BIOSECURITY SA
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# Compendium of branched broomrape research

## Section 1. Distribution and dispersal

A COMPILATION OF RESEARCH REPORTS FROM THE BRANCHED BROOMRAPE ERADICATION PROGRAM SOUTH AUSTRALIA

DECEMBER 2013

PREMIUM FOOD AND WINE FROM OUR CLEAN ENVIRONMENT

SOUTH AUSTRALIA

Government of South Australia
Primary Industries and Regions SA

# Compendium of branched broomrape research

Information current as of 3 December 2013



## Disclaimer

# Table of Contents



See also the following publications:

Secomb N. (2006) Defining the distribution of branched broomrape (*Orobanche ramosa* L.) by tracing the movement of potential vectors for the spread of seed. In Fifteenth Australian Weeds Conference Papers and Proceedings (Eds C. Preston, J.H. Watts, N.D. Crossman), Weed Management Society of South Australia, Adelaide, pp. 614-617.

Ginman, E. (2009) Dispersal biology of *Orobanche ramosa* in South Australia. Masters Thesis, University of Adelaide.

Ginman E., Prider J., Watling J., Virtue J., Leys J. (2013) The influence of ground cover on the dispersal of *Phelipanche mutelii* by wind. (in preparation).

Ginman E., Prider J., Matthews, J., Watling J., Virtue J. (2014) Sheep as vectors for *Phelipanche mutelii* seed dispersal (in preparation).

# 1. Potential distribution of *Orobanche ramosa* ssp. *mutelii* in Australia

*Phil Warren*

*Biosecurity SA*

*January 2013*

A prediction of the potential distribution of *Orobanche ramosa* subsp. *mutelii* in Australia was done using CLIMATCH (Bureau of Rural Scientists, September 2009). Climate matching was done using the Mediterranean (Carlon et al. 2008) and Australian distribution of *O. ramosa* subspecies (affinity *mutelii*) or synonomously, *Phelipanche mutelii*. This resulted in the selection of 925 climate stations (Fig. 1) that were used to produce the predicted distribution in Australia based on climate (Fig. 2). Figure 2 is a conservative prediction from CLIMATCH, but still shows that all major grain production areas, southern pastures, and temperate native herbs may be at risk from *O. ramosa* subsp. *mutelii* in Australia.

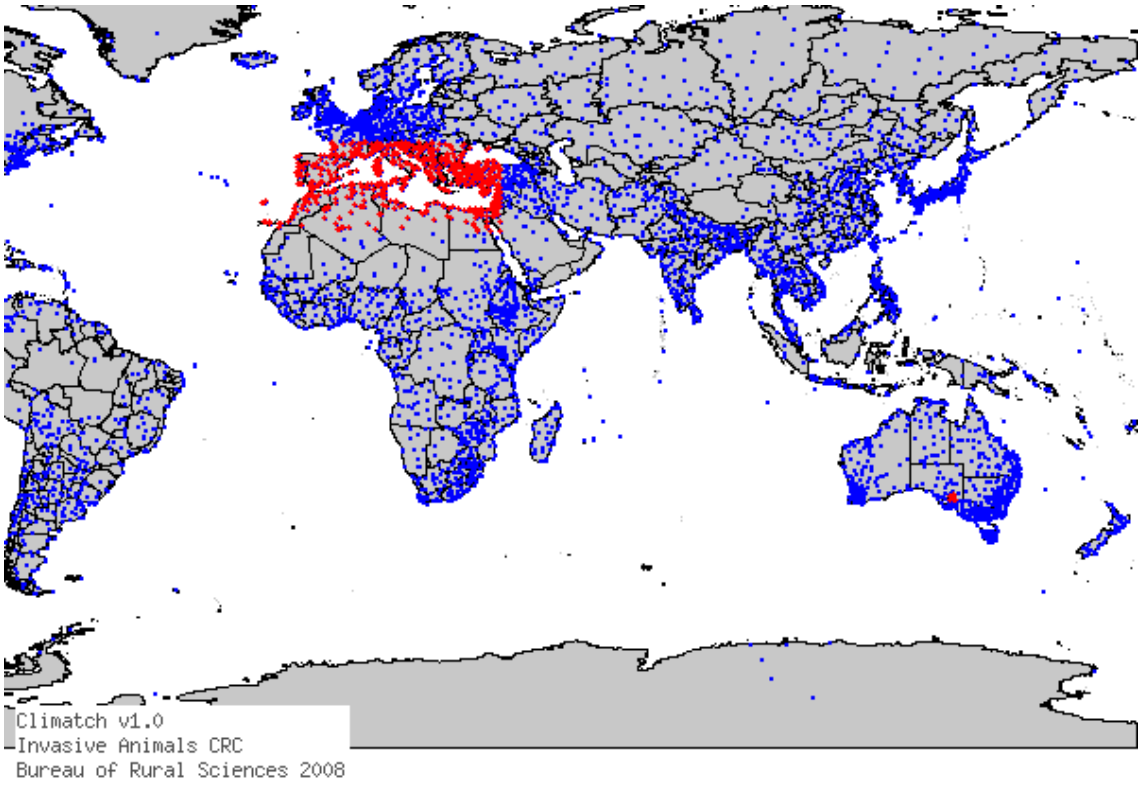



**Figure 1. Selected source climate stations used to predict occurrence in Australia**

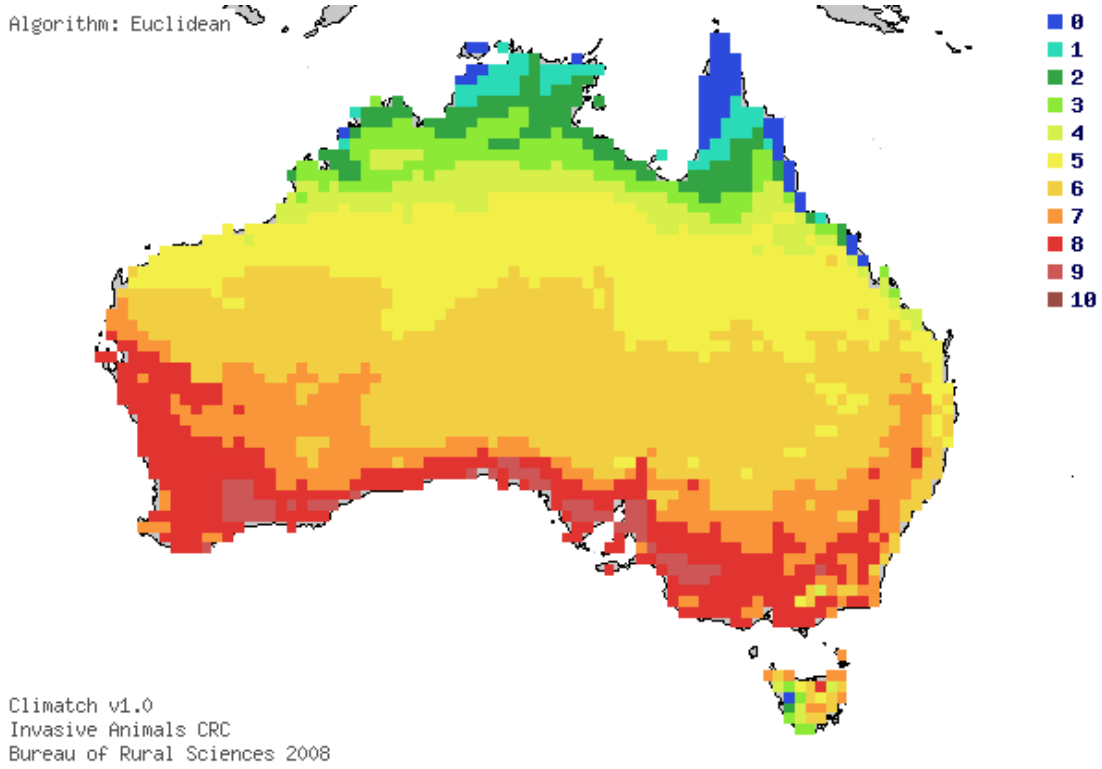



**Figure 2. Prediction of potential distribution of *Orobanche ramosa* subsp. *mutelii* in Australia.**

## Reference

Carlón, L., Gómez Casares G, Laínz M, Sánchez Pedraja O, Schneeweiss G.M.. (2008) More on some *Phelipanche* Pomel, *Boulardia* F. W. Schultz and *Orobanche* L. (Orobanchaceae) from the western Palearctic. Documentos Jardin Botanico Atlantico (Gijon) 6:1-128

# 2. Using GIS technology to predict the current and potential distribution of branched broomrape *(Orobanche ramosa)* in South Australia

*Nick Secomb*

*Branched Broomrape Eradication Program*

*August 2004*

## Abstract

The location of branched broomrape infestations were compared to soil landscape data supplied by Department of Water, Land and Biodiversity Conservation (DWLBC) GIS staff. Direct correlation can be observed between broomrape infestations and a number of parameters in the Soil Landscapes of South Australian Agricultural Areas data. In particular, strong correlation can be observed between broomrape infestations and root zone depth, water repellence, hardpan depth and potential for wind erosion.

## Introduction

Branched broomrape infestations have been found in a 70 km X 70 km area east of Murray Bridge. A Containment Area has been established which encompasses all known infestations (Figure 1).

Branched broomrape is a parasitic weed that hosts on many broadleaved plants. Branched broomrape cannot grow and then emerge without a viable host. Due to this reliance, infestations of branched broomrape can be difficult to find. If host plants have been controlled or removed, branched broomrape will not emerge. Cereal crops are grown in much of the Containment Area. Since broadleaved herbicides are used as part of the normal cereal growing process, many infestations of branched broomrape can go unobserved for a number of years. Analysis of known infestations was required to predict which areas would be at most risk to branched broomrape.

DWLBC's GIS unit has recently released digital data on the soil landscapes of South Australia. Different soil conditions have been recorded across the state. The soil characteristics recorded include soil acidity, soil alkalinity, drainage, hardpan depth and potential for wind erosion. In total, forty-three soil characteristics have been recorded.

## Methodology

All infestations found during the 1999, 2000, 2001 and 2002 surveys were accurately mapped using a Differential GPS unit. This data was used to produce an Arcview shapefile. This data was overlayed on top of the soils data provided by DWLBC's GIS unit. The attribute table of both shapefiles were joined, creating an infestation theme with all of the relevant soil attributes present in each infestation.

This data was then exported as a delimited txt file and the relative area of each soil attribute in the infested area was recorded. This data was compared to the area of each soil attribute across the entire quarantine area.

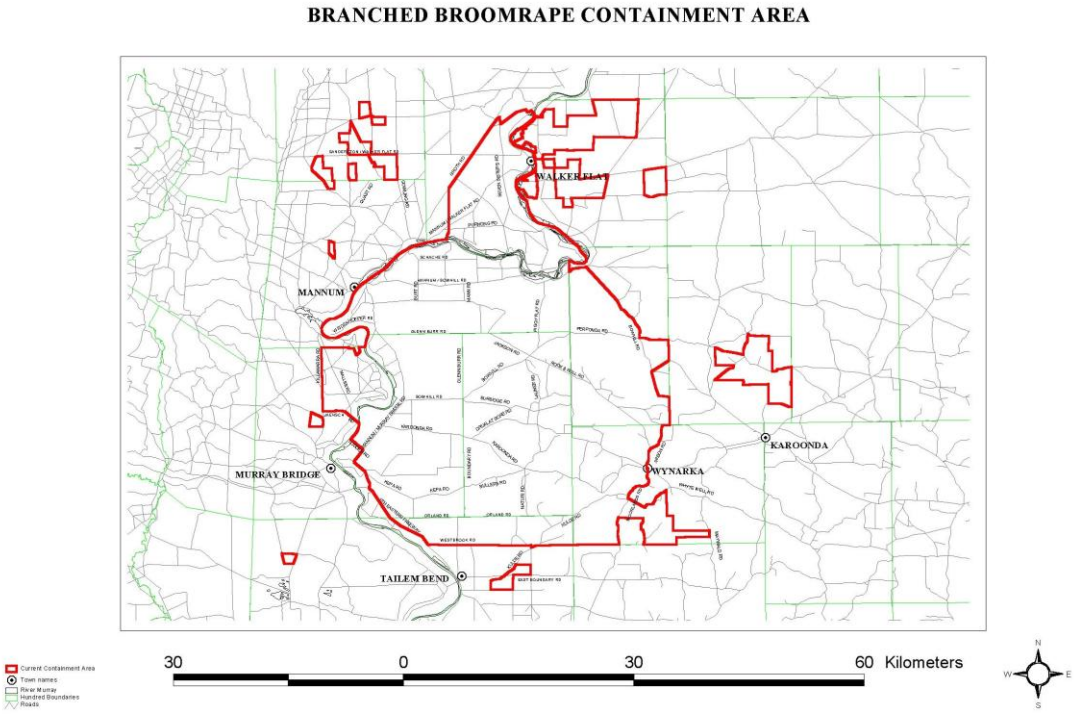
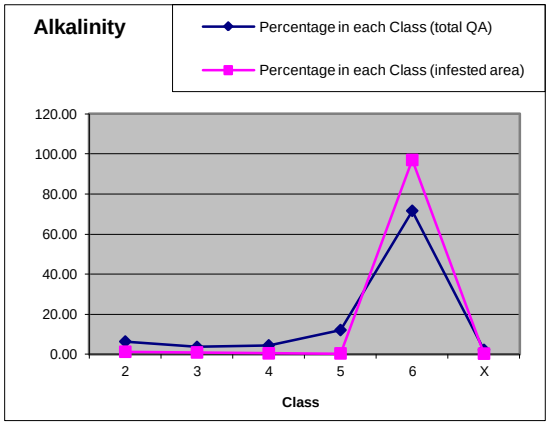
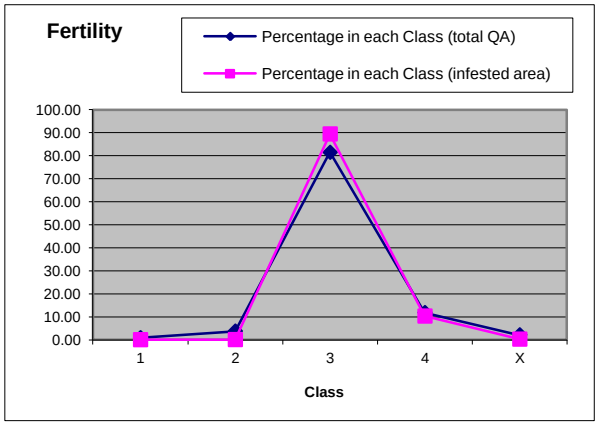
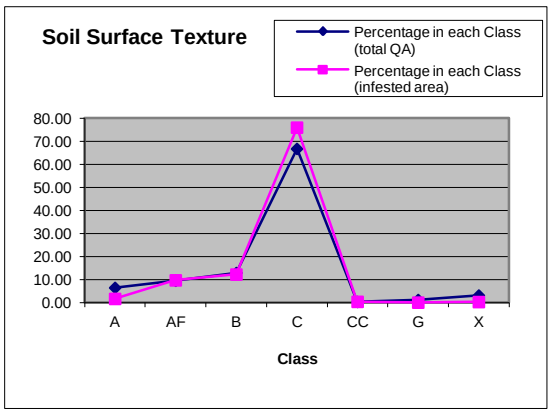


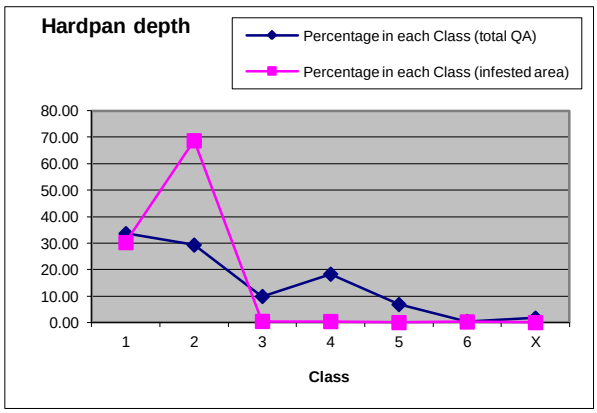

**Figure 1. Known distribution of *Orobanche ramosa* in South Australia as defined by the quarantine zone boundary.**

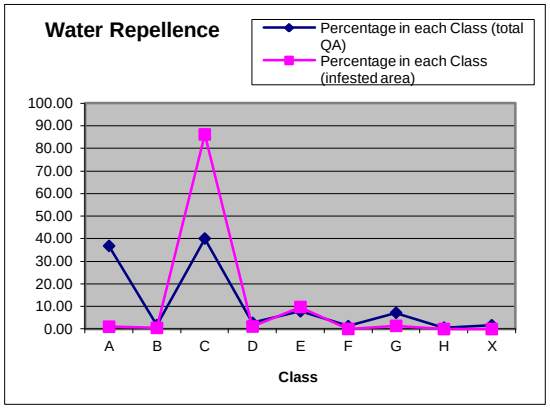
## Results

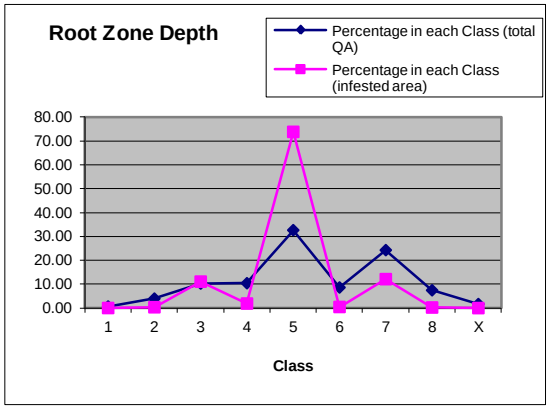


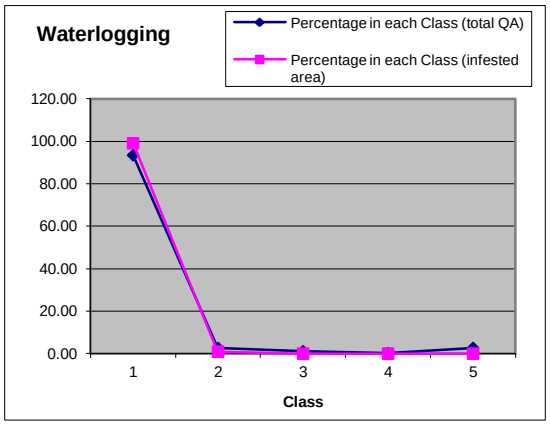



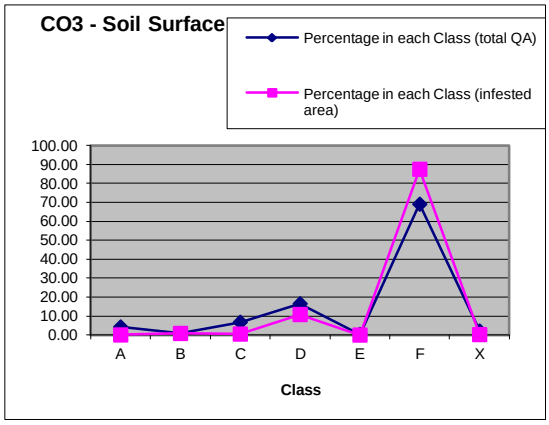



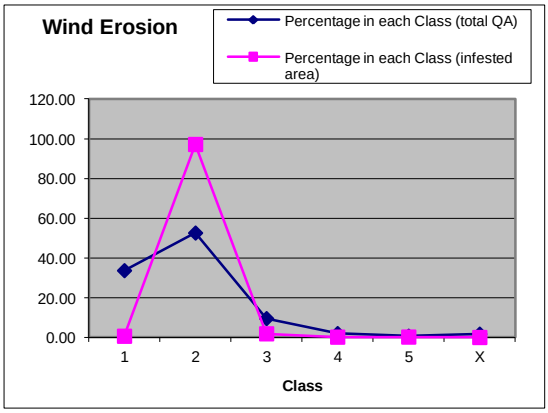



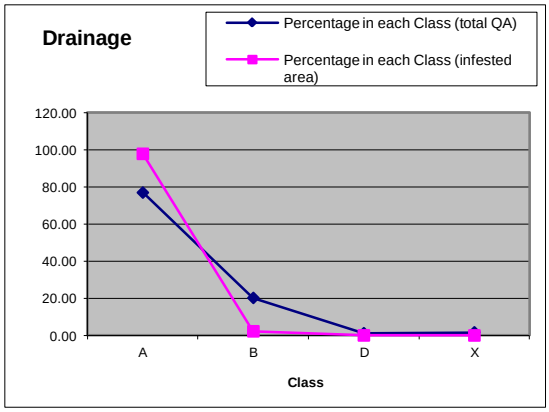



## Discussion

Of the ten soil attributes tested, five had a strong correlation with infestations of branched broomrape. Alkalinity, root zone depth, hardpan depth, water repellence and potential for wind erosion all had a strong correlation with known infestations.

This data could then be used to predict other high risk areas both within and outside of the current Containment Area.

# 3. Literature review: Branched broomrape - risk of endozoochory

*David Cooke*

*Animal and Plant Control Board*

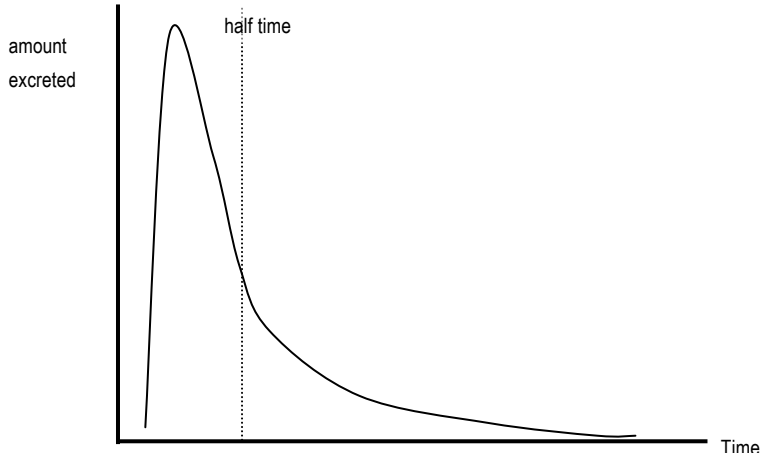
*April 2002*

## Introduction

There is direct evidence that *Orobanche* seed can be dispersed in the gut of grazing livestock (endozoochory) and excreted in a viable condition.

However, there is little quantitative evidence for the retention time of viable *Orobanche* seed in the gut. The best direct evidence is from Jacobsohn et al. (1987) who placed seed of *O. aegyptiaca* (closely related to *O. ramosa* in the section *Trionychon*) and two other *Orobanche* species in the rumen of rams and recovered viable seeds from the faeces. Most of the seeds were excreted in the second day after ingestion, and no viable seeds were recovered after the fourth day although faeces were collected and tested up to the seventh day.

Until experiments with *Orobanche ramosa* have been carried out, inferences can only be drawn from general studies of retention and viability using other types of seed.




## Retention time

Brandt & Thacker (1958) established a mathematical model for the movement of material through the gut of a mammal, based on a theory of constant flow through a volume in which complete mixing occurs. After an initial lag phase representing the minimum time in which material can pass through the gut, the rate of excretion of a marker substance is exponentially related to the time elapsed since ingestion of a single measured dose. This is because the concentration of the marker remaining in the gut is also decreasing exponentially. It is therefore possible to speak of a half-time by which 50% of the marker dose has been excreted.

However, this model is only an approximation, especially if applied to the whole gut contents (digesta). Digesta consists of a mixture of liquid and solid particles of various sizes that move at different rates. A key review of the subject by Warner (1981) concluded that mean retention time, defined as the average time of retention in the gut of all the elements of the digesta, is the best measure of digesta flow and compared various methodologies for determining it.

So half-time is the appropriate measure for a specific seed type in the gut, and mean retention time applies to the whole digesta.

Among herbivores in general, mean retention time is greater in larger animals and also greater in ruminants than in non-ruminants of similar size. Within a species, it varies with the age and condition of animals, type of feed, amount of feed and water content (Warner, 1981). In ruminants, it decreases with increasing food intake (Grovum & Hecker, 1973) and with increasing forage quality (Simao Neto et al., 1987).

The following table lists some experimentally determined mean retention times, in hours, as summarised in Warner (1981). The wide ranges of results for sheep and cattle reflect the number and diversity of studies on these species.

**Table 1. Mean retention times tend to be longer in cattle than in sheep, as noted by Atkeson et al. (1934)**

| | |
|---|---|
| rabbit | 15 |
| horse | 26-37 |
| grey kangaroo | 38-45 |
| goat | 43 |
| sheep | 31-103 |
| cattle | 54-127 |

.

The half-time of one component of the digesta, such as a seed, can be higher or lower than the mean retention time. Smaller particles generally move faster through the gut than larger ones (Warner, 1981). An estimate of the retention time based on movement of a soluble chemical marker is therefore likely to be lower than the half-time for a seed in the same animal. For example, Hartnell & Satter (1979) reported a retention time of 50 hours in cattle, with all traces of the marker gone within 120 hours.

Digesta does not move at the same rate in all sections of the gut. Some material gets delayed by being temporarily held in various *culs de sac,* i.e.:

- the rumen of sheep, goats and cattle
- the caecum
- folds of the ilium and colon

The caecum selectively removes large solid objects from the digesta flow, returning them at intervals. Storage in the caecum can account for the anomalous retention by sheep of an occasional seed of *Reseda lutea* for up to 12 days, or of *Solanum elaeagnifolium* up to 31 days reported by Heap & Honan (1993). In an extreme case, the large hard-coated seeds of the tree *Enterolobium* were retained by horses for up to 60 days (Janzen, 1981); these seeds are each about 12,000 times the volume of an *Orobanche ramosa* seed.

In two other case studies of seeds ingested by sheep, *Echium plantagineum* seed was mostly passed within 3 days (Piggin, 1978) and recovery of *Nassella trichotoma* peaked on days 3 and 4 and had fallen to a low level by day 7 (Cook, 1998).

In cattle, Gardener et al. (1993a) found half-times of 34-50 hours for retention of a range of 10 legume and 8 grass species, although occasional seeds were still being voided when the experiment was terminated at 160 hours.

These results support the model: the great majority of seeds are passed by the fourth day, but occasional seeds may lag for an indefinite time.

## Viability

Seeds can be damaged by:

- mastication, during ingestion and/or later rumination (Harmon & Keim, 1934; Piggin, 1978; Özer, 1979),
- microbial activity in the rumen (Simao Neto & Jones, 1987; Gardener et al., 1993b).
- digestive enzymes and acid in the stomach (Simao Neto & Jones, 1987; Gardener et al., 1993b).

If seeds imbibe sufficient water to begin germination or rupture the testa, they are rapidly destroyed by chemical and mechanical stresses (Janzen, 1981; Gardener et al., 1993a). This can occur anywhere along the gut.

Some of the studies cited above in relation to retention times also demonstrate a decrease in seed viability. Although Piggin (1978) found some *Echium* seeds passed by sheep after the third day, none of these were viable. Atkeson et al. (1934) and Harmon & Keim (1934) reported that the percentage germination of over 20 weed species decreased with time in the gut. Gardener et al. (1993) also found high decreases in viability in all of their 18 pasture species except for hard-seeded legumes.

However, Cook (1998) recovered a few viable seeds of *Nassella* after 7 days in sheep; and the solitary seeds of *Reseda* and *Solanum* voided by sheep after 12 and 31 days respectively were viable (Heap & Honan, 1993).

Seeds of *Orobanche aegyptiaca* remained viable for up to 4 days in the gut of sheep (Jacobsohn et al., 1987). *Solanum elaeagnifolium* and *Reseda lutea* both have resistant, sclerified testas and *Nassella* seeds are similarly protected by a sclerified lemma. Since *Orobanche* seeds are very small with a thin testa, they may be less well adapted to survive in the stomach than these larger seeds. This should be tested by further experiments. One approach would be to agitate some *O. ramosa* seeds in a warm solution of HCl plus pepsin and test samples at intervals for viability, as done for grass seeds by Ocumpaugh & Swakon (1993).

The use of fresh manure containing seeds excreted in a viable condition on fields is believed to spread *Orobanche*. However, if the manure is processed into granules or pellets, the high temperatures of this process kills a high percentage of seeds (Joel et al., 1988). Some seeds may survive because they are more resistant to heat shock when dry than if they are in an imbibed condition. Even the storage of manure containing weed seeds is known to reduce their percentage viability, down to virtually 0% after 3 months for most weeds (Atkeson et al., 1934; Harmon & Klein, 1934) although hard-seeded legumes survived longer (Özer, 1979).

## Conclusions

The indirect evidence suggests that viable seeds of *Orobanche ramosa* will not be excreted more than 6 days after ingestion by sheep, or more than 7 days by cattle. Experiments using *O. ramosa* are necessary to test this prediction.

## References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Harmon, G.W. & Keim, F.D. (1934) The percentage and viability of weed seeds recovered in the faeces of farm animals and their longevity when buried in manure. *J. Amer. Soc. Agron*. 26: 762-767.

Hartnell, G.F. & Satter, L.D. (1979) Determination of rumen fill, retention time and ruminal turnover rates in ingesta at different stages of lactation in dairy cows. *J. Animal Sci.* 48: 381-392.

Heap, J.W. & Honan, I. (1993) Weed seed excretion by sheep - temporal patterns and germinability. Proc. 10th Australian Weeds Conf. Brisbane 1.

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Janzen, D.H. (1981) *Enterolobium cyclocarpum* seed passage rate and survival in horses, Costa Rican Pleistocene seed dispersal agents. *Ecology 62*: 593–601.

Joel, D.M., Kleifeld, Y., Golan, S., Omry, F. & Cohen, E. (1988) Weed seeds in granulated dairy manures. *Phytoparasitica* 16: 373.

Ocumpaugh, W.R. & Swakon, D.H.D. (1993) Simulating grass seed passage through the digestive system of cattle: A laboratory technique. Crop Sci. 33: 1084-1090.

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Piggin, C.M. (1978) Disperal of *Echium plantagineum* L. by sheep. Weed Research 18: 155-160.

Simao Neto, M., Jones, R.M. & Ratcliff, D. (1987) Recovery of pasture seed digested by ruminants. 1. Seed of six tropical pasture species fed to cattle, sheep and goats. Aust. J. Exp. Agric. 27: 239-246.

Simao Neto, M. & Jones, R.M. (1987) Recovery of pasture seed digested by ruminants. 2. Digestion of seed in sacco and in vitro. Aust. J. Exp. Agric. 27: 247-251.

Warner, A.C.I. (1981) Rate of passage of digesta through the gut of mammals and birds. Nutrition Abstracts and Reviews Series B, 51: 789-820.

# 4. Assessment of branched broomrape seed (*Orobanche ramosa*) using in vitro digestion models

*Dr Dean Revell and Assoc. Prof. John Brooker*

*The University of Adelaide*

*October 2002*

## Rationale

Resolution is required on the rate of seed passage through the digestive tract and seed viability following consumption by sheep. The 'rate of passage' work requires a large amount of seed material to feed to sufficient sheep for statistically reliable (and industry believable) results. At this stage, this approach is problematic. Following recent discussions, it was considered that a preliminary set of questions that need to be answered are:

- Does rumen fermentation reduce seed viability?
- If so, what duration of residence time in rumen is required to render 100% of ingested seeds unviable?
- Does acid digestion (post-ruminally) affect seed viability?
- If so, what extent of acid digestion is required?

Given limited seed numbers for such experiments, and the initial desire to be able to control the experimental conditions, an *in vitro* approach is recommended. If it is established that branched broomrape seed is highly susceptible to microbial fermentation and/or acid digestion, the rate of passage of the seed is less of a pressure issue to quantify; ie, if seeds are very susceptible to damage, any seeds that are excreted more than a few days after ingestion *may* be classified with high confidence as being unviable. Final in vivo studies would be required in due course to confirm this prediction.

The methodology for *in vitro* microbial fermentation is well accepted, and established in John Brooker's microbiology laboratory at Roseworthy. The acid digestion methodology is also based on published literature (eg, Ocumpaugh & Swakon, 1993; Simulating grass seed passage through the digestive system of cattle: A laboratory technique. Crop Sci. 33, 1084-90).

## Proposed methodology – in brief

### 1. In vitro fermentation

Rumen samples collected from sheep (sheep grazing pasture, as similar as possible to those in the 'BB zone' will be used)

Add a precisely known number of seeds to incubation tubes containing rumen fluid, plus media to support microbial growth (50 mls each; three replicates per treatment).

Incubate for: 0 mins (ie, control), 12, 24, 48, 72, 96, 120, 144 and 168 hrs.

Stop fermentation at set times, and count seeds (recovery) and test for viability by germination tests.

### 2. Acid digestion

Seed agitated in warm solution of HCl and pepsin for 4 time periods (0, 5 mins, 20 mins, 60 mins, 2 hours, 12, 24 and 48 hours). Some preliminary work, with non-BB seeds will be undertaken to establish the procedure as a working technique in our laboratory.

Count seeds (recovery) and test for viability by germination tests.

### 3. Rumen fermentation plus acid digestion

To mimic the actual processes in an animal, seeds will be subjected to both rumen fermentation and acid digestion. It is conceivable that both processes (fermentation and acid digestion) are sufficient to render the seeds unviable, even if each alone is insufficient. A range of test conditions will be selected based on the results from steps 1 and 3.

## Summary of results

*Phil Warren*

*Branched Broomrape Eradication Program*

*July 2004*

The following graphs summarise the results of two series of "in vitro" or test tube experiments to find out how the seed of branched broomrape behaves as it passes through the gut of a sheep.

The experimental methods are well established as a close reflection of what actually happens in the animal.

The graphs show that after a certain period inside a sheep there is virtually no germination of branched broomrape seed.

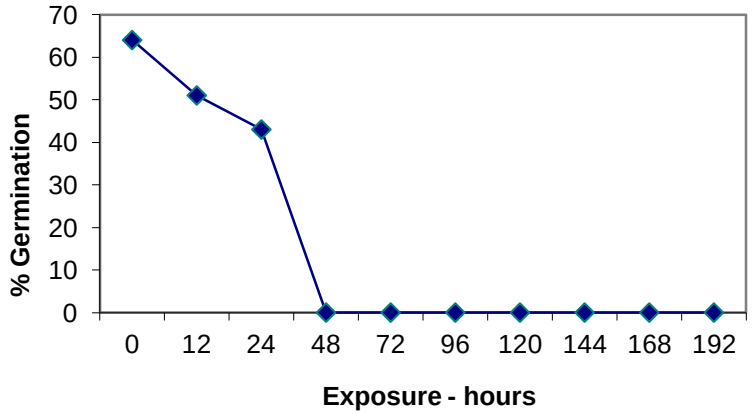



**Figure 1. Seed germination in the rumen or paunch part of the digestive system**

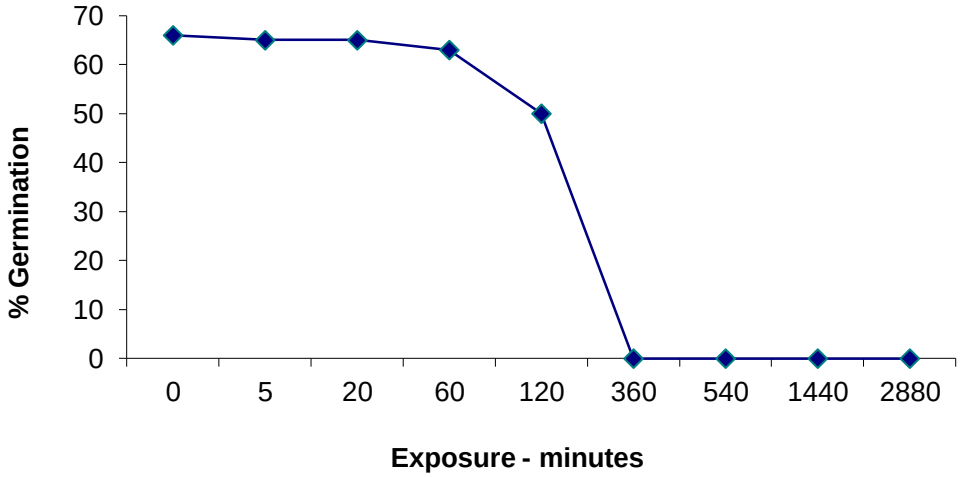



**Figure 2. Seed germination in the fourth or proper stomach part of the digestive system.**

The retention time of feed in the gut varies with feed quality, water content and age of animal and for a sheep can vary from around 31 to 103 hours.

The results show that it is unlikely for seed of branched broomrape to germinate after 48 hours in a sheep.

This has been translated into the protocols as the 48 hour withholding period in a yards or separate site when moving stock from an infested paddock.

## Inside the animal or on the outside

There is also the risk that seed is carried on the outside of an animal. There is no information available about this.

The protocol that requires an animal to be kept on a clean paddock for 15 days after it has been in the yards provides for this risk.

# 5. Dispersal of branched broomrape by sheep – preliminary results

*Emma Crossfield*

*University of Adelaide*

*August 2005*

Twenty four unshorn sheep were used to investigate dispersal of broomrape seeds by sheep. They were kept in pens at the Mannum Trial Site. These are the preliminary results that are described more fully in the thesis (Ginman 2009).

The body weight of the sheep changed over the course of the experiment. 15 sheep gained weight during the experiment, and 9 lost weight. The average initial weight was 41.79 kg, and the average final weight was 42.46 kg (

Figure 1).

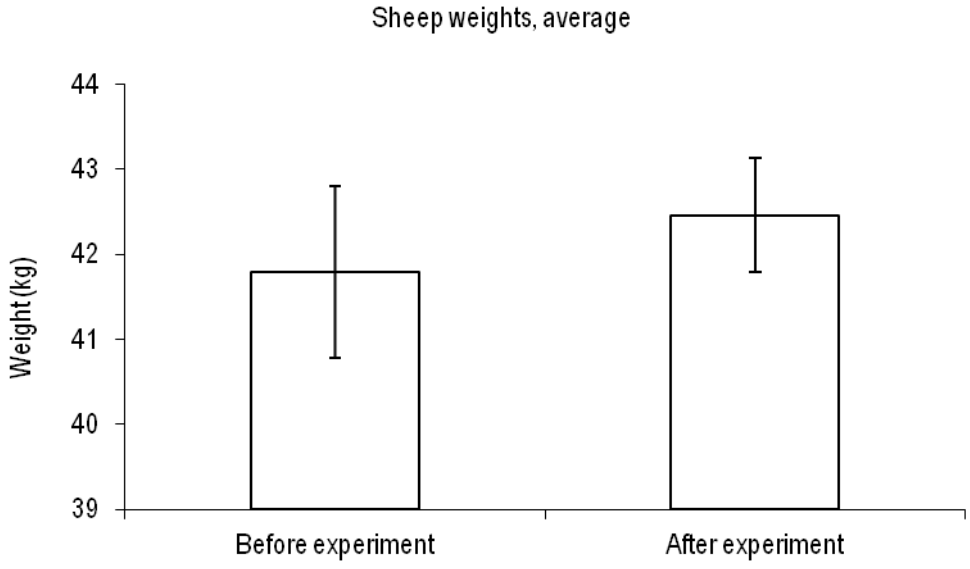


**Figure 1. Weights of sheep before and after experiment, Mannum SA, Feb-March 2005.**

## Experiment 1: Passive attachment of *Orobanche ramosa* seeds to the external surface of sheep

Soil cores showed that direct addition of seed caused an increase in the topsoil seed bank followed by a decrease in the seed bank over the time that the sheep were added (

Figure 2).

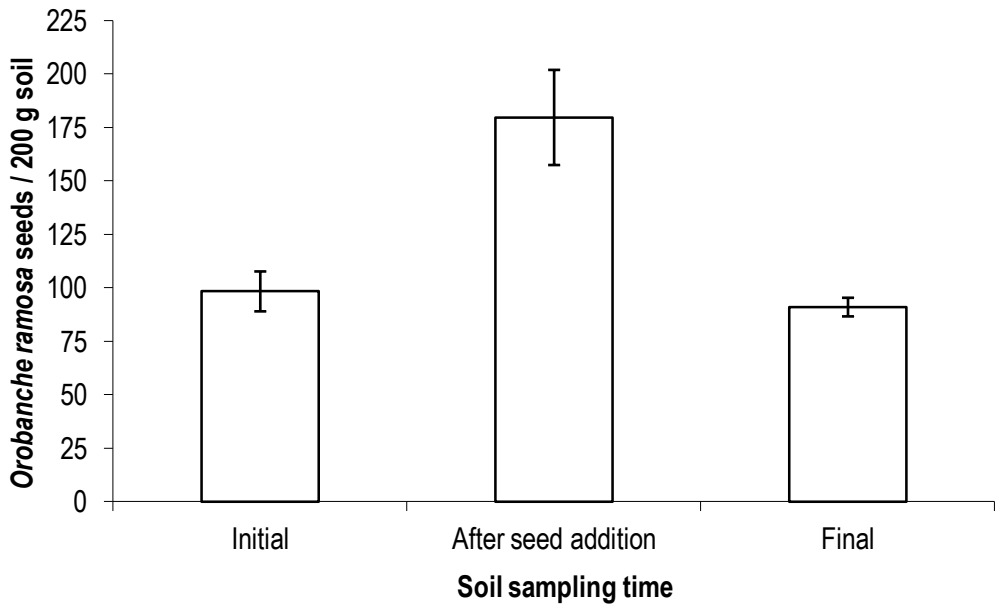



**Figure 2. Top soil seedbank for pens used in Passive uptake experiment, Mannum SA, Feb-March 2005.**

Seeds passively attaching to foot hair:

| Treatment | Sheep | Total seeds | ave seeds |
|---|---|---|---|
| D3 | A1 | 66 | 25.66667 |
| | B1 | 8 | |
| | C1 | 3 | |
| D5 | A2 | 2 | 22.33333 |
| | B2 | 1 | |
| | C2 | 64 | |
| D7 | A3 | 0 | 0.333333 |
| | B3 | 0 | |
| | C3 | 1 | |

The results indicate that it is possible for the sheep to pick up seeds on their feet (at least) but they seem to quickly drop off again (i.e. the reduction in seed numbers found at Day 7 c.f. Day 3 and Day 5).

## Experiment 2: Active attachment of *Orobanche ramosa* seeds to the external surface of sheep

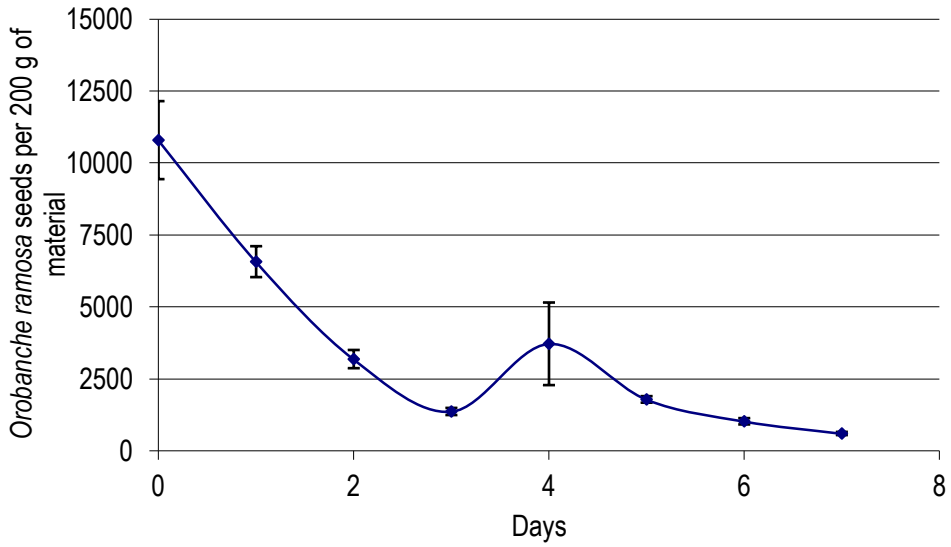



**Figure 3. PCR results from material collected on sheets under sheep pens during "active attachment" experiment.**

## Experiment 3: *Orobanche* seed gut-passage time for sheep

DNA analysis of sheep manure after drenching has revealed a clear pattern. Despite differences in the initial number of seeds introduced into the gut by drenching, seed numbers present in expelled manure peaked on Day 2, and then dropped sharply to a zero reading by Day 7-9 (

Figure 4).

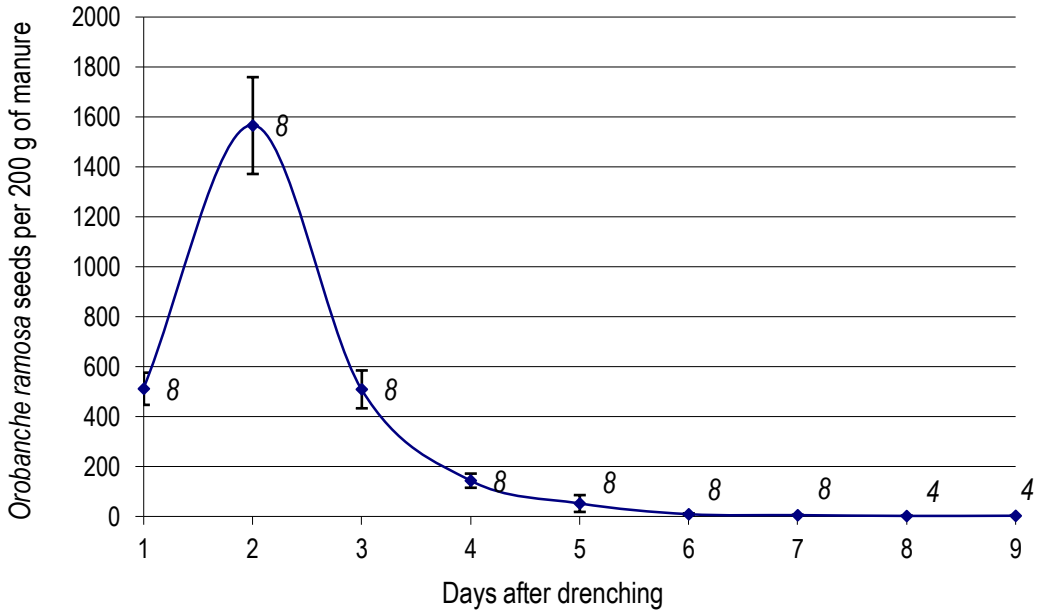



**Figure 4. Average gut passage time for *Orobanche ramosa* seeds through sheep, bars are standard errors. Labels on graph indicate number of sheep sampled on that day after drenching on day zero.**

# 6. Pilot Study: Primary dispersal seed shadow

*Emma Crossfield*

*University of Adelaide*

*February 2005*

## Aim

To assess:

- the efficacy of three different designs of seed traps for *O. ramosa* seed,
- how far *O. ramosa* can be dispersed by "normal" wind, and
- a suitable sampling period i.e. how many days do I need to leave the traps out.

## Background

There are many different seed (and pollen) trap designs used in the literature (Hoekstra 1965; Berner *et al.* 1994; Page *et al.* 2002), and it is hard to judge which will be most effective for *O. ramosa* as many studies do not document the size of seeds they are capturing in the seed traps, nor the reasons behind the choice of trap. I need to determine which is the best seed trap design based on five factors: (1) ability to catch seed; (2) cost of unit; (3) ease of deployment; (4) ease of retrieval; and (5) ease of analysis.

The three trap designs assessed were:

Sticky tape line. A continuous line of double-sided sticky tape attached to a strip of sheet metal for stability. It will start at the base of the plant (0 m) and run in a line straight (sticky side up) out to 1 m. To retrieve, place another piece of sticky tape over the top and label with a Texta (need to label which end is which i.e. 0 m and 1 m, and which side was facing up).

Vaseline slides. Glass microscope slides smeared with Vaseline (petroleum jelly) and placed on the ground at 25 cm intervals. To retrieve, place clean slide over the top and tape together. Used for pollen sampling (Hoekstra 1965).

Taped slides. Glass microscope slides with 7 cm strip of double-sided sticky tape placed in the centre. Slides placed on the ground at 25 cm intervals. To retrieve, place single sided sticky tape over surface.

## Materials & Methods

### Design

The experiment was set up on an 8 m x 8 m area of low pasture-type weeds, free of emerged *O. ramosa* plants. Three adult *O. ramosa* plants at point of seed drop and with viable seeds visible were transplanted together into the centre of the area. They were held in place with wire pins (approx 10 cm long) driven into the ground. The units were set up on six radii around the plants as shown in

Figure 5. When the actual experiment is conducted it will be all eight radii around the plant, not just the six that I did here.

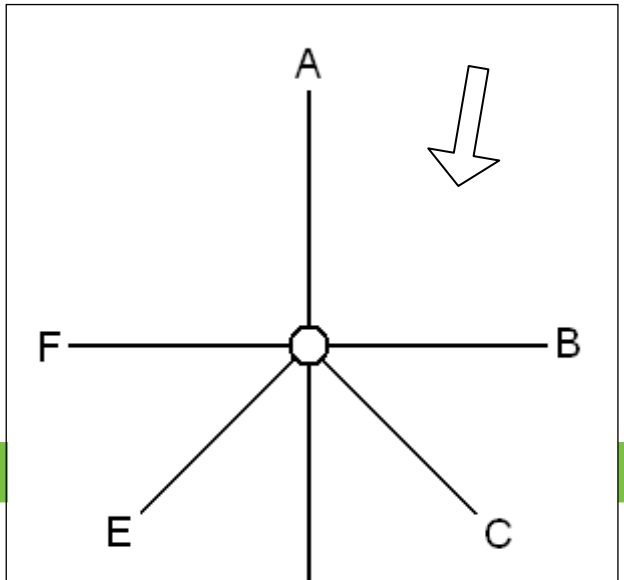


**Figure 5. Arrangement of radii around *O. ramosa* plants (circle) for pilot experiment. The arrow indicates the direction of the prevailing wind at the time of deployment.**

### Trap types

*Unit 1: Sticky tape line*

1 m x 5 cm sheet metal
1 m double-sided tape
1 m normal (single sided, translucent) tape
Texta for labelling
Double-sided tape was stuck to the metal in a long strip. The unit was placed on the ground with one end abutting the plant, the other end extending out on the radii. Backing paper was removed from the tape. Traps were left for 24 hours. To retrieve, normal tape was placed over double-sided tape to lock in sample. 0 m and 1 m were written on the appropriate ends of the unit with Texta. In the quarantine laboratory, a dissecting microscope (2.6x) was used to observe seeds.

*Unit 2: Vaseline slides*

Vaseline (white petroleum jelly)
Glass microscope slides
Normal (single sided, translucent) tape
Texta for labelling
Slides were coated in a thin layer of Vaseline, applied using my finger. The slides were placed along four radii at intervals of 25 cm. Traps were left for 24 hours. To retrieve, another clean slide was placed on top of the sample slide and the two were taped together and labelled with a Texta. In the laboratory, slides were separated and the seeds on each pair of slides were counted with the aid of a 2.6x dissecting microscope and a hand tally counter.

*Unit 3: Taped slides*

Glass microscope slides
Double-sided tape
Normal (single sided, translucent) tape
Texta for labelling
A strip of double-sided tape was stuck on the centre of each slide, and slides were placed at 25 cm intervals along each of the radii. Traps were left for 24 hours. To retrieve, the sticky surface of each slide was covered with a strip of normal tape, and labelled with a Texta. In the laboratory, the seeds on each slide were counted with the aid of a 2.6x dissecting microscope and a hand tally counter.

## Results

### Trapped seeds

Seeds were able to be trapped by each of the three trap designs. Seeds were not counted in the Sticky tape line because the surface of the metal which the tape was stuck on was too mottled to see seeds under the microscope, though it could be seen that seeds were caught by this method. The results of the Taped slides are shown in

Figure 6, while the results from the Vaseline slides are in Figure 8. In all cases, 90% of seeds in each radii were caught within 0.5 m of the plant. While the total number of seeds caught by each method were slightly higher for Vaseline Slides than Taped Slides, this difference was not statistically significant ($P = 0.2140$, d.f. = 38).

In some radii for the Taped slides, there were odd 'outlying' individual seeds, i.e. C 1.25 m, C 2.5 m, and D 1.5 m. These may have been seeds that were deposited on the slides by me when I was deploying the traps, e.g. seeds falling from my overalls.

## Scoring of methods

Scores are awarded by: (1) ability to catch seed; (2) cost of unit; (3) ease of deployment; (4) ease of retrieval; and (5) ease of analysis.

**Table 1. Scores awarded to each of the three methods for trapping *O. ramosa* seed. Criteria were scored on a scale of 1 to 10, 1 is the poorest score, 10 the best.**

| Method | Seed catching | Cost | Deployment | Retrieval | Analysis | Total |
|---|---|---|---|---|---|---|
| Sticky tape line | 8 | 6 | 7 | 6 | 6 | 33 |
| Vaseline slides | 10 | 8 | 5 | 3 | 3 | 29 |
| Taped slides | 8 | 6 | 10 | 8 | 8 | 40 |

## Sampling period

Traps were deployed for 24 hours. During this time a combined total of 4312 *O. ramosa* seeds were caught by the three trap types, and a distribution curve was produced for each of the radii (see

Figure 6, Figure 8 and Figure 8).

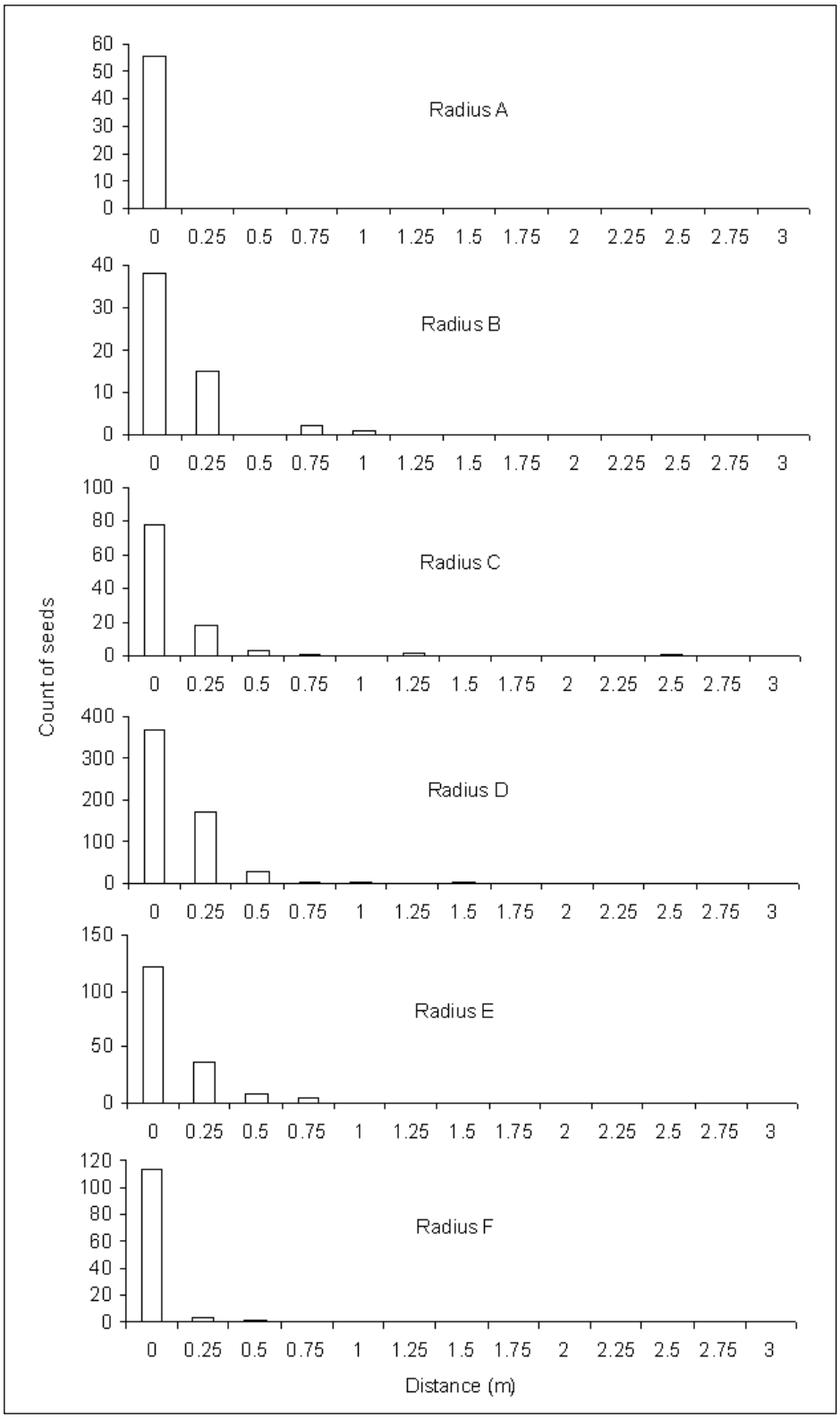



**Figure 6. Results of 'Taped Slides' pilot wind dispersal experiment. Radii are as shown in Figure 1. Note the scale on the y axis changes for each radii.**

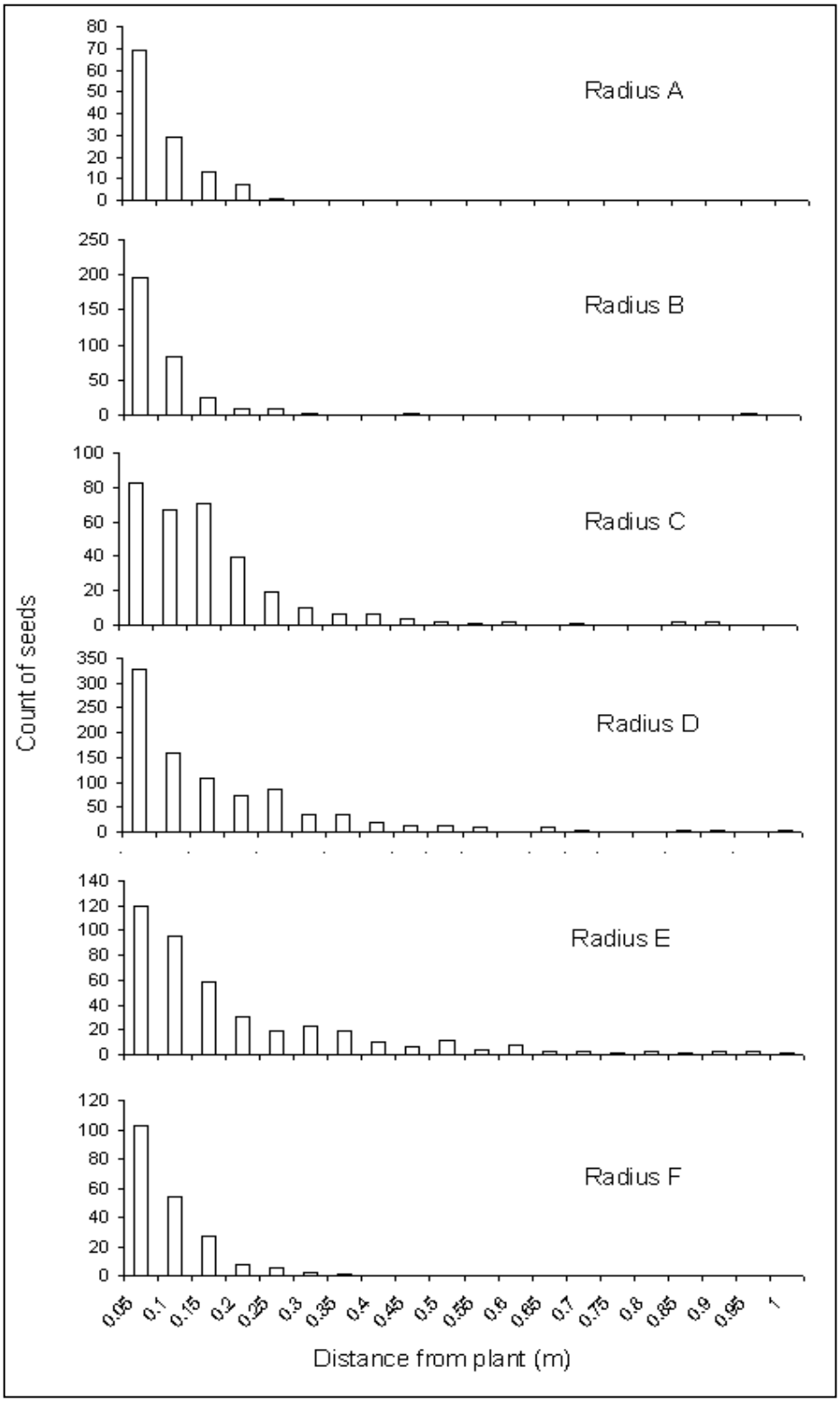



**Figure 7. Results of 'Sticky tape line' pilot wind dispersal experiment. Radii are as shown in**

**Figure 5. Note the scale on the y axis changes for each radii.**

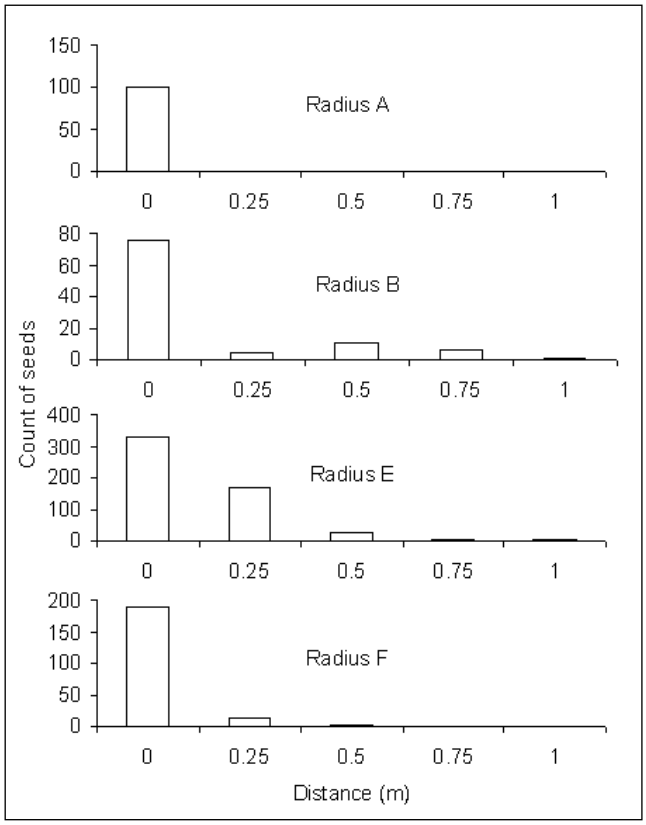



**Figure 8. Results of 'Vaseline Slides' pilot wind dispersal experiment. Radii are as shown in**

**Figure 5. Note the scale on the y axis changes for each radii.**

# Discussion

## Trapped seeds

The distribution curves for the dispersal of seeds by wind was as expected: a negative exponential line with the odd tail-end outlier (see Figure 6, Figure 8 and Figure 8). This pattern was shown by all three trapping methods. There is still the question/issue of compounded results by the capture of seeds close to the plant means that they are not available to be caught further away from the plant. The best way of testing this may be to use artificial seed releasers and count the total number of seeds available to be released.

### Scoring of methods

By far the easiest trap unit to use was the Taped slides. The only drawback was if sand was present on the slide it made it difficult to analyse under the microscope as the sand grains were similar in size to the *O. ramosa* seeds, which made analysis slower. Vaseline slides were messy and time consuming to deploy, messy to retrieve and allowed for error by me when analysing them in the lab (I cleaned the wrong side of a couple of slides, thereby destroying the data). Additionally the jelly melted quickly in the hot sun and there may be the chance of the liquid jelly running off the slide if it was positioned with a slope. The Sticky tape line was quick to deploy and retrieve but cumbersome to deal with in the lab.

### Sampling period

I am happy with a sampling period of 24 hours. It is practicable to achieve on a day to day field work basis, it provides enough seed to show a trend, and it allows for the seed release to be linked to the wind conditions (recorded with anemometer) over the sampling period. By linking several 24 hour periods together, I should be able to get a good picture of the trends over a few days as well as being able to resolve the finer details of the seed dispersal by wind.

## Conclusions

The Taped slides were the most efficient trap design, followed by the Vaseline slides and the Sticky tape line.

90 % of all seeds remained within 0.5 m of the plant. Wind speed and direction was not measured (but now have the equipment in place for the actual experiment).

24 hours seems to be a suitable sampling period, with a number of days linked together.

## New ideas arising from this pilot

### Trap design

After consulting with John Matthews, we thought it might be best to combine two units, the Taped slides and the Sticky tape line, to produce a continuous line of tape that was protected from the dirt (like the Sticky tape line) but is still easy to transport and look at under a microscope (Taped slides). 19 slides are placed in a 1.5 m aluminium rack, an aluminium strip wide enough to hold a slide, with 1 cm slides to protect the slides from the dirt (see

Figure 9). A single continuous strip of double sided tape is placed along the centre of all of the slides, secured at each end by sticking the tape to the aluminium. To deploy, racks are placed into position on 8 radii around the plant, and the backing paper from the tape is removed. To retrieve, racks are removed from the ground and normal tape is placed over the top of the double sided tape. Slides are labelled with a

Texta and cut into separate slides with a Stanley knife. By careful labelling I can tell which end of each slide was closest to the plant, and therefore get a measurement of the actual distance of the furthest seed. Slides are analysed under a dissecting microscope.

### Seed source

Because of the variability of seed numbers per plant, I am thinking of using an artificial seed source, that is some kind of platform on a stake, raised to the average height of broomrape seed pods, but not a Petri dish as I reckon the lip on the edge of it will affect how the seeds are blown off. This would also simplify the methods as it takes away the variable of having to choose plants that are at point of seed drop.

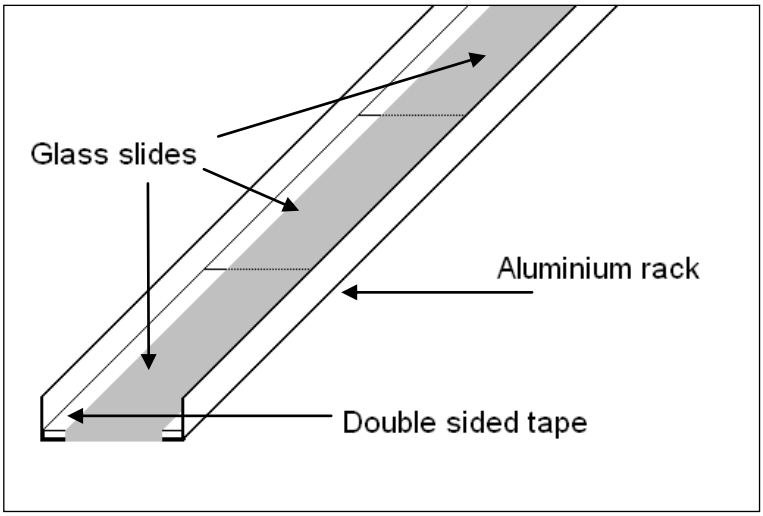



**Figure 9. Design of new slide rack, see text for details.**

## References

Berner DK, Cardwell KF, Faturoti BO, Ikie FO, Williams OA (1994) Relative roles of wind, crop seeds, and cattle in the dispersal of *Striga* species. *Plant Disease* **78**, 402-406.

Hoekstra PE (1965). A simple, inexpensive pollen trap. U.S. Forest Service Research Note SE-40. Asheville, North Carolina, U.S. Department of Agriculture, Forest Service: 3.

Page MJ, Newlands L, Eales J (2002) Effectiveness of three seed-trap designs. *Australian Journal of Botany* **50**, 587-594.

# 7. Using a portable field wind tunnel to investigate broomrape seed dispersal – preliminary results

*Emma Crossfield*

*University of Adelaide*

*August 2005*

Wind tunnels have been used for the last 20 years, under laboratory and field conditions, predominately to investigate erosion and crop damage by sandblasting. More recently wind tunnels have been used to examine dispersal of plant. Field based wind tunnels allow control over the wind velocity and direction applied to the propagules whilst maintaining realistic surface conditions. This study investigated the effects of wind speed and ground cover on seed dispersal of *O. ramosa*. A factorial field experiment of two ground cover treatments (stubble and bare ground) by three wind velocities was undertaken. Presented here are the preliminary results with full details in Ginman (2009).

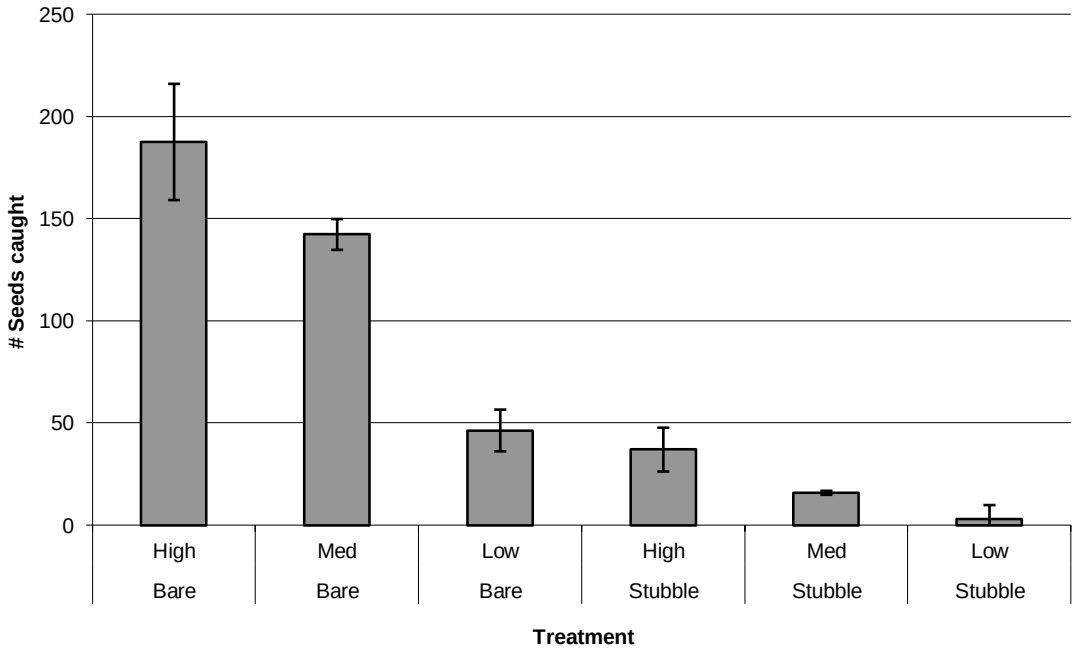



**Figure 10. Results of wind tunnel experiment, seeds trapped in Wind vane traps x2 at far end of tunnel (4.5m). Note that higher wind speeds yield more seeds, and bare soil allows more seeds to travel length of tunnel than stubble plots. But, in all cases seeds were trapped at far end of tunnel. Four replicates per treatment. Data are averages for each treatment, error bars indicate Standard Error.**

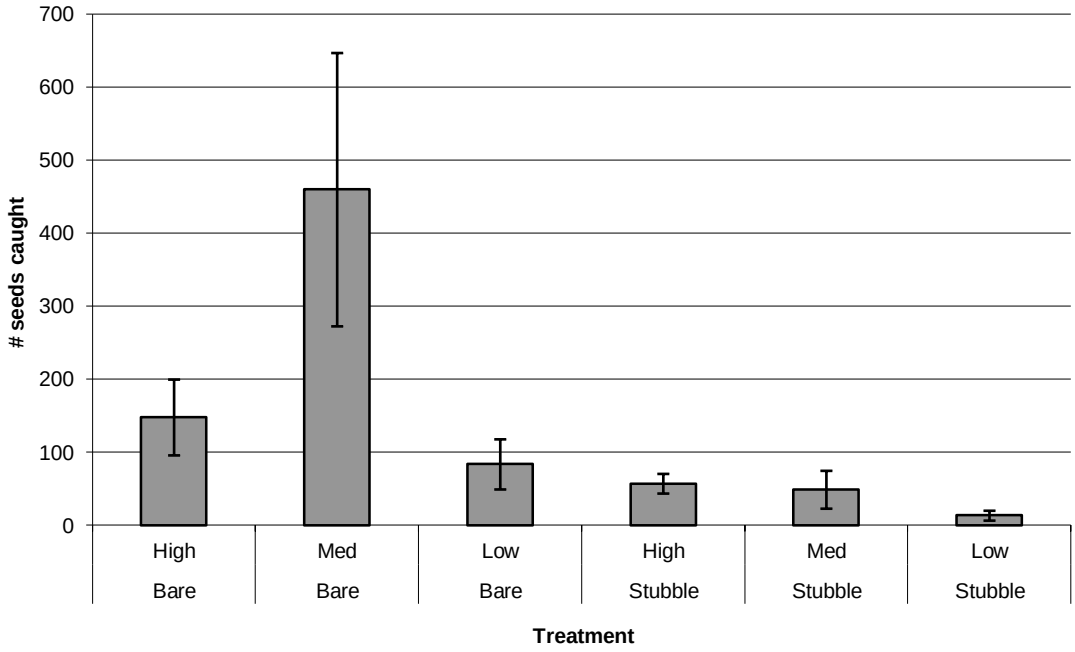
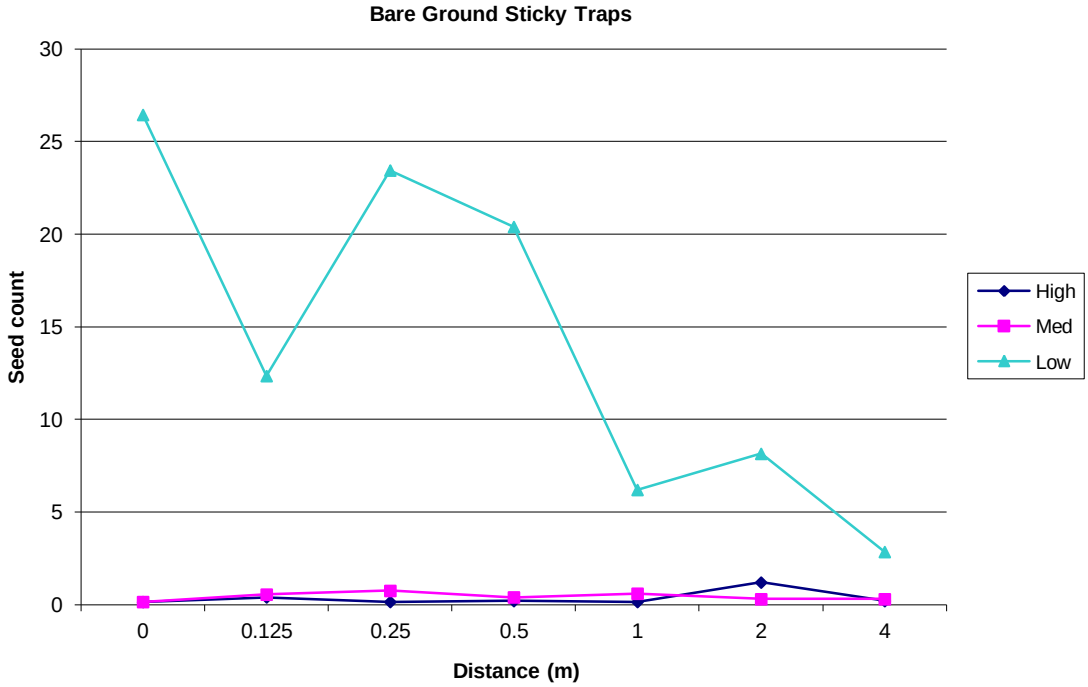



**Figure 11. Results of wind tunnel experiment, seeds trapped in Bagnold Trap at far end of tunnel (4.5 m). Results not as conclusive as Wind Vane Traps. Four replicates per treatment. Data are averages for each treatment, error bars indicate Standard Error.**



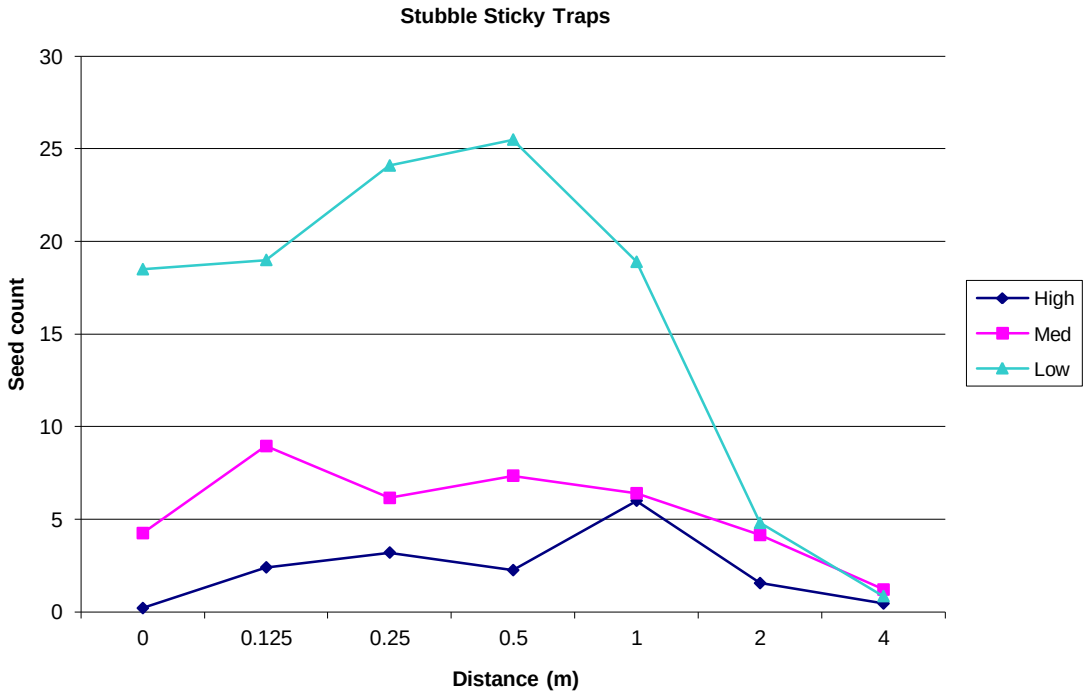




**Figure 12a & 3b. Results of Wind Tunnel experiment (top in bare ground, bottom is 20cm cereal stubble), seeds trapped via sticky traps (logarithmic placement down the tunnel, 5 reps at each distance). Lower wind speeds allow seeds to be trapped within tunnel, assume that higher wind speeds have completely removed seeds from system. Four reps per treatment. Data are averages for each distance and each treatment.**

The wind speeds used here are actually in terms of rpm on the fan: 1000, 1300, 1600 rpm. The resulting wind speeds were:

| Ground Cover | Treatment (wind) | RPM | Wind speed (m/s) |
|---|---|---|---|
| Stubble | High | 1600 | 24 |
| | Medium | 1300 | 21 |
| | Low | 1000 | 15 |
| Bare ground | High | 1600 | 17 |
| | Medium | 1300 | 16 |
| | Low | 1000 | 11 |

(I don't know (yet) why the stubble plots should generate wind speeds higher than the bare ground plots, as instinctively I would have thought this would be the other way around.)

## Take home message:

Wind can move *Orobanche* seeds much more easily that we may have thought. It takes less wind to move *Orobanche* seeds than sand grains, so if there is sand blowing around, the *Orobanche* seeds have already moved.

## Reference

Ginman, E. (2009) Dispersal biology of *Orobanche ramosa* in South Australia. Masters thesis, University of Adelaide.

# 8. Efficacy of washing to remove soil on potatoes

*Jane Prider and Andrew Craig*

*Biosecurity SA*

*May 2013*

## Summary

In a sample of potatoes commercially processed at a site in the former Quarantine Area:

- We found the equivalent of 14 g of soil and other loose material per tonne of potatoes
- Washing removes more than 99.93% of soil and other material adhering to harvested tubers
- All the particles found on washed potatoes were smaller than broomrape seed

## Introduction

Harvested commodities are one of the potential vectors for the spread of the parasitic weed, branched broomrape, as the very small seeds can contaminate produce or other waste materials associated with the harvested crop.

Ware potatoes pose minimal risk as processing removes soil and other material adhering to tubers and the end product is mostly destined for household use, where broomrape has very limited opportunities for establishment. Potatoes washed in a processing plant have no visible soil remaining (Fig. 1) but there is a lack of data on the quantity of soil and other material that remains not visible to the naked eye after processing.

To assess the risk associated with the movement of tubers from cropping zones where broomrape has been found, we estimated the soil adhering to washed and unwashed potatoes.

**Figure 1. Drying potatoes after washing at the processing facility.**

# Methods

## Estimates of branched broomrape soil seed bank in infested potato pivots

Soil samples were collected at a potato-producing property from 50 ha pivots where branched broomrape has previously been detected. There were ten pivots where infestations have been detected within the cultivated area of the pivot or in the inter pivot area less than 10 m from the cultivation line (Fig. 2). Sandy loam soils at Nildottie are representative of other Mallee soil associations that occur across the known range of branched broomrape in South Australia.

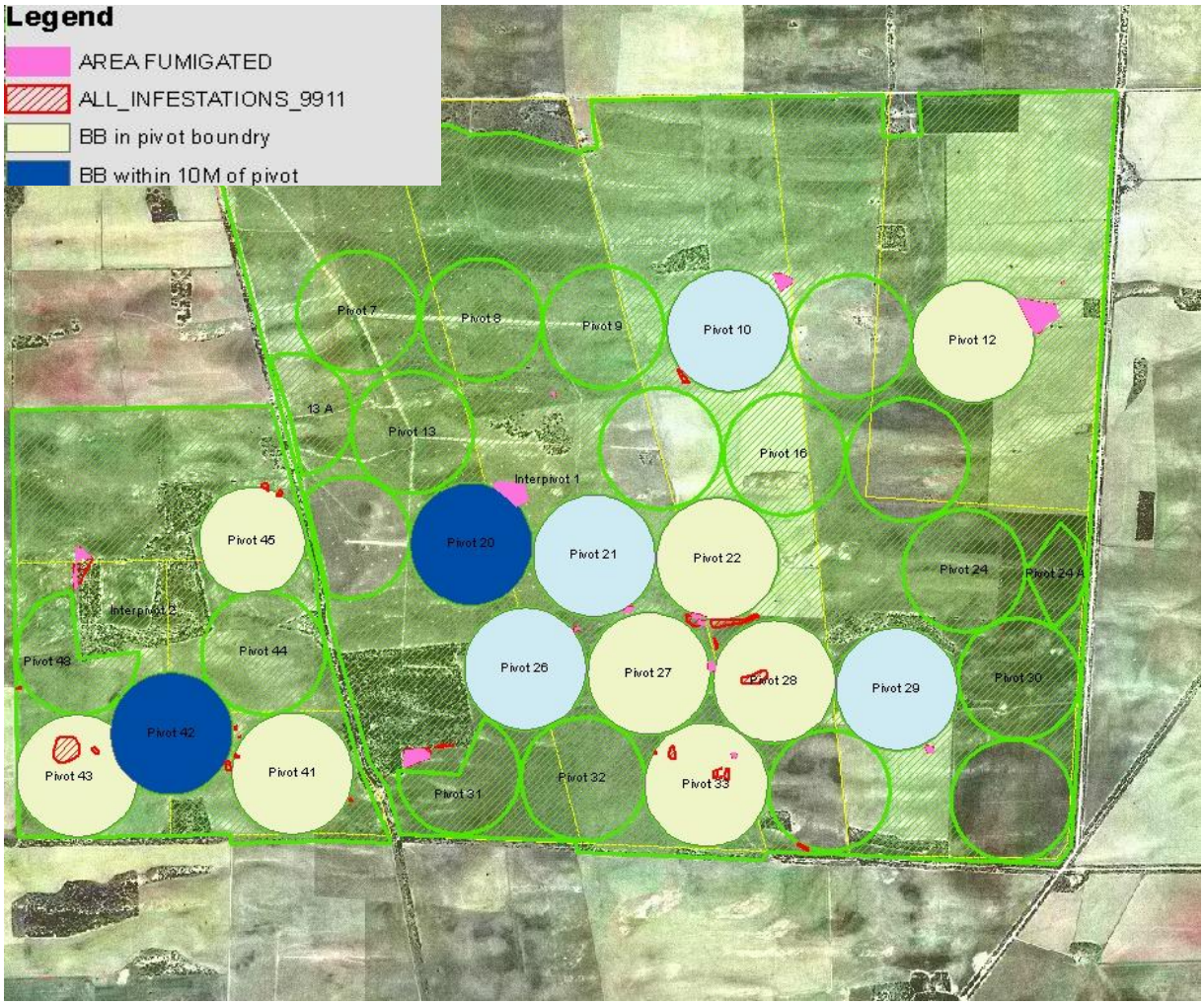



**Figure 2. Potato pivots at the property. Samples were collected from all pivots shaded yellow or dark blue.**

To estimate broomrape seed bank density, we collected soil samples from the infested area of the pivots or in the pivot adjacent to the infested inter-pivot area. We collected 25 soil cores in a 5 m by 5 m sampling grid. Cores were13 mm diameter and soil was sampled from 5 – 15 cm depth. Cores from each grid were pooled to form a single soil sample and five samples were collected from each pivot. This soil sampling method has been developed to minimise error in sampling the spatially variable broomrape seed bank (Prider et al. in press). The number of broomrape seeds was estimated for each sample using a quantifiable, recombinant DNA bioassay. This method has been commercially developed for quantifying broomrape seed and other soil pathogens in soil samples for surveillance and diagnostic purposes (Ophel-Keller et al. 2008, Prider et al. in press).

### Estimates of soil remaining after potato washing

Bags of washed and unwashed potatoes were sourced from the quarantine area on 19th April 2013. A 20 kg bag of washed tubers and a 20 kg bag of unwashed tubers were obtained from one property and a 10 kg bag of unwashed potatoes from another producer. At the first property (where soil samples were also collected) potatoes were grown in loamy sand to sandy loam soils (sandy loam site). The potatoes from the other site were grown on a texture-contrast soil (loam site), with a sandy loam topsoil over a loamy, calcareous subsoil. The potatoes were stored in a cool room at 10 °C.

The washed sandy loam potatoes were cleaned in a commercial-grade washing facility on-site. At this facility, harvested tubers are dropped into a dip tank which removes all loose soil. From here the tubers progress along a perforated tumbler through a tank partially filled with water. As the tubers pass along the tumbler they are alternately sprayed with jets of water or immersed in water in the tank. The rotation of the tumbler, rubbing together of the tubers and water removes adhered soil. An individual tuber takes approximately four minutes to pass along the tumbler. Packing occurs after tubers are rinsed, dried and graded.

To assess the soil remaining on washed potatoes, we found that dry brushing was the only method that did not remove large amounts of potato skin and starch, which was unable to be separated from soil particles.

Individual potatoes were dry-brushed with a 25 mm soft natural bristle brush to remove all loose material. Particular attention was given to removing loose material from around eyes and in cavities. Tubers were brushed until no visible loose particles were detected under a microscope. The material removed from a random selection of 1.5 kg from each bag of potatoes comprised a single sample. These samples were weighed separately and then combined to give a total for the entire bag of potatoes. The soil remaining in the bottom of the bag was also weighed and added to the total weight of material removed from potatoes in that bag.

## Results

### Seed bank

No branched broomrape seed was detected in any of the soil samples collected from pivots where broomrape had previously been detected nearby or within the pivot. As the detection limit for broomrape seed is 1 seed per 500 g soil sample it is inferred that the branched broomrape seed bank density is probably less than this. Because a total of 25 cores were sampled, the true probability of a core being infested is less than 0.0711 (http://statpages.org/confint.html), suggesting a seed density of no more than 0.14 seeds per kg.

### Soil on harvested tubers

We sampled 117 washed tubers. The average weight of each tuber was 126 g. We sampled 136 unwashed tubers from the sandy loam site and 51 from the loam site. The average weight of each tuber was 116 g and 150 g, from each source respectively. Soil could not be seen on the washed potatoes although under a microscope some small sand grains could be discerned on some tubers (Fig. 3). Soil could readily be observed on unwashed tubers (Fig. 4).

Dry-brushing of potatoes removed all loose material including potato skin particles, organic matter and soil (Fig. 5). Most of the sand grains removed were less than 0.5 mm long and all organic particles, with the exception of potato skin fragments, were less than the size of a broomrape seed, 0.2 mm long. No weed seeds, including broomrape seeds, were found on washed potatoes. The soil comprised only a part fraction of the volume of removed material from washed potatoes although the mass of this material would have been more than the skin particles.

Sand or soil particles comprised the majority of material removed from unwashed potatoes. Tubers from the loam site remained soiled after brushing as very fine soil particles adhered to the skin could not be removed.

The mass of the material removed from tubers is summarised in Table 1. This includes material that had fallen from tubers and was collected from the bottom of the bag.

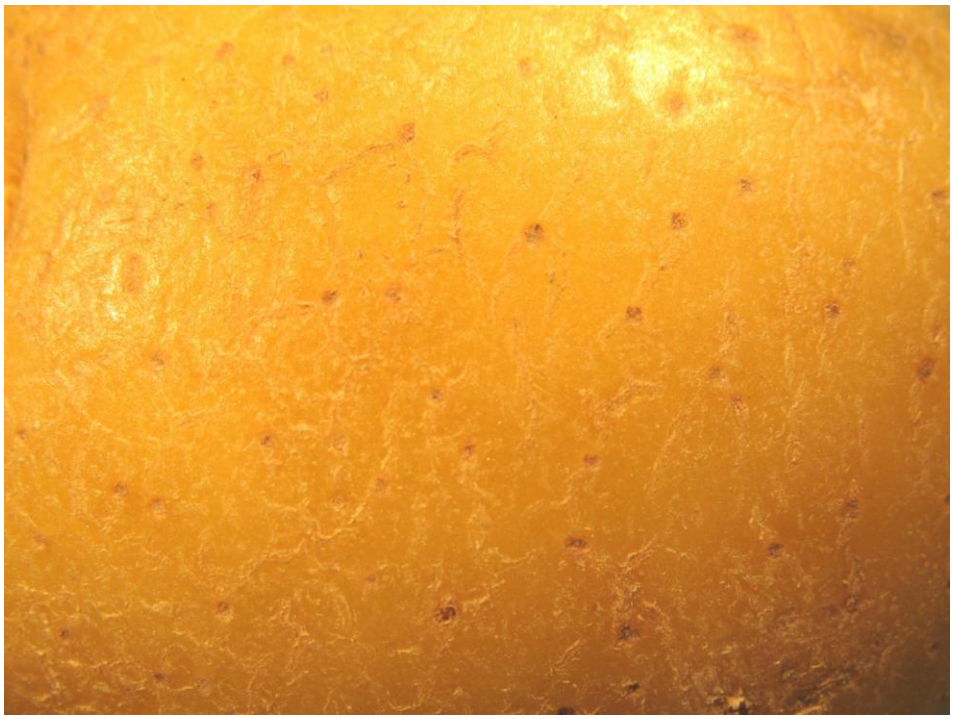


**Figure 3. Close-up photograph of washed tuber from Nildottie.**

**Figure 4. Dry soil particles adhering to the skin of an unwashed tuber from Nildottie. These particles were easily removed by brushing.**

**Table 1. Mass of loose particles brushed from tubers. These figures have been used to calculate the mass of soil on one tonne of harvested potatoes but this value also includes other loose material.**

| Source location | Type | Total weight of tubers sampled (kg) | Total weight of material removed (g) | g soil g potatoes$^{-1}$ | kg soil tonne potatoes$^{-1}$ |
|---|---|---|---|---|---|
| sandy loam | washed | 14.79 | 0.21 | 0.000014 | 0.014 |
| sandy loam | unwashed | 15.71 | 308.91 | 0.0197 | 19.7 |
| loam | unwashed | 7.62 | 20.58 | 0.0027 | 2.7 |

# Discussion

## Seed bank

Most branched broomrape finds at the sandy loam study site have been within interpivot areas (see Fig. 2). The largest within-pivot populations occurred in Pivots 33 and 43 in 2006 but broomrape has not occurred in these pivots since then. Reasonable control of broomrape can be achieved in potato crops with good control of weed hosts as potatoes are not hosts to the local broomrape species. Control of hosts would reduce the input of further seed into the seed bank. Our failure to detect broomrape seed in any soil samples from pivots and the low incidence of broomrape emergence within pivots indicates that seed bank density is low within pivots. Our estimate of a seed density of less than 1 seed per 0.5 kg soil assumes an even distribution of seeds in the soil profile. Seeds are very patchy in the soil profile so the seed density is likely to be quite variable.

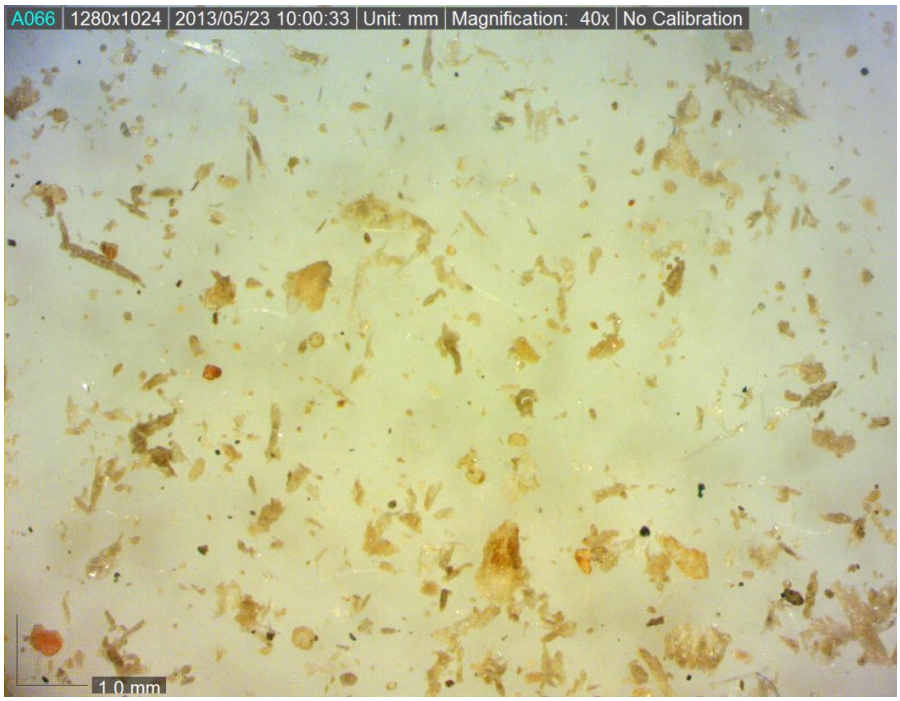



**Figure 5. Example of some of the material removed from a 1.5 kg sample of washed potato tubers from the sandy loam site. Small pieces of potato skin, other organic matter and sand grains can be seen. Scale bar is 1.0 mm.**

## Soil on harvested tubers

Our data show the washing process removes 99.93% of soil and other particles that adhere to tubers during the harvesting process. Our sampling gives an upper estimate of 14 g of soil per tonne of harvested tubers but this includes potato skin fragments.

The majority of soil and soil organic particles removed were smaller than the size of a broomrape seed, which is 0.2 mm long. No large pieces of organic matter of this size, other than potato skin, were found during the sampling (see Fig. 5).

There was no seed found on 14.79 kg of potatoes. We can therefore be 95% sure that the true seed rate on 14.79 kg is less than 3, or less than 1 seed per 5 kg of washed potatoes or less than 40 seeds per tonne. If 5000 t of potatoes are exported, we can guarantee that there would be less than 200,000 seeds.

Using the soil amount per potato as 0.014 kg/tonne and an upper bound of 0.14 seeds/kg, the upper bound for seeds per tonne of potatoes is 0.002. If 5000 tonne of potatoes are taken from infested pivots per year, no more than 10 seeds are likely to be transported per year. Not all the pivots are infested so the true value would be lower than that. Previous work has indicated that where broomrape is established, the success rate of a single seed is 0.0005 (based on a more or less stable population with each plant producing more than 2000 seeds). The likelihood of an establishment where branched broomrape occurs is therefore very low.

The sandy loam site potatoes are grown in a very sandy soil that is easily removed from tubers with a soft brush. No visible soil remained on the potatoes after brushing. There were clumps of soil accumulated in cavities on unwashed potatoes but no clumps of soil were found on washed potatoes that may harbour weed seeds. The loam site potatoes remained stained with soil after brushing but no soil clumps were found on these potatoes after brushing. No washed samples of potatoes from this source were available for sampling.

Unwashed potatoes from the sandy loam site had 19.7 kg of adhering soil per tonne of tubers. With a harvest of 45 tonnes of tubers per hectare, each harvested hectare of potatoes would yield 0.88 tonnes of soil. This is within the range of the mass of adhered soil measured on harvested potatoes in other studies, 0.2 to 3.6 tonnes/hectare (Ruysschaert et al. 2006, Ruysschaert et al. 2007). The variation in soil adhering to tubers is attributable mainly to soil moisture at harvest and soil type. Less soil adheres to tubers with an increasing sand content, decreasing clay content or drier soils.

A previous assessment of soil remaining on cleaned potatoes was not able to detect any remaining soil (Dr Trevor Wicks, Senior Plant Pathologist, SARDI Horticulture Pathology, 2008). Tubers were scrubbed with a soft toothbrush in water and the wash water was examined visually. Any soil that may have been present was obscured by starch and skin pieces. For this study we used a different approach that minimised potato skin and starch loss. Although our samples also comprised a large proportion of this material it was possible to make a conservative estimate of soil remaining on washed potatoes after commercial washing.

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# 9. Potential for broomrape seed to contaminate hay

*Branched Broomrape Eradication Program*

*October 2006*

Aim: to assess the risk of contamination of hay by branched broomrape seeds.

## Methods

1. Mannum Trial Site

Plants were collected from the Mannum Trial Site from vetch plots. Ten plants were cut 50 mm above ground level on five harvest dates at weekly intervals. The plant heights were measured and buds, flowers, capsules and seeds > 150 µm were counted. A subsample of seeds was tested for germination.

2. Paddocks

20 branched broomrape plants were collected from five paddocks on different properties. The plants were excavated from the soil and the depth of the attachment and height of the plant above ground level were recorded.

## Results

### Mannum Trial Site

Multi-stemmed plants were not collected at the first harvest date, with more multi-stemmed plants collected in the third to fifth weeks of harvest (Table 1). Stem heights were similar at all but the first harvest date; between 9.5 and 10 cm. More capsules were present at later harvest as the number of flowers and buds decreased. Seed number was highest at the final harvest. Capsules picked from all harvests produced germinable seed.

**Table1. Harvest data for branched broomrape stems from vetch crops at the Mannum Trial Site. Values are means ± 1 SE. Ten plants were harvested at each date**

| Harvest date | Number of stems | Stem height (mm) | Number of buds | Number of flowers | Number of capsules | Number of seeds |
|---|---|---|---|---|---|---|
| 19/09/2006 | 10 | 82 ± 4.7 | 2 ± 0.7 | 6 ± 1.3 | 2 ± 0.9 | 4151 ± 851 |
| 27/09/2006 | 13 | 96 ± 6.2 | 5 ± 0.7 | 5 ± 0.9 | 2 ± 1.2 | 3628 ± 1282 |
| 5/10/2006 | 24 | 98 ± 4.7 | 2 ± 0.4 | 2 ± 0.5 | 5 ± 0.9 | 3425 ± 840 |
| 10/10/2006 | 23 | 99 ± 5.4 | 1 ± 0.3 | 2 ± 0.4 | 6 ± 1.1 | 2822 ± 786 |
| 17/10/2006 | 19 | 98 ± 5.4 | 0 ± 0.1 | 0 ± 0.1 | 9 ± 1.1 | 7749 ± 2296 |

### Paddocks

Samples collected from paddocks the same season were between 6 and 9 cm tall with variation between sites (Fig. 1). There was also variation in the depth of the attachment at one site. Most attachments were between 4 and 5 cm depth but at Site C they were at 8 cm depth.

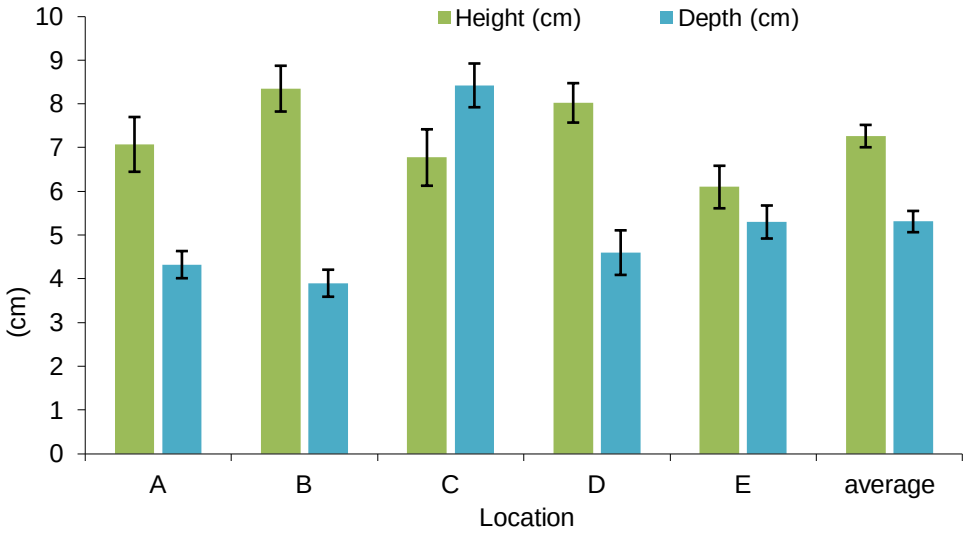


**Figure 1. Height of broomrape plants and depth to attachment to host in the 2006 season. Bars are means ± 1SE, n = 20.**

# 10. AFLP analysis of *Orobanche* species in South Australia

*Andrew Craig and Anna Williams*

*Branched Broomrape Eradication Program*

*December 2008*

## Introduction

The aim of this project was to determine the genetic variation of *Orobanche ramosa* (branched broomrape) within the Murray mallee quarantine area and determine whether the spread of *O. ramosa* has derived from a single or multiple origins. By developing a genetic profile across the population, the age and origin of the population can be determined. This will aid in more reliable identification of the *Orobanche* species, which is currently based on morphological characteristics, and help to refine field and research strategies.

Based on Amplified Fragment Length Polymorphism (AFLP) analysis of *Orobanche* species done elsewhere, this study would be expected to find 90-140 bands per primer pair with approximately 90% polymorphism. Therefore using four primer pairs, a potential 360-560 bands would be used to assess genetic variation of branched broomrape. The genetic distances seen between the samples will determine the likely site of origin and direction of spread, if any.

## Materials and methods

### Plant material

*Orobanche* populations were sampled from Adelaide, Port Noarlunga and within the Branched Broomrape Quarantine area around the Murray Bridge, Mannum and Bow Hill Murray Mallee region. Whole plants were collected, snap frozen in liquid nitrogen and stored at -30°C until ready for DNA extraction. Locations of population sites in the Quarantine Area are shown in Figure 1.

### DNA extraction

Total genomic DNA was isolated from *Orobanche* flowers, buds and stems following a standard plant extraction protocol. Briefly, plant material was frozen in liquid $N_2$ and crushed with a mortar and pestle and 100 mg suspended in 600µl of plant extraction buffer (1% sarkosyl,100 mM Tris-HC, 100 mM NaCl, 10 mM EDTA, 2% Polyvinyl-polypyrrolidine) in a 2 ml microfuge tube. 600 µl of phenol:choloform:iso-amyl alcohol (25:24:1) was added and tubes placed on an orbital mixer for ten minutes. Following a ten minute spin at 10,000 rpm the aqueous layer was removed and added to a fresh 2ml microfuge tube containing 600 µl of phenol:choloform:iso-amyl alcohol (25:24:1) and placed on an orbital mixer for five minutes before a second spin at 10,000rpm for ten minutes.

The DNA in the aqueous layer was precipitated in 600 µl of iso-propanol + 60 µl of 3M Sodium acetate (pH 4.8) with gentle inversion mixing for ten minutes and then spun at 10,000 rpm for ten minutes. The supernatant was removed and DNA pellet suspended in 1ml of 70% ethanol before a final spin of 5 minutes at 7,500 rpm. The DNA pellet was air dried and resuspended in 20 – 50 µl of R40 (TE Buffer +40µl/ml RNase A) and stored at -20°C.

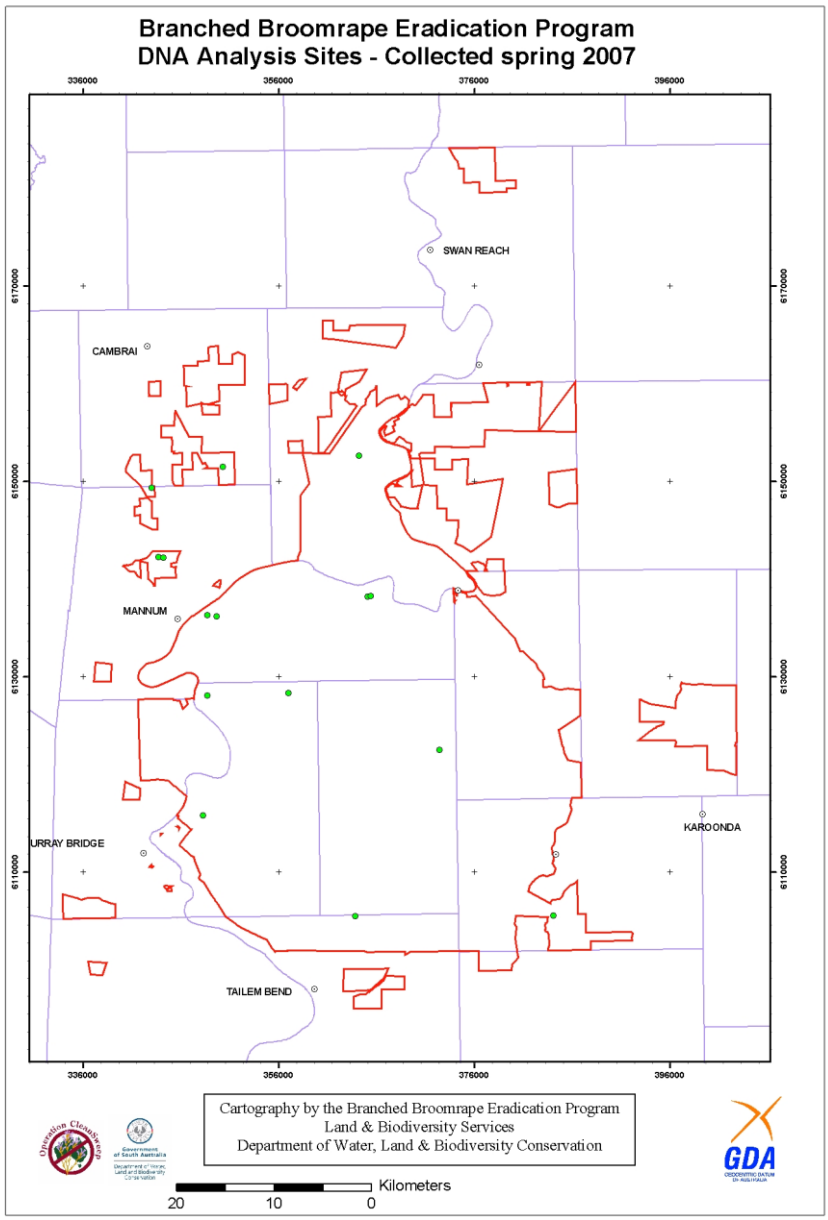


**Figure 1. Collection localities (green dots) within the Quarantine Area.**

## AFLP Assay

The AFLP assay protocol is based on Vos *et al*, 1995, AFLP: a new technique for DNA fingerprinting. Genomic DNA was digested with restriction enzymes PstI (5 units, New England Biolabs) and MseI (5 units, New England Biolabs) and ligated to PstI (5µM) and MseI (50 µM) adapters with T4 DNA ligase (400 units, New England Biolabs) in a 60 µl reaction. Pre-amplification PCR of 4 µl restriction-ligation products with primers Mse C (75 µg/ml) and Pst A (75 µg/ml) and Taq polymerase (5 U/µL) in a total 20 µl reaction was carried out on thermal cycler (PTC-100, MJ Research) with 20 cycles of 94°C for 30 seconds, 56°C for 1 minute and 72°C for 1 minute.

The pre-amplification product was diluted 1/20 for selective PCR using fluorescently labelled primers. Selective PCR was done with 25µg/ml PstI and 25 µg/ml MseI primers with 5 µl diluted Pre-Amplification mix in a total of 20 µl using a Touchdown PCR program. Touchdown program:

1) 94°C for 3 minutes
2) 94°C for 30 seconds
3) 65°C for 30 seconds (annealing)
4) 72°C for 1 minute
5) Repeat steps 2 to 4, reducing annealing temperature until 56°C is reached
6) 94°C for 30 seconds
7) 65°C for 30 seconds
8) 72°C for 1 minute
9) Repeat steps 6 to 8 for a further 34 cycles
10) 72°C for 10 minutes
11) Hold at 15°C

Adapters

| | |
|---|---|
| Pst1-1 | 5' CTC GTA GAC TGC GTA CAT GCA |
| Pst1-2 | 5' TGT ACG CAG TCT AC |
| Mse1-1 | 5 'GAC GAT GAG TCC TGA G |
| Mse1-2 | 5' TAC TCA GGA CTC AT |

Pre-Amplification Primers

| | |
|---|---|
| Pst1+A | 5' GAC TGC GTA CAT GCA GA |
| Mse1+C | 5' GAT GAG TCC TGA GTA AC |

Selective Primers

| | | |
|---|---|---|
| Mse1+CCC | 5' GAT GAG TCC TGA GTA A **CCC** | |
| Pst1+ ACG | 5' GAC TGC GTA CAT GCA G **ACG** | FAM |
| Mse1 +CAG | 5' GAT GAG TCC TGA GTA A **CAG** | |
| Pst1 +ATT | 5' GAC TGC GTA CAT GCA G **ATT** | HEX |
| Mse1 +CAG | 5' GAT GAG TCC TGA GTA A **CAG** | |
| Pst1 +CG | 5' GAC TGC GTA CAT GCA G**CG** | NED |

PCR products were separated on an Applied Biosystems 3730xl DNA analyser and polymorphic alleles scored using Applied Biosystems Peak Scanner and Genescan software.

## Results

Separation of the samples of *Orobanche minor* collected from West Beach and *O. cernua* var. *australiana* (Port Noarlunga) from samples collected in the quarantine area demonstrate that there is no close relationship between the three taxa of *Orobanche* present in South Australia or evidence of hybridisation (Fig. 2).

Samples collected from within the quarantine area showed no distinctive separation based on site (Fig. 3). Most samples formed a large cluster although the samples from Site 15 and to a lesser extent the samples from Site 8 were distinct from other sites. The spread of points indicates a high degree of genetic variation in the populations sampled, with samples collected from the same site not forming tight groupings. This would indicate that *Orobanche ramosa* is

not of recent occurrence in the region or has not recently spread to sites. The only site that would indicate some recent spread was Site 15, located in the southern extremity of the quarantine area. There was no evidence for a single recent introduction to the area.

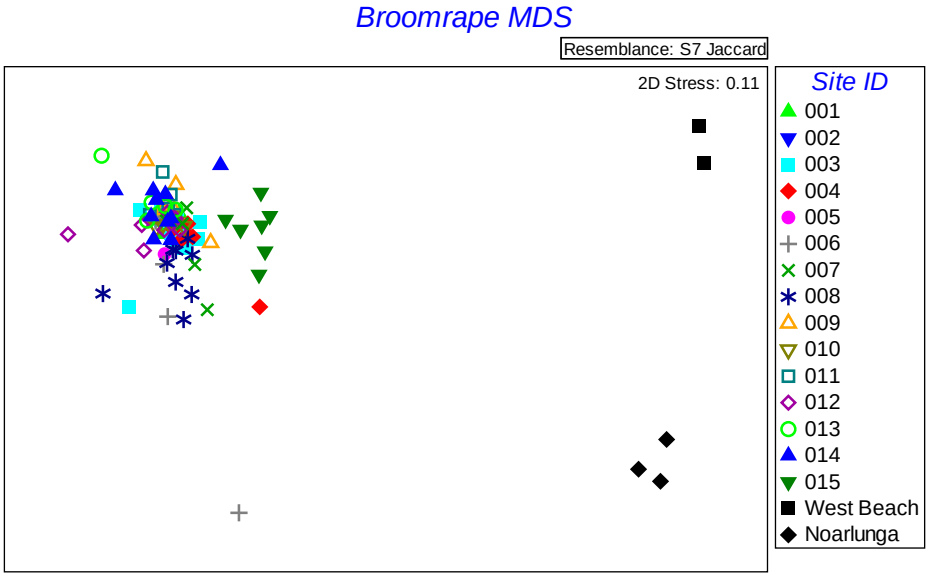


**Figure 2. Multi-dimensional scaling (MDS) plot of genetic separation of the three *Orobanche* taxa currently found in South Australia. The three clusters represent genetic distance between sampled plants. West Beach *(Orobanche minor*) and Noarlunga (*O. cernua* var. *Australiana*) are genetically distinct from each other and from the plants sampled in the quarantine area.**

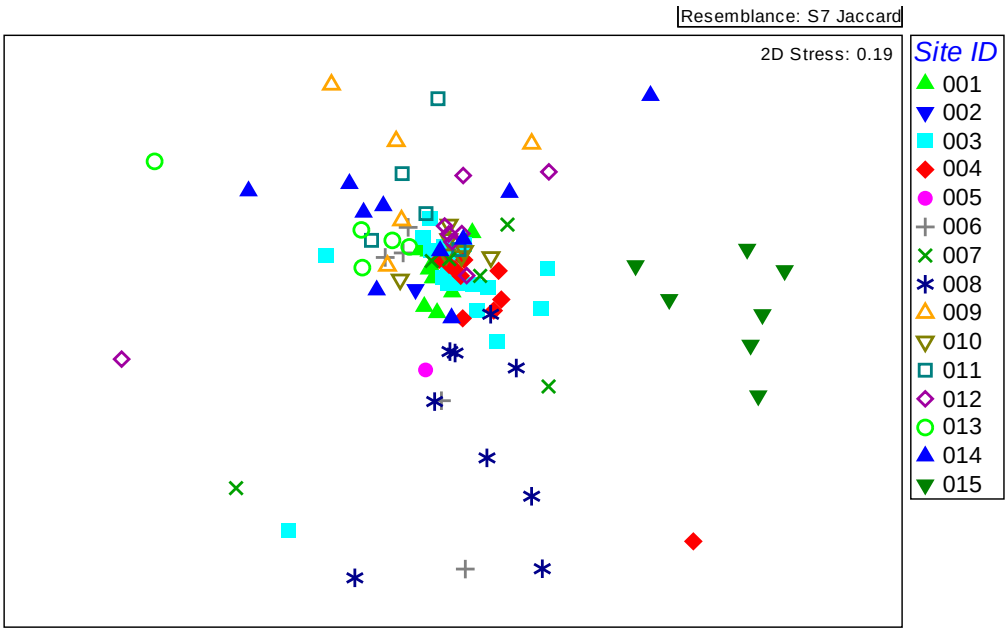


**Figure 3. Multi-dimensional scaling (MDS) plot of genetic relationship between *Orobanche ramosa* samples covering 15 sites within the quarantine area. The plot shows plants are genetically diverse within the sampled site and between sites. Site 15 shows some genetic clustering away from the other sites, indicating a recent spread, however generally the samples show diversity within paddocks.**