979 and *R. obtusifolius*, Van Assche and Van Lerberghe 1989), however this is a species specific trait.

Many secondary metabolites such as phenolics, tannins and alkaloids act as plant allelochemicals. *A. vesicaria* plants have been reported to be high in plant phenolics (Auld and Medd 1987). Leachate from bracts has been implicated in the inhibition of germination (such as for *Atriplex griffithii*, Ungar and Khan 2001; *Triticum taushii,* Gatford *et al.* 2002). This study found that the leachate from the valves of the fruiting body had an inhibitory effect on germination and subsequent growth of *A. vesicaria* when applied in high concentrations.

Acetosa vesicaria originated from desert and semidesert areas of North Africa, southwest Asia, southern Iran, Afghanistan and Pakistan. In desert environments, plant survival is strongly limited by temporal changes in water availability (Sayed 1998; Tielbörger and Valleriani 2005; Tobe *et al.* 2005). Seedlings are particularly vulnerable because their short roots are distributed in the shallow soil layers prone to quick drying. Precipitation moistens soil differently depending on soil depth. Even a light rainfall moistens surface soil but evaporates quickly, on the other hand, soil in deep layers will be moistened only after a heavier rainfall but is expected to persist longer due to a lower evaporation rate. Based on this it is expected that seed germination and seedling survival would be affected by the depth of seed burial in the soil. Contrary to this *A. vesicaria* seeds largely persist in the fibrous valves of the perianth on the soil surface. Though, it would appear that a certain amount of water is required to firstly infiltrate the perianth which is mostly hydrophobic and secondly to wash the germination inhibitor from the tissues. We found that there is some indication of a chemical germination inhibitor in the tissues of the perianth (Figs 21 and 22). Water is chiefly absorbed into the perianth from the damp soil. These are protective mechanisms to prevent precocious germination in low rainfall events.

4.0 Herbicide Studies

Competition from weeds for light, water and nutrients leads to reduced establishment and growth of native plants and is particularly problematic when trying to establish rehabilitation sites after disturbance such as mining and road building.

Manual weeding and development of biological agents are costly, time consuming and labour intensive methods of weed control. Application of herbicides is a less cumbersome and effective method if the correct chemical is selected. This research was carried out to select the most suitable herbicide/s for effective control of *A. vesicaria* with minimal effect (off-target damage) on native taxa.

Glyphosate

Glyphosate is the herbicidal active ingredient in Roundup® and many other herbicide brands that control a broad spectrum of plant species both annual and perennial including; grasses, sedges, broad-leaved weeds and other woody plants. Glyphosate is a non-selective, broad-spectrum herbicide that is the most widely used herbicide in the world (Weersink *et al.* 2005; Kolpin *et al.* 2006; Sorensen *et al.* 2006) and is routinely used to control weeds in agriculture, bushland and urban localities. Glyphosate is a foliar spray and Grangeot *et al.* (2005) reported that 50% of uptake is attained in three hours, and is directed towards the sink tissues (apical developing tissues and roots), by blocking the sikimic acid pathway in plants (Deng 2005; Dill 2005; Meriles *et al.* 2006). Glyphosate is considered environmentally friendly, with a high biogradability and a low leaching risk (Mamy and Barruysi 2005; Weersink *et al.* 2005; Sorensen *et al.* 2006), as it is generally rapidly and strongly adsorbed.

Metsulfuron-methyl

Metsulfuron-methyl (Brush-off®) is a residual sulfonylurea compound used as a selective pre- and post-emergence herbicide for broadleaf weeds and some annual grasses. It is a systemic compound with foliar and some soil activity and works rapidly after the plant takes it up. Metsulfuron-methyl acts by inhibiting cell division in the shoots and roots and is biologically active at low use rates. Sulfonylurea herbicides have been shown to affect both growth and reproduction of annual plant species (Kjær *et al.* 2006), because these herbicides reduce the transfer of

assimilation products out of the treated leaves and therefore the availability of these resources for further growth and reproduction of the plant.

Asulam

Asulam (Asulox®) is a selective post-emergent systemic carbamate herbicide used to control a variety of annual grasses and broadleaf weeds. Asulam has a relatively narrow spectrum because it mainly affects ferns, docks and bryophytes (Pakeman *et al.* 2006). Asulam has been successfully used to control *Panicum repens* (Hossain *et al.* 2002).

Chlorsulfuron

Chlorsulfuron (Glean®) is a triazine herbicide and is recommended for selective pre- or post-emergent control of broadleaf and some grass weeds. Chlorsulfuron inhibits spermidine accumulation in mitotic tissues of root tips (Deng 2005). "Excellent" control of *R. retroflexus* has been achieved with 30 g/ha Chlorsulfuron (Malik *et al* 2001).

Dicamba

Dicamba (Kamba®) is a pre- and post-emergent benzoic acid herbicide used to control annual and perennial broadleaf weeds (Soltani *et al.* 2006). This herbicide is a growth regulator type of herbicide and in sensitive plants can cause uncontrolled cell division and growth resulting in vascular tissue destruction. The mode of action is foliage translocation with some root absorption. The herbicide Dicama was the most effective, cheapest and least damaging to pastures in controlling *Marrubium vulgare* (McMillan 1990).

2,4-dichlorophenoxy acetic acid (*2,4-DB (Buticide)*)

2,4-D (2,4-dichlorophenoxy acetic acid) is a chlorinated phenoxy compound that function as a systemic growth regulator herbicide with complex mechanisms of action, once absorbed 2,4-D is translocated within the plant and accumulates at the growing points of shoots and roots where it inhibits growth. 2,4-D is a highly selective herbicide used to control many types of broad-leaved weeds and was the first successful selective herbicide developed. This herbicide was introduced in 1946 and is now the third most widely used herbicide. 2,4-DB is more selective than 2,4-D as

its activity is dependent on oxidation to 2,4-D within the plant and not all plants are capable of doing this.

Surfactants

Leaf cuticle and plasma membrane have been identified as barriers to herbicide activity (Aladesanwa and Oladimeji 2005) and the addition of a surfactant has proven to increase the efficacy in weed control (Aladesanwa and Oladimeji 2005; Molin and Hirase 2005). Surfactants increase spray droplet retention by plant foliage through reducing the surface tension of the spray solutions (Liu 2004), whereby efficient coverage, less wastage, and environment protection are achieved (Basu *et al.* 2002). There are several types of surfactants to improve the performance of herbicides and the majority are water soluble and non ionic such as Alkyl phenol ethylene oxide (Agral™). The introduction of organosilicone surfactants such as Pluse® in the late 1980's resulted in increased stomatal infiltration, translocation and rainfastness of herbicides (Zabkiewicz 2000). The influence of adjuvants on herbicide performance was reviewed by Liu (2004) who concluded that the effectiveness of surfactants depends of the chemical structure and concentration of the surfactant, the concentration and chemical properties of the herbicide and the leaf surface character of the plant.

4.1 Materials and Methods

4.1.1 Selection of field sites

After consultation with the Department of Environment and Conservation (DEC), KJ Environmental (spray contractor), Main Roads Western Australia and mining companies three sites in the Pilbara region of Western Australia (Fig. 28), known to be infested with *A. vesicaria* for several years, were selected:

- Site 1: Disused Main Roads quarry 90 km northwest of Newman (23°06.800S, 119°00.146E).
- Site 2: CALM bushland site near Joffre entry station in the Karijini National Park (22°32.049S, 118°06.272E).
- Site 3: Disused quarry near the Tom Price mine site (22°45.227S, 117°51.562E).

Also, during 2005 due to low rainfall and poor germination of *A. vesicaria* a fourth site was selected for the final field trial.

- Site 4: Plutonic mine main waste dump (25°19.26S, 119°26.46E), (Fig. 29).

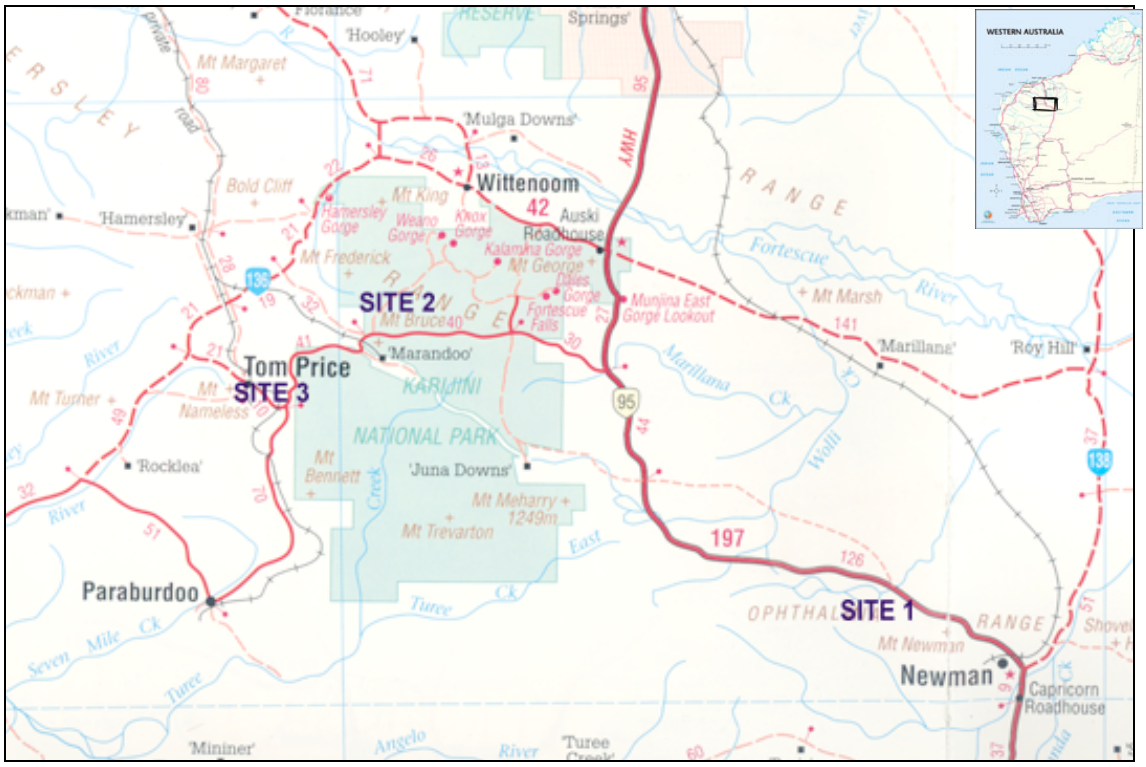

Figure 28. Location of field sites in the Pilbara region of Western Australia (Hema Maps 1998)

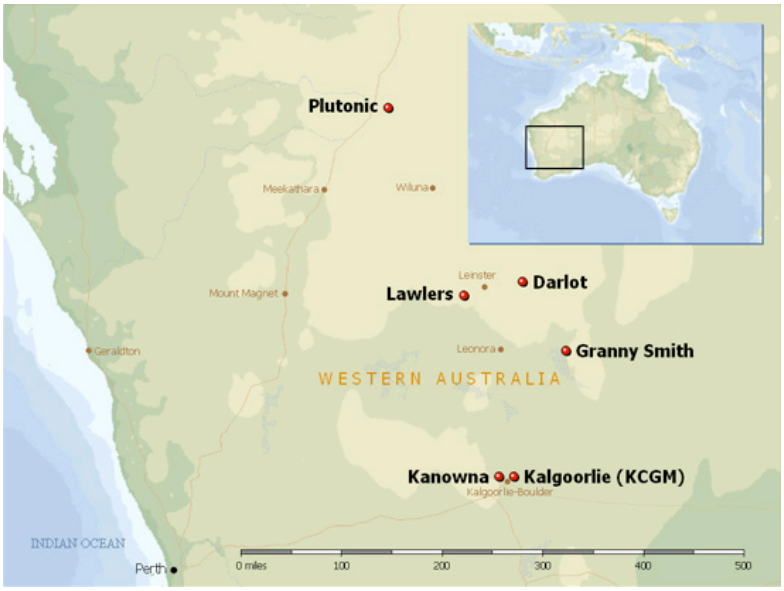

Figure 29. Location of Plutonic Mine (Site 4). (Barrick Global Operations 2006)

4.1.2 Selection of herbicides

One of the principle aims of this research was to find an effective herbicide to control *A. vesicaria* with the least impact on "off target" species. Glyphosate has been widely used for the control of *A. vesicaria*, however little research has been undertaken and the available information is anecdotal. High rates of metsulfuron-methyl have also been used in aerial applications causing considerable "off-target" damage. There have been reports of herbicide use on other docks such as *R. crispus* and *R. obtusifolius*, these herbicides include: glyphosate, paraquat, sulosate, asulam, fluroxypyr, 2,4-D, dicamba, MCPA.

For this research we have selected 875 g/kg glyphosate, asulam (400 g/L Asulox®), 2,4-DB (400 g/L Buticide®), metsulfuron-methyl (600 g/L Brush-off®), dicamba (Kamba®) and chlorsulfuron (750 g/L Glean®) and trials were conducted using various concentrations of each herbicide and two surfactants Agral® and Pulse®.

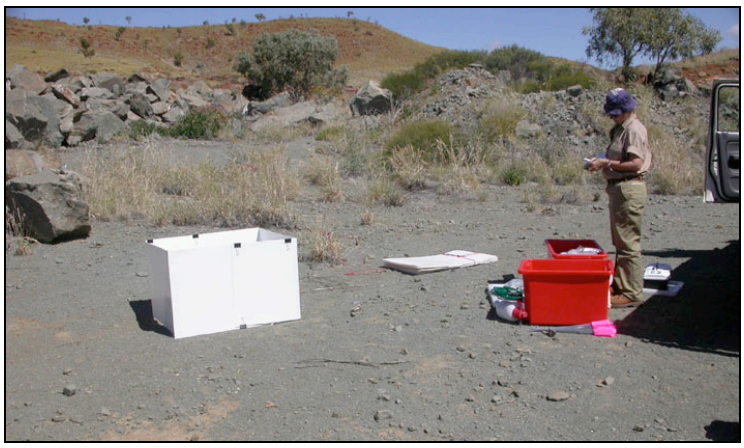

Figure 30. Open-ended 1x1 m box used for field herbicide spray trials. Site 1, August 2003.

4.1.3 Field trial spray plots

To prevent spray drift (1x1 m) field trial spray plots were contained within an open-ended box, formed using Coreflute® (Fig. 30). For easy identification each corner was marked on the ground using different coloured fluorescent spray paint and plot numbers. Three replications were carried out for each treatment using randomly selected plots. Herbicide concentrations were prepared accordingly and subsequently 125 ml was evenly delivered to each 1x1 m plot using a two litre hand held spray pack. A preliminary assessment of *A. vesicaria* was made prior to herbicide application including plant numbers, general health and flowering in each plot and weed control assessment was carried out six weeks after treatment. All data was analysed for statistical significance by analysis of variance. Fisher's least significant difference ($P<0.05$) was used to determine significant differences between treatments.

4.1.4 Pot trials

Pot trials were undertaken at Kings Park and Botanic Garden in Perth, Western Australia. Seed was germinated in petri dishes in germination incubators as previously described. After germination they were transferred to individual 50 ml pots filled with Kings Park pasteurised seed sowing mix (two parts jarrah sawdust, one part nursery sand, 1/2 part coarse river sand, lime 1 kg/m^3, dolomite 800 g/m^3), placed in the glasshouse and kept well watered until the roots were well developed. The surviving plants were then transferred to 130 mm pots using Kings Park

standard potting mix and placed outside in the nursery to harden off and grow on. The plants were watered daily with a timed irrigation system.

Treatments were replicated three times with 10 pots per replication. Herbicide concentrations were prepared accordingly and subsequently 125 ml was evenly delivered to each 1x1 m plot containing the 10 pots with a two litre hand held spray pack using the same open-ended box used in field trials (Fig. 30). All data was analysed for statistical significance by analysis of variance. Fisher's least significant difference ($P<0.05$) was used to determine significant differences between treatments.

4.1.5 Field trial 1

Field trial one was conducted in August 2003. Herbicide treatments and rates are presented in Table 5. Efficacy of the herbicide was graded: 0= no response; 2= healthy but flowering arrested; 3= leaves browning; 4= all leaves brown, stem green, upright; 5= no leaves, flowers, stem flexible, 6= dead and dry. Flowering status was also graded: 0= no flowering, 1= flowering arrested, 2= mature dry flowers, 3= mature fresh flowers, 4= new immature flowers.

Table 5. Herbicide treatments and application rates for field trial one.

Herbicide	Treatment rates			
Controls	Water	Pulse (P)	Agral (W)	Pulse+Agral
Glyphosate (G)	1 ℓ/ha	1 ℓ/ha+P	1 ℓ/ha+W	1 ℓ/ha+P+W
Glyphosate	3 ℓ/ha	3 ℓ/ha+P	3 ℓ/ha+W	3 ℓ/ha+P+W
Glyphosate	6 ℓ/ha	6 ℓ/ha+P	6 ℓ/ha+W	6 ℓ/ha+P+W
Metsulfuron-methyl (M)	2.5 g/ha	2.5 g/ha+P	2.5 g/ha+W	2.5 g/ha+P+W
Metsulfuron-methyl	5 g/ha	5 g/ha+P	5 g/ha+W	5 g/ha+P+W
Metsulfuron-methyl	7.5 g/ha	7.5 g/ha+P	7.5 g/ha+W	7.5 g/ha+P+W

4.1.6 Field trial 2

Field trial two was conducted in May 2004, herbicide treatments and rates are presented in Table 6. Efficacy of the herbicide was graded: 0= new growth and flowers; 2= new growth; 3= alive and healthy (no new growth or flowers); 4=green, slightly stressed with new growth; 5=green, very stressed with new growth; 6=red or burnt leaves and new growth; 7=dead leaves and new growth; 8= green and very stressed; 9=red or burnt and very stressed; 10= dead with flexible stems; 11= dead and dry.

Table 6. Herbicide treatments and application rates for field trial two.

Herbicide	Treatment rates			
Glyphosate (G)	G3 ℓ/ha	G3 ℓ/ha+P	G3 ℓ/ha+W	G3 ℓ/ha+P+W
Metsulfuron-methyl (M)	G5 g/ha	M5 g/ha+P	M5 g/ha+W	M5 g/ha+P+W
Glyhosate+Metsulfuon-methyl (G+M)	G3 ℓ/ha + M5 g/ha	G3 ℓ/ha + M5g/ha +P	G3 ℓ/ha + M5g/ha+W	G3 ℓ/ha + M5g/ha+P+W
Asulam (A)	A2.5 ℓ/ha	A2.5 ℓ/ha+P	A2.5 ℓ/ha+W	A2.5 ℓ/ha+P+W
Asulam	A5 ℓ/ha	A5 ℓ/ha+P	A5 ℓ/ha+W	A5 ℓ/ha+P+W
Asulam	A7.5 ℓ/ha	A7.5 ℓ/ha+P	A7.5 ℓ/ha+W	A7.5 ℓ/ha+P+W
Chlorsulfuron (C)	C5 g/ha	C5 g/ha+P	C5 g/ha+W	C5 g/ha+P+W
Chlorsulfuron	C10 g/ha	C10 g/ha+P	C10 g/ha+W	C10 g/ha+P+W
Chlorsulfuron	C15 g/ha	C15 g/ha+P	C15 g/ha+W	C15 g/ha+P+W
Controls	water	Pulse (P)	Agral (W)	Pulse+Agral

4.1.7 Field trial 3

Field trial three was conducted in August 2004, herbicide treatments are presented in Table 7. Efficacy of the herbicide was graded as for field trial two.

Table 7. Herbicide treatments and application rates for field trial three.

Herbicide	Treatment rates			
Glyphosate (G)	G3 ℓ/ha	G3 ℓ/ha+P	G3 ℓ/ha+W	G3 ℓ/ha+PW
Metsulfuron-methyl (M)	G5 g/ha	M5 g/ha+P	M5 g/ha+W	M5 g/ha+P+W
Glyhosate+Metsulfuon-methyl(G+M)	G3 ℓ/ha + M5 g/ha	G3 ℓ/ha + M5g/ha +P	G3 ℓ/ha + M5g/ha+W	G3 ℓ/ha + M5g/ha+P+W
Asulam (A)	A7.5 ℓ/ha	A7.5 ℓ/ha+P	A7.5 ℓ/ha+W	A7.5 ℓ/ha+P+W
Asulam	A10 ℓ/ha	A10 ℓ/ha+P	A10 ℓ/ha+W	A10 ℓ/ha+P+W
Asulam	A15 ℓ/ha	A15 ℓ/ha+P	A15 ℓ/ha+W	A15 ℓ/ha+P+W
Controls	Water	Pulse (P)	Agral (W)	Pulse+Agral

4.1.8 Field trial 4

Due to the low rainfall and poor germination of *A. vesicaria* field trial four was conducted at Plutonic mine site (Fig. 28) on the main waste dump. As there was only one site to conduct trials, each treatment was replicated five times using methods previously stated. Field trial four was conducted in September 2005 and herbicide treatments and rates are presented in table 8. Efficacy of the herbicide was graded as for field trial two.

Table 8. Herbicide treatments and application rates for field trial four.

Herbicide	Treatment rates	
Controls	Water	Pulse (P)
Glyphosate (G)	G3 ℓ/ha	G3 ℓ/ha+P
2,4-DB (Buticide) (B)	B2 ℓ/ha	B2 ℓ/ha+P
2,4-DB (Buticide)	B3 ℓ/ha	B3 ℓ/ha+P
Dicamba (Kamba) (K)	K0.3 ℓ/ha	K0.3 ℓ/ha+P
Dicamba (Kamba)	K0.6 ℓ/ha	K0.6 ℓ/ha+P

4.1.9 Pot trial 1

This trial was undertaken to test the efficacy of glyphosate on *A. vesicaria* in a reasonably controlled environment; glyphosate concentrations are as for Field Trial one (presented in Table 5). Recorded data for each plant include, height to the most distant green node, flowering and relative health before and after treatment. After six weeks the plants were harvested; wet and dry weights recorded and all seed collected for germination trials.

4.1.10 Pot trial 2

This trial was undertaken to test metsulfuron-methyl on *A. vesicaria;* metsulfuron-methyl concentrations are as for Field Trial one (presented in table 5). Recorded data for each plant include, height to the most distant green node, flowering and relative health before and after treatment. After six weeks the plants were harvested, wet and dry weights recorded.

4.1.11 Pot trial 3

This pot trial was designed to determine if various watering regimes affect the efficacy of glyphosate on *A. vesicaria*. Ten pots 130 mm containing dry BGPA pasteurised potting mix were individually weighed watered and allowed to drain then reweighed to determine the average field capacity. For seven days prior to herbicide treatment plants were watered daily 20%, 40%, 60%, 80% or 100% of field capacity. After seven days of prescriptive watering half of the plants were sprayed with water (control) and half were sprayed with 3 ℓ/ha glyphosate plus 2 ml/ℓ Pulse as previously described.

Plants were kept in the glasshouse and watered according to the prescription on a daily basis. Data was collected weekly and included: herbicide efficacy, flowering, colour of leaves, emergence of new leaves, leaf fluorescence, leaf chlorophyll

concentration and colour of stems. After six weeks all plants were harvested and wet and dry weights recorded. Only leaf chlorophyll concentration, wet and dry weight data are presented in this report. Leaf chlorophyll was measured with a SPAD-520 chlorophyll meter (Minolta, Osaka, Japan) on the youngest fully extended leaves of each plant.

4.1.12 Germination of seed collected from herbicide treated plants

Fruiting perianths were collected from each treatment of the glyphosate pot trial for a seed germination trial. Germination trials were conducted as outlined previously. The mean length of each germinant was recorded as a measure of vigour. All data was analysed for statistical variance. Germination percentages were arcsine-transformed prior to analysis (untransformed data appears in all tables and figures). Fisher's least significant difference ($P<0.05$) was used to determine significant differences between treatments.

4.2 Results

4.2.1 Field trial 1

There was no significant difference in *A. vesicaria* response to herbicide treatments between the three sites, the results presented in Table 9 and Figure 31 is from the trial at Tom Price quarry. Effective control of *A. vesicaria* was only apparent when treated with glyphosate.

Table 9: Flowering and percentage control *Acetosa vesicaria* in response to herbicide application

Herbicide treatment	% Control by numbers	Flowering response*
G1	0 a	1.00±0 a
G1+P	94±5.9 b	0.33±0.33 a
G1+W	0 a	2.33±0.88 ab
G1+P+W	61±28.7 b	0.33±0.33 a
G3	87±8.3 b	1.67±0.88 ab
G3+P	100±0 b	0.33±0.33 a
G3+W	73±20.4 b	2.00±1.00 ab
G3+P+W	99±0.6 b	0.67±0.33 a
G6	100±0 b	0.33±0.33 a
G6+P	100±0 b	0 a
G6+W	70±30.3 b	1.00±0.58 a
G6+P+W	100±0 b	0.33±0.33 a
M2.5	0 a	1.00±0 a
M2.5+P	11±11.0 a	1.00±0 a
M2.5+W	0 a	1.00±0 a
M2.5+P+W	0 a	1.00±0 a
M5	0 a	1.00±0 a
M5+P	0 a	2.00±0.99 ab
M5+W	7±6.7 a	1.67±0.67 ab
M5+P+W	0 a	0.67±0.33 a
M7.5	0 a	1.00±0 a
M7.5+P	0 a	1.00±0 a
M7.5+W	0 a	0.67±0.33 a
M7.5+P+W	0 a	2.00±1.00 ab
Water	0 a	3.33±0.33 b
Pulse (P)	0 a	3.33±0.33 b
Agral (W)	0 a	3.00±0 b
P+W	0 a	3.33±0.33 b

*Flowering response was scored 0= no flowering, 1= flowering arrested, 2= mature dry flowers, 3= mature fresh flowers, 4= new immature flowers

There was significantly greater response (P<0.001) to the higher rates of glyphosate particularly when combined with Pulse. The surfactant Agral appeared to decrease the efficacy of glyphosate (Fig. 31). Flowering was arrested in response to metsulfuron-methyl but growth of new leaves was not.

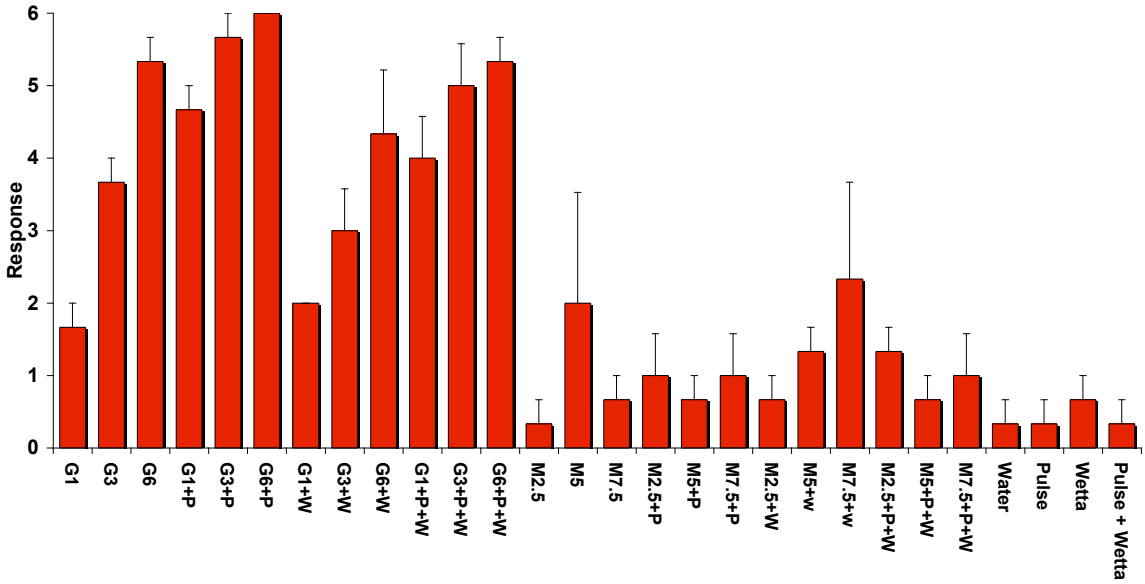

Figure 31. Herbicide efficacy based on *Acetosa vesicaria* response in a field trial. Vertical bars represent ± standard error of the mean (n=3).
Where: 0= no response; 2= healthy, flowering arrested; 3= leaves browning; 4= all leaf brown, stem green, upright; 5= no leaves, flowers, stem flexible, 6= dead and dry. Treatments are presented in table 5.

4.2.2 Field trial 2

Glyphosate (3 ℓ/ha) was significantly (P<0.001) more effective than the other three herbicides when applied to *A. vesicaria* and the efficacy was not enhanced when combined with metsulfuron methyl (Fig. 32). Chlorsulfuron was no more effective than the controls. Addition of Agral appeared to decrease the efficacy of glyphosate but not significantly. Both glyphosate and metsulfuron-methyl inhibited formation of flowers and arrested the maturation of immature perianths. There were significant variations in response between the three trial sites (Fig. 32) but the overall response was the same.

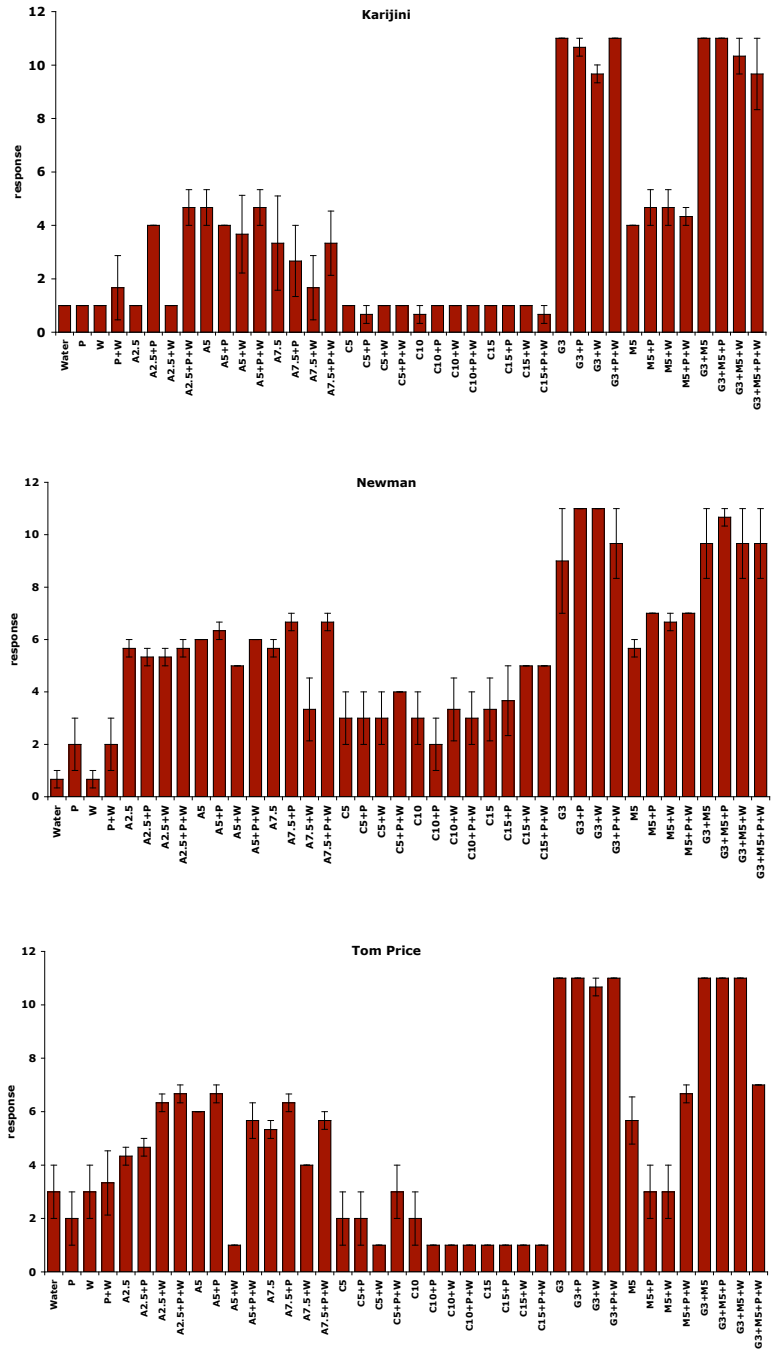

Figure 32. Herbicide efficacy based on *Acetosa vesicaria* response in a field trial. Vertical bars represent ± standard error of the mean (n=3). Treatments are presented in Table 7.Where: 0= new growth and flowers; 2= new growth; 3= alive and healthy (no new growth or flowers); 4=green, slightly stressed with new growth; 5=green, very stressed with new growth; 6=red or burnt leaves and new growth; 7=dead leaves and new growth; 8= green and very stressed; 9=red or burnt and very stressed; 10= dead with flexible stems; 11= dead and dry.

4.2.3 Field trial 3

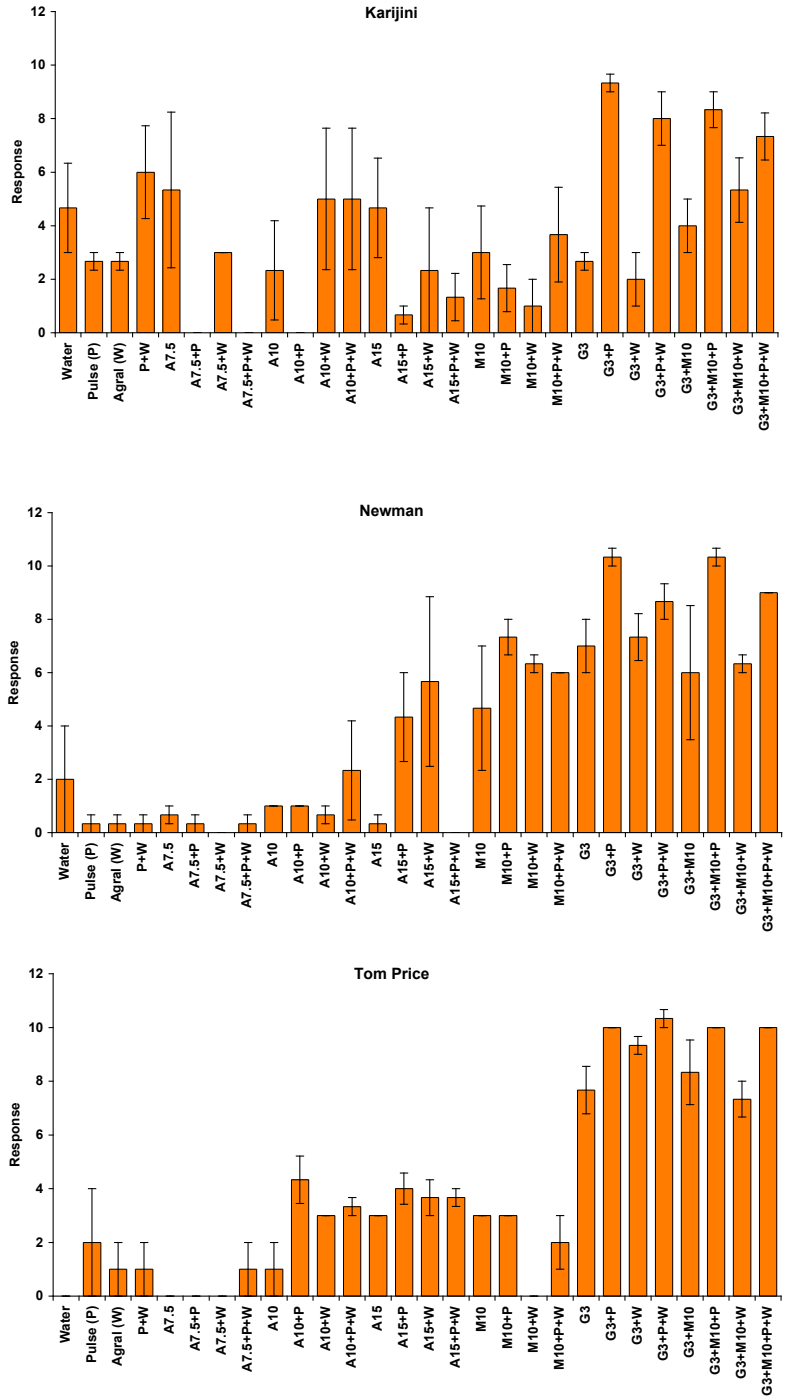

Figure 33. Herbicide efficacy based on *Acetosa vesicaria* response in a field trial. Treatments are presented in Table 7. Vertical bars represent ± standard error of the mean (n=3). Where: 0= new growth and flowers; 2= new growth; 3= alive and healthy (no new growth or flowers); 4=green, slightly stressed with new growth; 5=green, very stressed with new growth; 6=red or burnt leaves and new growth; 7=dead leaves and new growth; 8= green and very stressed; 9=red or burnt and very stressed; 10= dead with flexible stems; 11= dead and dry.

Acetosa vesicaria plants were significantly (P>0.001) more responsive to the herbicide glyphosate particularly with Pulse added (Fig. 33). The increased rates of asulam and metsulfuron-methyl (from Field Trial 2) did not result in an increased

response to these herbicides. There were marked differences between the three sites, especially at the Karijini National Park site (Site 2) as the control plants were remarkably stressed. Addition of Agral significantly reduced the efficacy of glyphosate.

4.2.4 Field trial 4

The efficacy of glyphosate with the addition of Pulse was significantly (P<0.001) greater than any of the other herbicide treatments (Fig. 34). Dicamba (K) and 2,4-DB (B) both had significantly greater effect than the controls, however some plants within plots sprayed with these herbicides, regardless of the treatment concentration, produced new growth and flowers (Fig. 34, Table 10).

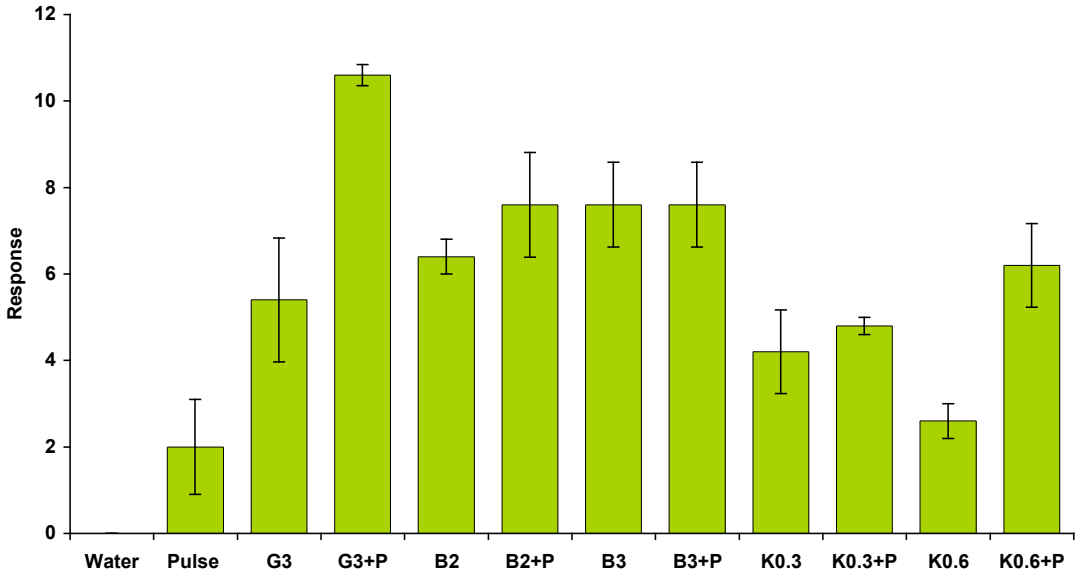

Figure 34. Herbicide efficacy based on *Acetosa vesicaria* response in a field trial. Treatments are presented in Table 8. Vertical bars represent ± standard error of the mean (n=5). Where: 0= new growth and flowers; 2= new growth; 3= alive and healthy (no new growth or flowers); 4=green, slightly stressed with new growth; 5=green, very stressed with new growth; 6=red or burnt leaves and new growth; 7=dead leaves and new growth; 8= green and very stressed; 9=red or burnt and very stressed; 10= dead with flexible stems; 11= dead and dry.

Table 10. Production of new flowers six weeks after herbicide treatment

Treatment	New flowers (%)*	
Water	100±0	a
Pulse	54±15.1	b
Glyphosate	26±18.9	bc
Glyphosate + Pulse	0	c
2,4-DB (2ℓ/ha)	2±1.7	c
2,4-DB (2ℓ/ha) + Pulse	7±4.4	c
2,4-DB (3ℓ/ha)	3±2.2	c
2,4-DB (3ℓ/ha) + Pulse	4±1.9	c
Dicamba (0.3ℓ/ha)	17±14.5	bc
Dicamba (0.3ℓ/ha) + Pulse	41±19.6	bc
Dicamba (0.6ℓ/ha)	54±31.7	b
Dicamba (0.6ℓ/ha) + Pulse	19±38.8	bc

4.2.5 Pot trial 1

Leaves of *Acetosa vesicaria* appeared burnt immediately following application of Pulse (P), with and without glyphosate. Leaves from treatments with Pulse and glyphosate (regardless of the dosage) became increasingly red (Fig. 35) and eventually died. Burnt leaves of control treatment containing Pulse (no herbicide) did not redden and die but the burnt spots remained (Fig. 36).

Figure 35. The red and chlorotic appearance of *Acetosa vesicaria* leaf three weeks after the application of glyphosate and Pulse.

Plants from all the control treatments (no herbicide) and the lowest rate of glyphosate produced new flowers (Table 11). Plants treated with the lower two rates of glyphosate (1 ℓ/ha & 3 ℓ/ha) together with the surfactant Agral (W) did not produce new flowers but immature perianths continued to mature. Six weeks after application, with the exception of 1 ℓ/ha glyphosate (G1), 1 ℓ/ha glyphosate plus Agral (G1+W) and 3 ℓ/ha glyphosate plus Agral (G3+W), all herbicide treatments resulted in a

significantly (P<0.001) lower plant fresh weight, with most of them dying (Table 11), this corresponded to a significantly lower dry weight for these plants.

Figure 36. The burnt appearance of *Acetosa vesicaria* leaf three weeks after the application of Pulse.

Table 11. Responses of *Acetosa vesicaria* to three concentrations of glyphosate with and without the addition of the penetrant Pulse (P) and/or the wetting agent Agral (W).* Glyphosate treatments are presented in Table 5.

Treatment	Wet weight (g)	Dry weight (g)	Height (mm)	Flowering
Water	16.1 a	5.3 a	138.0 a	3.0 a
Pulse (P)	16.0 a	5.2 a	126.7 ab	3.0 a
Agral (W)	15.0 a	5.4 a	132.7 a	3.0 a
P+W	15.8 a	5.8 a	121.3 ab	3.0 a
G1	15.4 a	5.3 a	113.3 b	2.2 b
G3	4.3 b	2.8 bd	7.3 c	0.3 c
G6	2.9 b	2.5 b	0 c	0 c
G1+P	2.0 b	1.8 b	0 c	0 c
G3+P	2.5 b	2.4 b	0 c	0 c
G6+P	2.5 b	2,4 b	0 c	0 c
G1+W	14.0 a	6.5 c	77.0 d	1.9 bd
G3+W	8.5 c	3.3 d	44.7 e	1.2 d
G6+W	4.4 b	2.5 b	12.3 c	0.4 c
G1+P+W	2.7 b	2.2 b	1.0 c	0 c
G3+P+W	2.9 b	2.4 b	0.7 c	0 c
G6+P+W	2.5 b	2.1 b	0 c	0 c

*Means in a column followed by the same letter are not significantly different (P=0.05)

Plants from all the control treatments continued to grow as did some plants from the 1 ℓ/ha glyphosate (G1), 1 ℓ/ha glyphosate plus Agral (G1+W) and 3 ℓ/ha glyphosate plus Agral (G3+W) treatments. Others plants from these treatments appeared to be dying and after four weeks new shoot growth appeared near the base of the plant, but all glyphosate treated plants showed visual symptoms of injury (Fig. 37). All plants treated with glyphosate combined with pulse died regardless of the concentration.

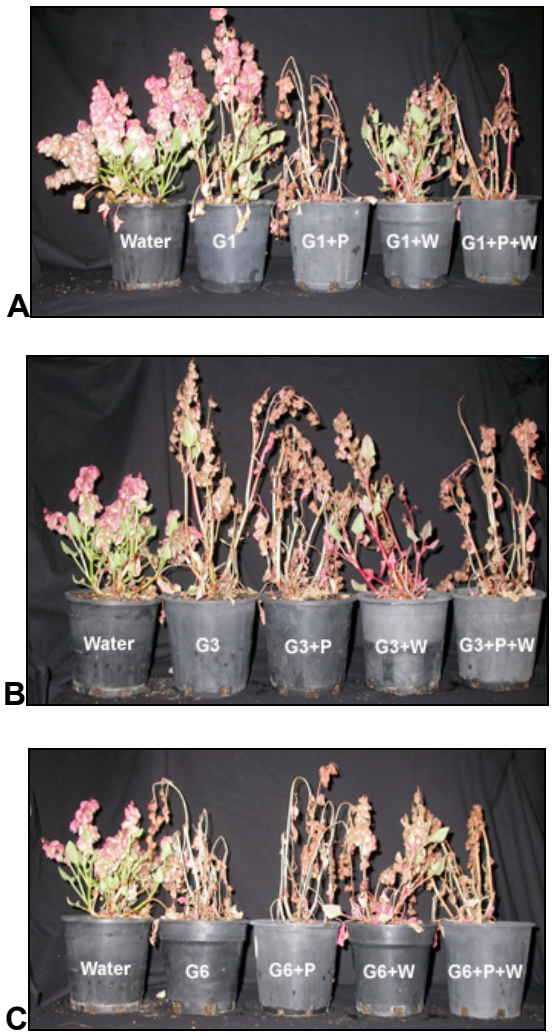

Figure 37. Efficacy of glyphosate treatment on *Acetosa vesicaria* in a pot trial. A. 1 ℓ/ha glyphosate; B. 3 ℓ/ha glyphosate; C. 6 ℓ/ha glyphosate.

4.2.6 Pot trial 2

Efficacy of metsulfuron-methyl was poor with or without the addition of Pulse (P) and/or Agral (W). The most effective treatment was 7.5 g/ha metsulfuron-methyl with the addition of both Pulse (P) and Agral (W) (Table 12). Pulse treated *A. vesicaria* leaves appeared burnt immediately following application. With the addition of metsulfuron-methyl, particularly the highest concentration, leaves became very red and many of these leaves eventually dried and fell from the plant. Plants from all treatments were able to recover and produce new growth and, with the exception of three treatments (M5+P, M7.5+P & M7.5+P+W), produce new flowers.

Table 12. Efficacy of metsulfuron-methyl treatment on *Acetosa vesicaria*, with and without the addition of the penetrant Pulse (P) and/or the wetting agent Agral (W). *Metsulfuron-methyl treatments are presented in table 5.

Treatment	Wet weight (g)	Dry weight (g)	Height (mm)	% New Flowers
Water	41.1 a	19.4 a	349.7 a	67 a
Pulse (P)	35.6 a	18.9 a	287.6 a	60 a
Agral (W)	38.4 a	19.2 a	324.5 a	70 a
P+W	37.6 a	18.7 a	298.5 a	63 a
M2.5	31.0 b	18.3 ab	170.3 b	10 b
M5	25.2 b	18.1 b	80.6 c	7 b
M7.5	26.4 b	17.8 b	154.2 b	3 b
M2.5+P	27.8 b	17.6 b	153.0 b	7 b
M5+P	25.6 b	17.9 b	97.3 bc	0 b
M7.5+P	23.3 bc	17.7 b	70.0 cd	0 b
M2.5+W	34.8 b	17.7 b	213.7 b	37 ac
M5+W	30.9 b	18.2 b	177.3 b	20 bc
M7.5+W	25.8 bc	17.8 b	117.0 b	3 b
M2.5+P+W	28.1 b	17.6 b	129.7 b	17 bc
M5+P+W	24.6 bc	17.2 bc	101.7 bc	3 b
M7.5+P+W	20.1 c	16.3 c	43.7 d	0 b

*Means in a column followed by the same letter are not significantly different (*P*=0.05)

4.2.7 Pot trial 3

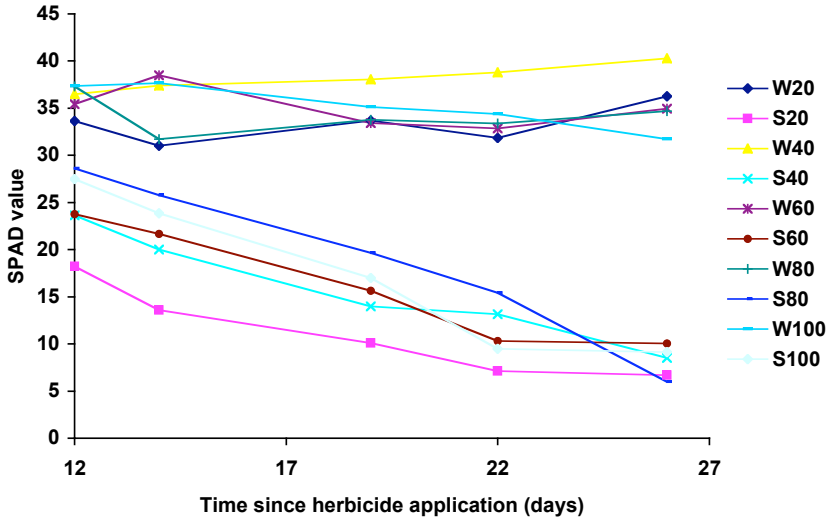

Figure 38. SPAD value as a measure of chlorophyll content for *Acetosa vesicaria* leaves over time since application of 3 ℓ/ha glyphosate with 2 ml/ℓ Pulse (S) or water (W) to plants grown in pots and watered with different amounts to simulate water stress.

Prior to harvesting plants there was no significant difference in the chlorophyll content (SPAD value) of herbicide sprayed plants (S) within any of the water treatment regimes and the decline in chlorophyll content was similar for all herbicide treated plants regardless of the watering regime (Fig. 38). The chlorophyll content of

leaves sprayed with water (W) (no herbicide) remained reasonably stable for the duration of the experiment with some variation between watering treatments.

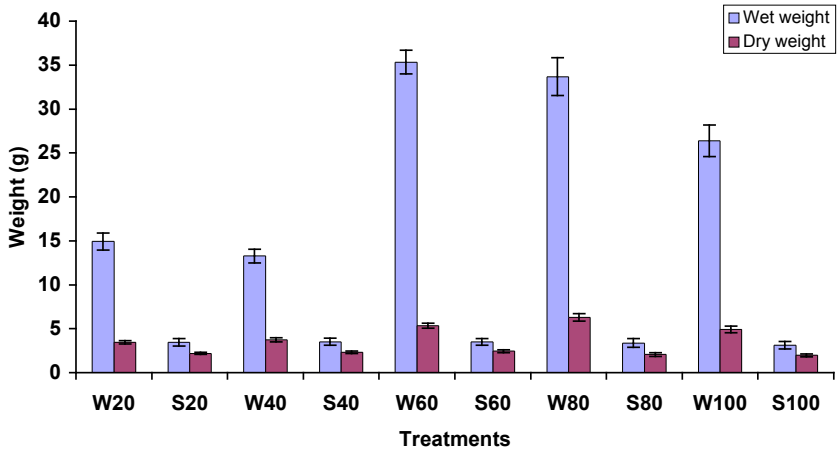

Figure 39. Wet and dry weights of *Acetosa vesicaria* plants 30 days after application of 3 l/ha glyphosate with 2 ml/l Pulse (S) or water (W) to plants grown in pots and watered with different amounts to simulate water stress. Vertical bars represent ± standard error of the mean (n=3).

All herbicide treated plants died and correspondingly there was no significant difference between the wet weights of herbicide sprayed plants (S) regardless of the water regime. Plants not sprayed with herbicide (W) were severely affected when watered at 20 and 40 percent of field capacity and the corresponding wet weights were significantly lower (P<0.001) than the other three watering regimes. Plants watered at 100% field capacity also had a significantly lower wet weight than those watered at 60 and 80 percent of field capacity (Fig. 39).

4.2.8 Germination of seed collected from herbicide treated plants

Glyphosate significantly reduced (P<0.001) the germination percentage of *A. vesicaria*, particularly 6 ℓ/ha glyphosate with the addition of Pulse (G6+P) (Fig. 40). Agral (W) reduced the effectiveness of glyphosate when combined with the higher two application rates.

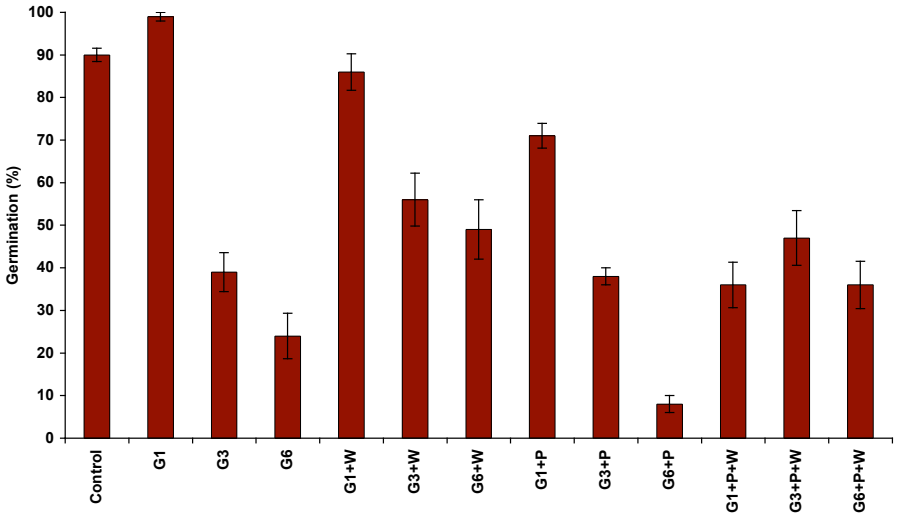

Figure 40. Germination of seed collected from glyphosate treated plants (pot trial 1). Treatments are presented in table 5. Vertical bars represent ± standard error of the mean (n=5).

Glyphosate reduced the vigour (length) of seedlings in all treatments with the exception of 1 ℓ/ha glyphosate plus Pulse (G1+P) these germinants were significantly (P<0.001) more vigorous than all other treatments including the control (Fig. 41)

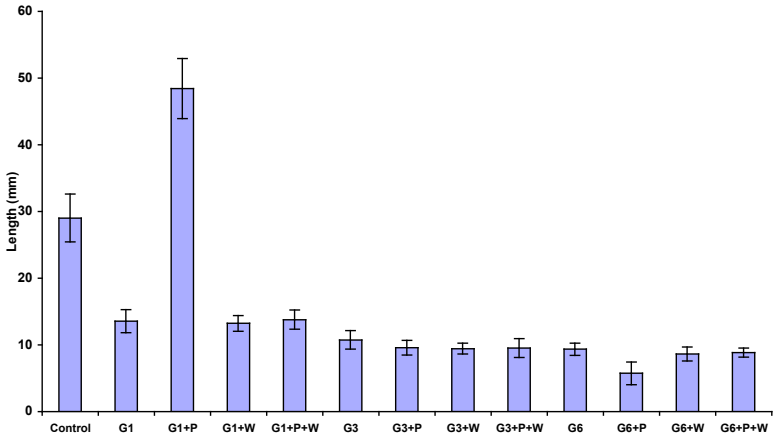

Table 41. Length of germinants (Fig. 39) ten days after commencement of experiment. Vertical bars represent ± standard error of the mean (n=5).

4.3 Discussion

The results of this research have clearly demonstrated that glyphosate is the most effective tested herbicide for control of *A. vesicaria.* This is somewhat surprising as we expected that one of the selective herbicides, such as asulam used in control of docks, would have had at least some measure of effectiveness and having less impact on native plants. In all experiments glyphosate was the only herbicide to achieve effective control especially with the addition of the surfactant Pulse®.

Increasing weed control using glyphosate or other herbicides with a surfactant has varied with the type of surfactant, the growth stage and weed species under evaluation (Molin and Hirase 2005). Cationic surfactants have been found to be more effective than non-ionic surfactants in increasing the efficacy of glyphosate in weed control (Aladesanwa and Oladimeji 2005) and can increase the efficacy when growing under environmental stresses such as limited moisture. Our research established the application of Pulse® an organosilicone surfactant increased the efficacy of glyphosate on *A. vesicaria* particularly when applied to mature flowering plants. We also demonstrated Agral® a non-ionic surfactant did not increase the efficacy of glyphosate, metsulfuron-methyl, chlorsulfuron or asulam; furthermore this surfactant appeared to reduce the action of glyphosate.

In this research we found that the highest concentration of glyphosate (6 ℓ/ha) without the addition of a surfactant was as effective as 3 ℓ/ha glyphosate with the addition of Pulse® for controlling *A. vesicaria* (Tables 9 and 11, Figs. 31 and 37). Also, the addition of Pulse® to glyphosate reduces the germinability and subsequent vigour of *A. vesicaria* (Figs. 40 and 41). As stated by Leaper and Holloway (2000), glyphosate salts can be quite effective when applied alone (without adjuvant), providing the dose is high enough. However, to reduce the 'off-target' damage to native taxa and to decrease pesticide inputs into the environment it is important for herbicide concentrations to be as low as possible and the addition of adjuvants such as Pulse® make this possible.

Despite the benefits of using the lowest application rate to effectively control *A. vesicaria*, 6 ℓ/ha glyphosate plus Pulse is four times more efficient in reducing germination numbers than 3 ℓ/ha glyphosate plus Pulse (Fig. 40). What's more, there

are some concerns regarding the success of rates lower than 6 ℓ/ha glyphosate on larger flowering plants. Schatral (2001) reported on viability of *A. vesicaria* seed collected from glyphosate treated plants and recorded a high viability for both the treated and untreated seeds using a tetrazolium test. Tetrazolium viability tests do not always correspond with germination results (for example *Bressica rapa* ssp. rapa; Poulsen *et al.* 2006). Additionally, *A. vesicaria* seed collected from Newman LIA, Plutonic Mine and West Angelas Bore were sites previously treated with glyphosate (pers com. K. Walker), these seed had significantly lower viability than the three collection points for fresh seed (Fig.10).

Initial experiments demonstrated that metsulfuron-methyl arrested flowering (Table 9) and despite a poor impact on the plant (Table 9) we considered it was worth investigating this herbicide further. Unfortunately despite increasing the concentration (Fig. 33) combining metsulfuron-methyl with glyphosate (Figs. 32 and 33) and conducting controlled pot trials (Table 12) there was no increase in the efficacy of metsulfuron-methyl on *A. vesicaria*.

Although the results have not been shown, it is important to note that glyphosate has a severe impact on many native plants. In one field trial we recorded 48 species of native plants and, although not all species occurred in each spray plot, clearly the impact of glyphosate on native species was severe, whereas asulam, metsulfuron-methyl and chlorsulfuron had very little impact on the native species. Brown and Brooks (2003) have also reported minimal impact to Western Australian native species when testing the influence of metsulfuron-methyl and chlorsulfuron.

Under favourable environmental conditions a lower concentration of herbicide will kill most target weeds, however under less than ideal conditions such as low rainfall a higher dose will be required and may result in unsatisfactory control (Medd *et al.* 2001). Surprisingly, our results have shown that water stress does not decrease the efficacy of glyphosate (Figs 38 and 39), though it must be stated that this test was undertaken in the controlled environment of the glasshouse and we are unsure how the results will translate to the field. The SPAD 502 Chlorophyll Meter instantly measures the amount of chlorophyll content, a key indicator of plant health. Our results demonstrate that regardless the amount of water supplied to the plants all

glyphosate treated plants had a significant decline in plant health over a 16 day period compared to the controls (Fig. 38), corresponding to a significant decline in dry matter (Fig. 39). Despite this result, it would be prudent to undertake herbicide spraying when the plants are in good health as most literature suggests reduced efficacy of herbicides when applied to plants that are affected by water-deficit stress (Abbott and Sterling 2006; Dhareesank *et al.* 2005; Medd *et al.* 2001).

Effective weed control during the early period of the growing season is critical to successful control of *A. vesicaria* for several reasons; the simplest being the ease and uniformity of spray application on seedlings rather than 1x1 m mature flowering plants. The lower application rate of 3 ℓ/ha glyphosate plus Pulse may be applied to seedlings with excellent results. Despite the reduction of germinable seeds following application of glyphosate, it is preferable to spray prior to flowering to effectively reduce the potential soil stored seed bank.

It is important to note that chemical control is only one part of the strategy to control *A. vesicaria*. High levels of seedling recruitment have been observed after rainfall and as previously mentioned these plants can flower and produce seed when very small. Disturbance tends to promote germination and establishment of infestations of *A. vesicaria* and without follow up control of germinants further spread of this serious environmental weed is certain.

5.0 Conclusion and Recommendations

Acetosa vesicaria can flower and set seed in adverse environmental conditions such as drought. The seed have two colour morphs; a larger pale seed and a smaller dark seed. Initially the pale seed germinates more readily, however after a period of after-ripening there is no difference in germination between the colour morphs. There is no physical dormancy preventing germination because water uptake (imbibition) is rapid. Germination of fresh seed is very low, but 12 weeks after seed fall from the parent plant the percentage of seed germination reaches a maximum (*ca* 80%), with 50% of seed able to germinate after four weeks. There is a chemical inhibitor in the tissues of the perianth preventing precocious germination. An adequate rainfall event is required to wash this inhibitor from the tissues for germination to occur, particularly when the seed is fresh.

Acetosa vesicaria seeds germinate in higher numbers with alternating temperatures 5^{o}C and 18^{o}C, which relate to natural winter conditions that are more conducive to growth of this species. Light, although not essential, increases germination and is most likely because the seeds are contained within the perianth that lies on the soil surface. The relative ageing experiment has demonstrated that *A. vesicaria* seed viability is average for geosporous species.

Seeds remain within the fruiting valves (perianth) and form dense mats that slowly break down over time to fibrous husks. *A. vesicaria* seeds respond with greater germination numbers to the stimulant gibberellic acid. Butenolide is more effective as a germination stimulant when sprayed on seed contained within the perianth on the soil surface. Unfortunately, butenolide is under a development licence held by the University of Western Australia (UWA), Murdoch University and BGPA and is unavailable for commercial use at this time. *A. vesicaria* seeds enclosed within the perianths are principally dispersed by wind, water and human activity.

Of all the tested herbicides only glyphosate is effective for controlling *A. vesicaria* particularly when the surfactant Pulse® is added. Many glyphosate-based herbicides have a surfactant added but we recommend purchasing glyphosate without a surfactant. This research has demonstrated that the surfactant Agral® decreases the effectiveness of glyphosate.

Glyphosate can be applied when *A. vesicaria* plants appear water stressed and be equally effective, however label recommendations state that plants should not be sprayed under drought stress. Unfortunately glyphosate has a detrimental effect on the native flora. We recommend spot spraying where possible and hand pulling of isolated plants to reduce the impact on native vegetation.

Our recommendations for spray applications of glyphosate for control of *A. vesicaria* are:

- For small plants less than 15 cm tall 3 ℓ/ha glyphosate 360 plus 2 ml/ℓ Pulse®. Alternatively; for the dry formulation with a concentration of 875 g/kg, 1 g/ℓ glyphosate plus 2 ml/ℓ Pulse®; or 0.04 ml/ℓ Glyphosate 360 plus 2 ml/ℓ Pulse®. One litre of these mixtures will cover 8m^2.
- For larger flowering plants 6 ℓ/ha glyphosate 360 plus 2 ml/ℓ Pulse®. Alternatively; for the dry formulation with a concentration of 875 g/kg, 2 g/ℓ glyphosate plus 2 ml/ℓ Pulse®; or 0.08 ml/ℓ glyphosate 360 plus 2 ml/ℓ Pulse®. One litre of these mixtures will cover 8m^2.

Large scale spraying operators should deliver the same rates per hectare, however the amount of carrier (water) used will be dependent on the type of equipment used, such as delivery systems and jet types.

The most effective time to apply glyphosate to *A. vesicaria* is early in the growing season because:

- Seedlings are more responsive to the herbicide.
- It is easier to apply a uniform application of the herbicide.
- A lower concentration of 3 ℓ/ha glyphosate 360 may be applied reducing the detrimental effect on the native vegetation.
- Seed production will not occur thus diminishing the seed bank.
- Follow-up applications are recommended with each rain event.

As fire has a huge impact on vegetation and is considered to be a “disturbance” we recommend that the impact of fire on the seed bank and germination be examined. Butenolide is a chemical derived from smoke that has shown to enhance germination, however the question of how the heat of fire affects the dense mats of

seed and plant material must be answered. We suspect that it will be a useful management tool to deplete a large amount of the seed with follow up herbicide treatment, but this proposition requires supporting evidence.

Prevention is always the best policy with weeds. Clean all machinery before entering an uninfected area, and always wash machinery down after use. Inspect seed batches for weed seed before use. Keep staff and the public informed about weeds and encourage them to report occurrences to environmental staff or land management officers. GPS sites for a data base so all infestations can be documented and visited on a regular basis. With time and perseverance it is possible that this weed will be kept under control, but there must be a united approach across land tenure and amongst all land management agents.

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