Passage and survival of *Acaciella angustissima* (Mill.) Britton & Rose and *Aeschynomene paniculata* Willd. ex Vogel seed through the sheep gut

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Summary *Acaciella angustissima* (syn. *Acacia angustissima)* (white ball acacia) and *Aeschynomene paniculata* Willd. ex Vogel (pannicle joint vetch), were rejected for release after their identification as potential weeds in pasture evaluation trials. These plants are now targeted for control and, where possible, eradication from old experimental sites across Queensland. It is suspected that domestic livestock, feral and native animals contribute to the movement of these seeds through the ingestion and defecation of viable seeds across the landscape. This aspect was explored by feeding the intact seeds of these two species to sheep in metabolism cages. Sheep faeces were collected each day for 5 days after which time the faeces were sieved and the surviving intact seeds were then collected, counted and germination tests undertaken. The results show that seeds of both species pass through sheep with most seeds being passed after 48 h with a percentage of these seeds being viable. Of the number of seeds fed, 4.25% were recovered for *A. angustissima* and 1.4% for *A. paniculata*. Seed recovered from the faeces had 0% and 13% germination for *A. angustissima* and *A. paniculata* respectively, but with additional post-digestion hot water scarification germination increased to 75% and 33% for *A. angustissima* and *A. paniculata* respectively. This paper discusses these results and the implications for the possible spread of these species across the northern Australian landscape.

Keywords Weed seeds, hardseed, legumes, digestion.

INTRODUCTION

Acaciella angustissima (syn. *Acacia angustissima)* (white ball acacia) and *Aeschynomene paniculata* (pannicle joint vetch) were rejected for release as pasture plants after they were identified as potential weeds in pasture evaluation trials where they were shown to be prolific producers of hardseed, widely adapted and relatively unpalatable (Cox *et al.* 2007). In the case of *A. angustissima* soil seed banks of 2322 seeds m^{-2} have been reported (Gardiner *et al.* 2008). These plants are now targeted for control and, where possible, eradication from old experimental sites across Queensland. However, these species have not been well studied and a better understanding of aspects of their ecology would be beneficial for strategising their control. Aspects of seed passage through the gastrointestinal tract of sheep and germination was the focus of this research as it is suspected that livestock, feral and native animals contribute to the movement and spread of these seeds across the landscape.

Acaciella angustissima is a bipinnate shrub legume from the Americas (Rico Arce and Bachman 2006) while *A. paniculata* has compound (pinnate) leaves and is an erect herbaceous legume native to tropical America. It has been noted germinating in cow manure (Csurhes 2009).

Other leguminous species with hard seeds are known to survive digestion and be dispersed by ruminants and in some cases have gone on to become established and develop into being environmental weeds (Paynter *et al.* 2003). The germination of hardseeded legumes is enhanced by scarification. Gardiner (1992) found for example that the germination of *Desmanthus* improved from 6% to 75% with scarification and also that after digestion in nylon bags in the rumen of steers some seed remained viable but had a germination of only 2.5%. However, with the addition of post-digestion scarification, germination was enhanced to 68% and it was concluded that digestion destroyed all soft seed leaving only the very hard seeds, which required additional scarification to germinate. Simao Neto and Jones (1987) also found that hard seeds were largely resistant to digestion. However, Thomson *et al.* (1990) showed that the germination of hard seeded legumes may increase with passage through sheep while Simao Neto *et al.* (1987) found seed recovery was affected by animal species, diet quality, hardseededness and seed size.

MATERIALS AND METHODS

Five merino wethers with a mean live weight of 35 kg were housed in individual metabolism cages at the School of Veterinary and Biomedical Sciences, James Cook University, Townsville, Australia. The wethers were acclimatised to the housing and had an *ad libitum*

high quality diet of lucerne pellets, hay and water and gained weight.

Due to limited seed stocks each animal was fed 10 g of seed of each species on day 1. The seeds were actively eaten by the sheep and a very high percentage of the seed was consumed. All faeces from each sheep were collected individually via a faecal collection apparatus each day for 4 days. The faeces were dried in a forced air over at 40°C and stored. A 20% by weight subsample of each sheep's daily faecal pellets was then rehydrated in a bucket of water and emasculated by hand and washed through a set of sieves ranging in size from 4 mm to 0.5 mm. The resulting fine faecal pieces and seeds were then sun-dried and put through a variable speed fan winnower, which separated the seeds from the faecal matter. Under a magi lamp seeds were separated into species and counted.

Germination studies used the maximum number of available intact seeds that were recovered from the faeces on day 2. Seeds were placed in 9 cm diameter Petri dishes containing two sheets of Whatman filter paper No.1001090. The filter paper was moistened daily. The Petri dishes were laid out in blocks on a laboratory bench top with ambient temperatures averaging 27.5°C. The seed treatments tested were as follows for each species over 7 days: (1) Control (intact untreated seeds), 25 seeds × 3 replicates; (2) Control plus hot water scarification (intact seeds soaked in 80°C water for 5 min), 25 seeds × 3 replicates; (3) Seeds recovered from faeces, 4 or 5 seeds × 3 replicates; and (4) Seeds recovered from faeces plus hot water scarification (as above), 4 or 5 seeds × 3 replicates. Due to small numbers of seeds fed and recovered, four seeds of *A. angustissima* and five seeds of *A. paniculata* were used per Petri dish.

The experiment was laid out as a randomised complete block with three replicates of each combination of the three factors (eight combinations in total). The number of seeds recovered from the faecal matter each day was analysed using analysis of variance (ANOVA) and where appropriate, pairwise comparisons were made using the 95% least significant difference (LSD).

The number of seeds germinated at day 7 was analysed using a generalised linear model (GLM), assuming a binomial distribution and a probit link function. All statistical analyses were conducted in GenStat, version 12.

RESULTS

Acaciella angustissima had 87 and *A. paniculata* 238 seeds per gram on average.

The number of seeds recovered peaked at day 2 (48 h) for both species with some seeds being passed and collected each day. There was a significant difference between the number of seeds recovered each day for *A. paniculata* ($P = 0.024$) but not for *A. angustissima*. For *A. paniculata* day 2 has a significantly higher recovery count than all other days, with no significant differences identified between days 1, 3 and 4.

Of the number of seeds fed, 4.25% were recovered for *A. angustissima* and 1.4% for *A. paniculata*. There was a significant difference in the mean recovery percentage for the two species, with *A. angustissima* having a significantly higher mean percentage recovered than *A. paniculata* ($P < 0.001$). The interaction between days and weed species was not significant.

Germination Hot water scarification significantly increased germination percentage of both species ($P = 0.023$) from 3% to 80% and 3% to 53% for *A. angustissima* and *A. paniculata* respectively for the intact untreated seeds. Seed recovered from the faeces and not subjected to post-digestion hot water scarification had 0% and 13% germination for *A. angustissima* and *A. paniculata* respectively. These germination proportions increased to 75% and 33% for *A. angustissima* and *A. paniculata* respectively when subjected to post-digestion hot water scarification.

DISCUSSION

A small percentage of ingested seeds of both species pass through the sheep gastro intestinal tract intact and a percentage of these are viable with some germinating readily and some remaining hard but viable. It is suggested therefore that ruminants are potential vectors of these weeds. The majority of the seeds of both species are expelled in the faeces of sheep at 2 days after ingestion, which was similar to results reported by Simao Neto *et al.* (1987) for other legume species. However, the percentage of our seeds recovered was lower than the 10% mean recovery of viable seeds ingested by sheep as reported by Simao Neto *et al.* (1987). Our results verify that both species are hard-seeded and that scarification significantly increases germination percentage.

The results presented here enhance our knowledge of aspects of the ecology of these weeds and potentially may assist in the control of and limit the spread of these species. Grazing could be used to, for example, harvest seed and then confine animals to yards especially 2–3 days after grazing. Grazing could be used to reduce the number of viable seeds reaching the soil seedbank or it could perhaps be recommended to avoid grazing during seeding to prevent seeds being spread through the livestock to other paddocks or regions. Where livestock are grazing and moving over extensive areas such as is the case in northern Australia there is some risk of them spreading these weed species across the landscape.

It would be useful in the future to explore the palatability of these seeds under field conditions and the germination and establishment of these species in faeces in the field. This trial may have benefited by feeding larger quantities of seeds and or sampling larger faecal subsamples as the number of seeds recovered was low.

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