

Research reports

Germination of *Hymenachne amplexicaulis* and *H. acutigluma* under contrasting light, temperature and nitrate regimes

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Abstract

A laboratory experiment compared germination of the invasive exotic grass *Hymenachne amplexicaulis* (Rudge) Nees and the native *H. acutigluma* (Steud.) Gilliland. Seeds of both species were exposed to combinations of light (constant dark, alternating dark/light or constant light), temperature (constant or alternating) and nitrate regimes (with or without the addition of KNO₃). Three seed lots of *H. amplexicaulis* (fresh, two and four months old) and one of *H. acutigluma* (fresh seed) were tested.

A significant temperature × light × nitrate × seed lot interaction occurred. At a constant temperature very few seeds of either *H. amplexicaulis* or *H. acutigluma* germinated, regardless of the light regime or addition of KNO₃. Generally, maximum germination occurred under a combination of alternating temperature, the presence of light (either constant or alternating) and the addition of KNO₃. The exception was four month stored *H. amplexicaulis* seed, which reached maximum germination without the need for KNO₃. Fresh seeds of both *H. amplexicaulis* and *H. acutigluma* exhibited similar germination requirements.

These findings suggest that conditions that buffer seeds from light and/or temperature fluctuations could reduce germination and possibly extend the life of seed banks of both *H. amplexicaulis* and *H. acutigluma*. Conversely, for land managers trying to control the exotic *H. amplexicaulis*, activities that create more favourable conditions for germination may help deplete seed banks faster.

Introduction

In the Northern Territory the native grass, *Hymenachne acutigluma* (Steud.) Gilliland, is used as fodder by some land managers who have seasonally inundated wetland areas on their properties (Calder 1981, Charleston 2006). The ability of *H. acutigluma* to grow in water during the wet season enables an abundant supply of feed to be available for grazing animals during the drier times of the year once the water has receded (Humphries *et al.* 1991).

In more recent years, artificial ponded grazing systems based on these natural occurrences have been developed, particularly in Queensland, in order to supply nutritious fodder during the dry season (Wildin 1989, Charleston 2006). One of the principal grasses recommended for planting is the exotic *H. amplexicaulis* (Rudge) Nees, a native of Central and South America.

Whilst *H. amplexicaulis* provides valuable fodder and is more than capable of fulfilling the role for which it was introduced, it has escaped from these ponded systems and is now posing concerns to other agricultural enterprises, particularly the sugar industry (Charleston 2006). Furthermore, Humphries *et al.* (1991) have identified it as one of Australia's most serious environmental weeds because of its ability to degrade tropical wetlands and destroy breeding habitats of waterbirds. Such detrimental impacts have led to its classification as a Weed of National Significance (Thorpe and Lynch 2000).

Hymenachne amplexicaulis is a perennial grass that can grow up to 2.5 m tall (Csurhes *et al.* 1999, Charleston 2006). It can form extensive colonies spreading on dry land or floating on water (Wildin 1989). It

is able to grow in water of more than one metre depth (Csurhes *et al.* 1999) and the flooding and drying cycles that frequently occur throughout the year are conducive to broadscale regeneration by seed (Wildin and Chapman 1987).

Large quantities of seed are produced following flowering which generally occurs from April to June in Queensland, although plants have been known to flower between March and September in very wet years (Charleston 2006). The seeds are spread largely by water (Csurhes *et al.* 1999) however waterbirds such as magpie geese (*Anseranas semipalmate*) may also be vectors (Charleston 2006).

H. amplexicaulis is also capable of vegetative reproduction, and stem sections can be spread by flooding, farm machinery (such as cane harvesters), and attempted mechanical control methods (Csurhes *et al.* 1999). Its reproductive ability, combined with the fact that it grows in ponded areas, means *H. amplexicaulis* has the potential to become a successful and widely spread weed as it can be carried through both man made and natural water systems. It is most successful growing in tropical low lying freshwater wetland areas that receive sediment run-off from croplands (Csurhes *et al.* 1999), although it has also been observed in streams without nutrient enrichment. In 1997, *H. amplexicaulis* was reported to have invaded at least 1000 hectares in the wet Tropics Bioregion of North Queensland (Low 1997).

Currently, there is limited ecological data available to fully determine the weediness of *H. amplexicaulis*. Any information that can be found is usually anecdotal, although Kibbler and Bahnisch (1999a,b) undertook some detailed quantitative assessments on the physiology of both *H. amplexicaulis* and the native *H. acutigluma*. They found that *H. amplexicaulis* is highly adapted to flooded conditions through its capacity for rapid elongation of the stem, production of new leaves at the water surface, the presence of aerenchyma throughout its organs, and the production of adventitious roots. These features allow it to survive and grow in deep water and produce large amounts of biomass. Whilst *H. acutigluma* displays similar features it is not so proficient at maintaining photosynthetic leaf area during flooding and it grows best in warmer climates (such as the Northern Territory) where it is able to maintain a high photosynthetic rate. In cooler climates (such as central Queensland) *H. amplexicaulis* appears to be able to maintain a higher photosynthetic rate than *H. acutigluma*, making it potentially weedier and capable of occupying a larger geographic range (Kibbler and Bahnisch 1999b).

This aim of this paper was to examine the germination of *H. amplexicaulis* and *H. acutigluma* under different light and

temperature regimes with and without the addition of nitrate. For *H. amplexicaulis*, comparisons were also made between different aged seed lots collected from the same site in order to test whether germination requirements varied with seed maturity. Information from this study will help identify both the conditions that are most conducive to high germination rates and those that may limit germination. Differences between the native *H. acutigluma* and the exotic *H. amplexicaulis* may also help explain the weediness of *H. amplexicaulis* and contribute to the development of effective management strategies.

Materials and methods

Seed samples

All seed samples were collected within the Wet Tropics region of northern Queensland. *H. amplexicaulis* seed was obtained from a drainage ditch approximately 20 km east of El Arish (17°47'S, 146°04'E). Three sampling trips were made, each two months apart (October 1999, December 1999 and February 2000). Germination trials commenced in February 2000, making seed lots at the commencement of the experiment 0, 2 and 4 months old. *H. acutigluma* seed was collected from a drain within the Innisfail township (February 2000) (17°31'S, 149°01'E) on the same day as the last *H. amplexicaulis* seed lot was collected so as to enable inter-species comparison. At the time, a location containing both *H. acutigluma* and *H. amplexicaulis* could not be found. However, inspection of climatic records for the two sites indicated that the climatic conditions under which plants were growing were similar and both species had abundant moisture, being located along continually flowing drains. Nevertheless, the literature reports examples where the environmental conditions under which seeds have developed and matured have had an influence on germination (Roberts and Boddrell 1984, Baskin and Baskin 1998), and any interpretation of results needs to take consideration of this potential environmental influence.

Seeds were removed from the seed heads by pulling inflorescences gently through a slightly closed hand. This ensured that the seed collected was mostly loose, mature seed ready to dislodge. For each seed lot, seeds collected from individual seed heads were thoroughly mixed to produce a single working sample. Eight representative sub-samples of 50 seeds were then taken to determine the percentage of empty, filled and viable seeds. Seed weight (after drying in an oven at 80°C for 48 hours) of individual seed lots was also obtained from four different sub-samples of 100 seeds. After collection, all seeds were stored in the dark (within brown paper bags) in an air-conditioned room kept at a constant temperature of 25°C until commencement of the trial.

Experimental design

A split plot experiment replicated four times was undertaken, with temperature regime allocated to main plots, light regime to sub-plots and seed lots and nitrate status to sub-sub plots. There were two temperature regimes (Constant (25 ± 1°C) or alternating (12 hour cycles, of 30/20 ± 1°C)) and four light regimes (constant light, constant dark (samples read daily under green light), constant dark (samples read only at the end of the trial), and alternating light (12 hour cycles of dark/light)). Sub sub plots comprised the four seed lots wetted up with either distilled water or a solution of potassium nitrate (KNO₃).

Two dark treatments were included to enable estimation of germination rates. Whilst enclosing Petri dishes in aluminium foil and not reading until the end of the trial ensures that seeds are exposed to no light, daily germination recordings can not be collected. Alternatively, recording daily germination under green light (Williams 1983a, Baskin and Baskin 1998) does allow germination rates to be determined, but there is a slight possibility that some stimulation of germination may still occur for some species (Baskin and Baskin 1998). Comparisons of final germination of both dark treatments were used as an indication of whether the green light treatment was representative of the dark and could be used for calculation of germination rates.

Controlled environment cabinets that contained three separate compartments that could be assigned to specific light regimes were used. Cabinets were chosen randomly to have either constant or alternating temperatures and individual compartments were randomly allocated lighting regimes. The two dark treatments were placed in the same compartment. Cabinets were run and monitored for one week prior to commencement of the experiment to ensure that selected temperature and light regimes were being imposed.

Each experimental unit (sample) comprised 50 randomly selected seeds placed on two Whatman™ No 4 filter papers in 90 × 14 mm Petri dishes. The filter papers were initially moistened with either distilled water or a solution of 0.2% potassium nitrate (KNO₃). Potassium nitrate is known to promote germination of dormant seeds under some conditions (Egley 1986, Whalley 1987, ISTA 1996). Any additional watering throughout the trial period was with distilled water only. To enable moisture to be available for the 21 day period for seeds in total dark, filter papers were placed on top of inverted watch glasses (85 mm) located within the Petri dishes. The edge of the filter paper sat in a reservoir of water and acted as a wick, leaving seeds continuously moist but not sitting in free water. Those experimental

units placed in constant and alternating light were enclosed in clear plastic bags (to prevent drying out from occurring) and placed in a single layer on the respective shelves. Those placed in total dark and read daily under green light were placed in black plastic bags, and those placed in dark and read only at the end of the trial were wrapped in aluminium foil and placed in black plastic bags.

Germinated seeds (identified by radicle emergence) were counted and removed each day for 21 days. Seeds that did not germinate within this time were checked for viability using the tetrazolium method (Moore 1985). A germination rate index was used to compare the rate at which seed lots germinated. The index was calculated as the time taken to reach 50% of maximum germination (Gramshaw 1972).

Statistical analysis

Statistical analysis using analysis of variance was performed on arcsine-transformed data that was later back-transformed for presentation in figures and tables. Fisher's protected LSD test was used to identify differences between treatments and interactions.

Results

Seed lots

There were significant differences ($P < 0.05$) between the three *H. amplexicaulis* seed lots with regard to their weight and the quantity of empty, viable and dead seeds (Table 1). The amount of empty seed varied from as low as 11% to as high as 50% for the two month old and fresh seed lots, respectively. For the fresh and two month old seed lots, caryopses that contained an embryo exhibited a high level of viability, with an average of only 4% dead seed. In contrast, the four month old seed lot had five times more dead seed.

The *H. acutigluma* seed displayed similar characteristics to the *H. amplexicaulis* seed lot collected at the same time in terms of the amount of empty seeds, but differed in the proportion of viable and dead seeds. *H. acutigluma* seed contained on average five times more dead seed than that of *H. amplexicaulis*.

Seed germination

There were no significant differences ($P > 0.05$) in total germination between the two dark treatments or the two light treatments (constant and alternating light). Therefore this data has been pooled and the results presented as either dark or light.

A significant temperature × light × nitrate × seed lot interaction ($P < 0.05$) occurred for seed germination (Table 2). At a constant temperature, very few seeds of either *H. amplexicaulis* or *H. acutigluma* germinated, regardless of whether they received light or KNO₃. The only exception

was four month old *H. amplexicaulis* seed, which demonstrated increased germination in response to light.

Under an alternating temperature regime, germination of fresh and two month old *H. amplexicaulis* and fresh *H. acutigluma* seed lots in the dark was not significantly different to that for constant temperature if germinated in water only. Four month old *H. amplexicaulis* seed, on the other hand, was stimulated to germinate in the dark by alternating temperatures.

The addition of either KNO_3 or light significantly increased germination of all seed lots under an alternating temperature regime. Maximum germination, however, for most seed lots occurred when both factors were present. The exception was four month stored *H. amplexicaulis* seed, which reached maximum germination under an alternating temperature regime when light was present, but did not require the addition of KNO_3 (Table 2).

Germination rate

Because of low germination responses to many of the imposed treatments, comparison of germination rates between the four seed lots was restricted to those treatments that promoted optimum germination (i.e. alternating temperatures and the presence of light). For these, there were significant differences ($P < 0.05$) in the rate at which seeds germinated (Figure 1). All of the three *H. amplexicaulis* seed lots differed significantly, with the highest germination rates recorded for two month old seed. There was no significant difference between freshly collected seeds of *H. amplexicaulis* and *H. acutigluma*. Across all seed lots the earliest that germination commenced following exposure to moisture was four and a half days, with peak germination generally occurring over the following 36 hours.

Discussion

In most instances, exposure to a combination of light, alternating temperature and KNO_3 stimulated maximum seed germination of both *H. amplexicaulis* and *H. acutigluma*. The only exception was *H. amplexicaulis* seed that had been collected and stored for the longest duration (four months) prior to testing. This seed lot reached its maximum germination under an alternating temperature when light was present and did not require the addition of KNO_3 .

Light, alternating temperatures and nitrate ions are widely recognized for their ability to stimulate the germination of many weed seeds. For example, Steinbauer and Grigsby (1957) studied the germination requirements of weed species from 15 families. They found that more than 75% germinated better in alternating rather than constant temperatures, 35% germinated better in light than in darkness

and 50% germinated better in dilute solutions of nitrate as compared with water.

A requirement for diurnal temperature changes is ecologically significant in that the more dependent a species is on temperature change the less likely it is to

germinate, enabling enforced dormancy. This effectively increases the opportunity for freshly fallen seed to be incorporated into the seed soil population and not germinate straight away (Williams 1983a).

Table 1. Seed characteristics of *H. amplexicaulis* and *H. acutigluma* seed lots. Values within columns followed by the same letter are not significantly different ($P < 0.05$).

| Seed lots | Collection date | 100 seed dry weight (g) | Empty seed (%) | Viable seed (%) | Dead seed (%) |
|------------------------------------|-----------------|-------------------------|----------------|-----------------|---------------|
| <i>H. amplexicaulis</i> (fresh) | February 2000 | 0.0275 b | 50 a | 46.5 b | 3.5 b |
| <i>H. amplexicaulis</i> (2 months) | December 1999 | 0.0417 a | 11 c | 85 a | 4 b |
| <i>H. amplexicaulis</i> (4 months) | October 1999 | 0.0308 b | 36 b | 42.8 b | 21.2 a |
| <i>H. acutigluma</i> (fresh) | February 2000 | 0.0399 a | 47 a | 33.4 c | 19.6 a |

Table 2. The effect of temperature, lighting regime and seed status on germination (% of viable seed) of *H. amplexicaulis* and *H. acutigluma*. Values followed by the same letter are not significantly different ($P < 0.05$).

| Seed lots | Constant temperature | | Alternating temperature | |
|------------------------------------|----------------------|-------|-------------------------|-------|
| | Dark | Light | Dark | Light |
| H_2O | | | | |
| <i>H. amplexicaulis</i> (fresh) | 0 k | 1 k | 1 k | 24 i |
| <i>H. amplexicaulis</i> (2 months) | 0 k | 0 k | 3 k | 51 e |
| <i>H. amplexicaulis</i> (4 months) | 1 k | 27 hi | 32 gh | 62 cd |
| <i>H. acutigluma</i> (fresh) | 1 k | 0 k | 7 jk | 22 i |
| KNO_3 | | | | |
| <i>H. amplexicaulis</i> (fresh) | 0 k | 1 k | 10 j | 87 b |
| <i>H. amplexicaulis</i> (2 months) | 0 k | 3 k | 29 ghi | 99 a |
| <i>H. amplexicaulis</i> (4 months) | 3 k | 36 fg | 59 d | 67 c |
| <i>H. acutigluma</i> (fresh) | 0 k | 5 jk | 40 f | 88 b |

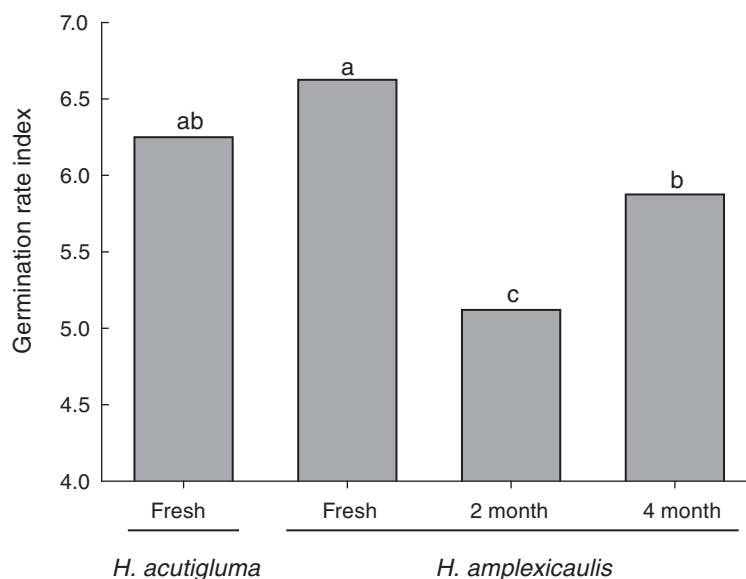


Figure 1. Germination rate of fresh *H. acutigluma* and fresh, two month and four month old *H. amplexicaulis* seeds. Bars followed by the same letter are not significantly different at $P < 0.05$.

Enforced dormancy could occur for both *H. amplexicaulis* and *H. acutigluma* because of the seasonally inundated habitats in which they grow. Having a layer of water on top of the soil may cause anaerobic conditions and/or prevent high or fluctuating temperatures necessary for germination from occurring. For example, Grime *et al.* (1981) suggest that seedling establishment around lakes, ponds, marshes and ditches is usually restricted to late spring when the water has receded and surface layers become exposed to air and increased radiation.

The failure of seed to germinate in darkness is common to species of disturbed habitats and marshland areas suggesting that light requirement of freshly dispersed seed prevents the germination of buried seed and initiates germination on disturbance (Grime *et al.* 1981). Preliminary results from a longevity study being undertaken on *H. amplexicaulis* support the findings from the current study. Significantly more viable seed remained in buried seed lots after four years compared with those located on the soil surface (Charleston 2006).

Nitrate is one of the factors known to influence dormancy under field conditions (Vincent and Roberts 1977, Derkx and Karssen 1993, Bell *et al.* 1999). *H. amplexicaulis* is suited to growing in areas of fluctuating water levels and where there is high nutrient and sediment runoff from nearby farms (Csurhes *et al.* 1999). Results from the current study suggest that this influx of nutrients, which would include nitrates, may promote the germination of hymenachne seed.

Although any of the three factors tested (light, temperature, nitrate) can have an effect on their own, the literature reports many instances where positive interactions between these factors have occurred (Roberts 1973, Vincent and Roberts 1977, Roberts and Benjamin 1979, Vincent and Roberts 1979, Saini *et al.* 1987). Vincent and Roberts (1977) suggest that experiments carried out in which only one of the three factors is investigated in isolation may be very misleading, particularly when an attempt is being made to interpret field results on the basis of laboratory experiments.

Saini *et al.* (1987) in a study of field pennycress (*Thlaspi arvense*) found that a combination of a transfer from low to high temperatures, light and nitrate induced 100% germination. Less than 1% germination occurred under light and dark conditions at a constant temperature, irrespective of whether KNO₃ was present or absent. Seed was released from dormancy when there was a change from a low to high temperature or daily alternating temperatures. Similarly, Williams (1983b), in a study of grassland plants, found that with one exception, none of the factors gave maximum germination individually; a

combination of at least two and occasionally three of the factors were required to give maximum germination. It was suggested that differential responses between species could have been associated with their level of relative dormancy. This could also explain the different responses that occurred between the three seed lots of *H. amplexicaulis*. The oldest seed lot germinated more readily in sub-optimal conditions and needed fewer factors present for maximum germination to occur. While this is the most probable explanation, there is a possibility that the environmental conditions under which the different seed lots matured could have also affected the level of innate dormancy and/or germination (Roberts and Boddrell 1984, Baskin and Baskin 1998). After all there were some characteristic differences between seed lots of *H. amplexicaulis* and between fresh seeds of *H. amplexicaulis* and *H. acutigluma* in terms of their size, and proportions of empty, dead and viable seeds. Further studies in this area are warranted, particularly given that there are now known sites that contain both *H. amplexicaulis* and *H. acutigluma* plants which would allow more direct comparisons to be made.

The findings from this study indicate that both the native *H. acutigluma* and the exotic *H. amplexicaulis* have similar germination requirements. Whether this also occurs for other key seed characteristics, such as longevity, warrants further investigation. For *H. amplexicaulis* the findings also provide an insight into possible management options that could be used to expedite rundown of the seed bank. For example, removal of above ground vegetative material through burning where possible may expose the soil to greater temperature fluctuations and higher light intensities and stimulate germination of the seed bank.

Acknowledgments

The authors acknowledge the assistance provided by staff at the Tropical Weeds Research Centre, especially Stephen Setter for collecting most of the seed. Special thanks also to Dr Joe Scanlan and Dr Dane Panetta for reviewing the manuscript.

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Fruit carrying characteristics of travellers into a quarantine zone in New South Wales in 1999/2000

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Abstract

Data was collected from 11 955 travellers entering the Fruit Fly Exclusion Zone. There was a continuing decline in the proportion of travellers with fruit however carriers continued to carry about six items of fruit as in past years. Generally the highest fruit carriage occurred at the start of the program and we postulate that regular travellers know that fruit fly road blocks do not occur in winter, and these travellers were caught with fruit when the program restarts each spring. Travellers originating from near the road block site were less likely to carry fruit. Retirees were more likely to carry fruit although fruit carriage by all traveller types declined compared with previous years. The most commonly carried fruit were pome (mainly apples), tomatoes, citrus, bananas, stone fruit and tropical fruit in that order. The continuing decline in the proportion of traffic carrying fruit was seen as a contributor to the overall fruit fly management of the Zone.

Introduction

Fruit fly cost the Australian horticulture industry \$128.7 million for the five year period July 2003 to June 2008 (Oliver 2007). Many strategies are used to maintain market access for the horticultural industry including pest eradication and prevention of re-entry into pest free regions after eradication campaigns. Strategies to prevent re-entry include pest monitoring, host and pest exclusion, community awareness and incursion controls. Roadside inspections to check vehicles for hosts of Queensland fruit fly (*Bactrocera tryoni* Froggatt) (Qfly) has been widely used in all states of Australia (Ballantyne 1992, Madge *et al.* 1997, Dominiak *et al.* 1998, Sproul 2001, Cantrell *et al.* 2002). In previous NSW programs, different host interception strategies were trialled and evaluated to further decrease the amount of fruit being carried from the Risk Reduction Zone (RRZ) into the Fruit Fly Exclusion Zone (FFEZ) (Dominiak *et al.* 2001, Dominiak and Barchia 2005a).

The effectiveness of the host exclusion program can be increased if the incursion risk factors are identified enabling vehicle inspections to target high risk times or

travel groups. If these risks are not known, road side inspections of the general public must adopt one of two philosophies. Either inspections must aim to inspect all traffic (but at a significantly high cost), or alternatively, a smaller proportion of traffic is assessed and it is accepted that a larger proportion of traffic escapes inspection. This second strategy can be made more effective if travellers are intrinsically encouraged not to carry fruit.

Some risks are already known and these risks come from diverse sources. Different types of fruit pose different risks (Dominiak *et al.* 1998, Dominiak and Barchia 2005a). Infested tomatoes and stone fruit were more frequently detected Qfly hosts. Cherries are stone fruit, and if infested, may support high numbers of larvae for their comparatively small size (Jessup personal communication). However cherries and pome fruit (apples and pears) are generally grown in colder areas and are unlikely to be infested. Bananas are usually harvested in a green condition and hard green bananas are not susceptible to fruit fly attack. Backyard fruit was regarded as high risk because of the general lack of care in its production, compared with fruit supplied to supermarket stores. Traveller types also pose different risks. Retirees are consistently the traveller type that mostly frequently carries fruit, followed by families, although the percentage of fruit carriage continues to decline with all traveller types. Trip origins and destinations significantly influence the risk of introducing infested fruit. Travellers originating from or going to the FFEZ and RRZ carry fruit less frequently while travellers from Queensland and inland New South Wales carry fruit much more frequently. Different highways have different profiles for fruit carriage, traveller type and types of fruit (Dominiak *et al.* 1998, 2000a,b, 2001, 2005a,b, Dominiak and Barchia 2005a).

In New South Wales (NSW), the TriState Fruit Fly Committee conducts an awareness campaign for both travellers and residents of the RRZ and FFEZ. In the past, this campaign has been delivered into five states. But even this campaign needs to be targeted if it is to efficiently use financial resources. This paper reports on the NSW