Dispersal biology of *Orobanche ramosa* in South Australia

Master of Science

Thesis

Emma L. Ginman

School of Earth and Environmental Science

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Chapter 4. Wind tunnel investigation of *Orobanche ramosa* dispersal by wind

Introduction

The seed morphology of *O. ramosa* suggests that wind is likely to be a dispersal mechanism (Howe and Smallwood 1982): seeds are small in size (3-6 µg), have a complex surface morphology (see Figure 3.1), but lack elaborate wings and plumes often associated with wind dispersal. Kuijt (1969) suggested that the concave cavities on the surface of *Orobanche* spp. seeds may assist them to lift up in wind.

The size of *Orobanche* spp. seeds places them in the 'dust propagules' category, along with the Orchidaceae family (Arditti and Ghani 2000; Cousens et al. 2008). While *Orobanche* spp. seldom appear in dispersal literature, the Orchideaceae are comparatively well studied. For example Arditti and Ghani (2000) reviewed the physical characteristics and fecundity of 20 orchid genera and related those to likely dispersal mechanisms (mainly wind and water). Murren and Ellison (1998) documented the size and shape of the orchid *Brassavola nodosa* (cigar-shaped seeds, 1.60 µg) and examined wind dispersal in a laboratory setting. *B. nodosa* seeds were found to travel up to 7 m at very low wind velocities (mean wind velocity of 1.25 m s^{-1}, height unreported), and greater numbers of seed travelled longer distances when the height of release increased. Orchid seed dispersal was also investigated by Machon et al. (2003) who found that most of the *Spiranthes spiralis* (L.) Chevall. seeds were detected within 0.15 m of the source. They also noted a low recovery rate of seeds in traps, trapping around 2% of the estimated total seeds available. The wind conditions at the time of trapping were not reported (Machon et al. 2003).

Species of *Striga* parasitise graminaceuos crops and have similar fecundity, seed longevity and seed size to *Orobanche* spp. (Musselman and Press 1995). Berner et al. (1994) trapped seeds of *Striga hermonthica* (Del.) Benth. at a distance of 12 m from the host plant. They also found seeds in traps within a *S. hermonthica* infestation at 2 m height, but none at 3 m and none placed at similar heights outside the infestations. The wind conditions at the time of trapping were also not reported (Berner et al. 1994).

Bullock and Clarke (2000) measured wind dispersal of the small-seeded *Calluna vulgaris* and *Erica cinerea* in English grasslands. They found that the vast majority of seeds were trapped within 0.8 m of the parent bush. However their main aim was to document long-distance dispersal, and the maximum found was in a trap at 80 m from seed source (Bullock and Clarke 2000).

Wind tunnels have been used for the last 20 years, under laboratory and field conditions, predominately to investigate erosion (Findlater et al. 1990; Raupach and Leys 1990; Leys and Raupach 1991; Shao et al. 1993; Leys et al. 1996; Eldridge and Leys 2003) and crop damage by sandblasting (Bennell et al. 2007). More recently wind tunnels have been used to examine dispersal of plant propagules (Eldridge and Leys 1999; Gravuer et al. 2003; Dauer et al. 2006; Baker and Beck 2008). Field based wind tunnels allow control over the wind velocity and direction applied to the propagules, whilst maintaining realistic surface conditions.

This chapter investigates the effects of wind speed and ground cover on seed dispersal of *O. ramosa*.

Methods

A factorial field experiment of two ground cover treatments (stubble and bare ground) by three wind velocities was undertaken.

Field site

The study site was located 71 km east of Adelaide, near Mannum, South Australia, (34.91°S, 139.40°E) (see map Figure 1.1 in Chapter 1), on a farming property within the South Australian Branched Broomrape quarantine zone.

The field experiment was conducted from 28 February to 3 March 2005.

Site preparation

The experimental field site was a 34.5 x 20 m area of wheat (variety ‘Krichauff’) sown approximately seven months prior. The field site was divided into two sets of 12 experimental plots, each 5.5 x 1.5 m, with a 1.5 m buffer alongside each plot and a 9 m wide roadway down the centre of the field site separating each strip of 12 plots (Figure 4.1).

Plots were divided into four replicate blocks, and within those blocks ‘stubble’ or ‘bare’ plots were randomly assigned. Stubble plots were cut to 0.2 m height with a sickle-bar mower attached to a tractor. The resultant stubble was sparse, irregularly spaced, and ≤ 0.2 m in height (Figure 4.2). Bare plots were mowed with a hand-pushed rotary slasher and cultivated with a tyne plough pulled by a tractor, resulting in a surface of loose soil, free of vegetation cover. Bare ground is classed ‘erodible’ and cereal stubble ‘stable’ in the event of a dust storm (Butler et al. 1995).

Wind tunnel

A field-based, portable wind tunnel was used to apply a variety of wind velocities (*u*) with the resultant seed dispersal measured by a number of trapping techniques (described below). Raupach and Leys (1990) describe the wind tunnel design in detail including flow conditioning, and a schematic diagram taken from that paper is reproduced in Figure 4.3. The tunnel consisted of a working section of 7.2 m in length, 0.9 m high and 1.2 m wide. The first 2 m of the length of the working section was covered by a wooden board as part of the flow conditioning; the remaining 5.2 m length was the experimental section open to the ground. A 56 kW diesel engine turned the 1.5 m axial fan, both mounted on a trailer. A hydraulic crane also mounted on the trailer was used to lower the working section of the tunnel onto the ground. The wind tunnel was capable of producing wind velocities of up to 50 km h^{-1}.

Pitot-static tubes (Dwyer Instruments, Inc., Michigan City) were located 1 m from the open end of the tunnel where wind velocity (u_z) was measured at six heights above the ground surface (z = 0.05, 0.10, 0.15, 0.20, 0.25 and 0.60 m).

Wind velocities

Horizontal wind velocity (*u*) within the tunnel was applied with three wind treatments, by setting the turning speed of the fan to 1000, 1300 and 1600 rpm. These three wind treatments were assumed to equate to low, medium and high wind velocities. Thus the 2-factorial experiment contained six treatments (bare-low, bare-medium, bare-high, stubble-low, stubble-medium, and stubble-high) randomly allocated within four replicate blocks.

For each experimental plot the wind tunnel was run for 60 s once the target fan speed had been reached and after addition of the seeds.

The roughness of a surface affects how wind passes over it. The rougher the surface, the more friction or drag is created, thus slowing down the wind velocity near the surface. For a given wind velocity, the friction created by the surface will reduce the velocity of the wind to zero at some height above that surface; surface roughness length (z_0) is a calculated value for that height, reported in mm. Surface roughness length was calculated for all wind tunnel runs to describe the surface roughness generated in the experimental plots, and for use in the calculation of the wind velocity at 10 m above the surface.

Friction velocity (u_*) is a measure of the velocity gradient adjacent to a surface, reported in m s^{-1}.

Surface roughness length (z_0) and friction velocity (u_*) were derived from tunnel measurements of velocity (u) and height of measurement (z) using Equation 1, from Leys and Raupach (1991):

$$u_z = \left(\frac{u_*}{k}\right) \times \ln\left(\frac{z}{z_0}\right)$$ **Equation 1**

where:

z is the height above mean ground level (m),

$u_{(z)}$ is the wind velocity at height z (m s^{-1}),

u_* is the friction velocity (m s^{-1}),

k is the von Karman constant (assumed to be 0.4), and

z_0 is the surface roughness length (also called aerodynamic roughness) (calculated in m, reported here in mm).

As described above, wind velocities are affected by surface roughness, so direct comparisons between wind velocities at heights within the tunnel for bare ground and stubble plots are generally unrealistic. To make comparisons between treatments with different degrees of roughness (such as bare ground versus stubble plots), measured wind velocities were extrapolated to equivalent wind velocities at 10 m (the Australian Bureau of Meteorology standard for reporting wind velocities in the field). The wind velocity measured in the tunnel at 0.2 m was converted to the equivalent wind velocity at 10 m (u_{10}) using the z_0 ratio (Equation 2) as described in Leys et al. (1996):

$$\frac{u_a}{u_b} = \ln\left(\frac{a}{z_0}\right) \div \ln\left(\frac{b}{z_0}\right)$$ **Equation 2**

where symbols are defined as for Equation 1, in addition:

u_a is the wind velocity at height *a* (in this case 10 m), and

u_b is the wind velocity measured at height *b* (in this case measured within the tunnel at height of 0.2 m).

As z_0, *a* and *b* are known, the ratio of wind velocity at 10 m to the wind velocity at 0.2 m (u_a/u_b) can be determined. u_a can then be calculated by multiplying u_b by the ratio. Height of 0.2 m is chosen for calculation of the z_0 ratio as it is considered to be the highest measured wind velocity (measured by Pitot tube) within the boundary layer (the layer of reduced velocity air immediately adjacent to a surface) produced by the stubble.

Seed traps

Four trap types were used: a Bagnold trap, wind vane samplers, sticky slide traps, and tube traps. All four trap types were deployed and used for each wind run. Traps were positioned on each experimental plot without disturbing the ground surface, the tunnel was then positioned over the top of the deployed traps. Soil cores were also taken after each run. It was assumed that traps were independent of each other, and were not affecting other traps. Seeds caught in traps were manually counted in the laboratory.

Bagnold traps and wind vane samplers are commonly used in wind tunnel experiments and natural soil erosion surveys in Australia (Leys and Raupach 1991; Leys et al. 1993; Leys et al. 1996; McTainsh 1999). These traps have been tested for their sediment trapping efficiency and the Bagnold trap is deemed to oversample the total mass (i.e. 102% ± 5%) while the wind vane samplers have a trapping efficiency of 86-95% (Shao et al. 1993). The trapping efficiency of the sticky slide traps and tube traps was not known.

Bagnold trap

A Bagnold trap was used to calculate seed transport rate down the tunnel. The Bagnold trap is considered to be the ‘classic’ horizontal sand flux sampler for estimating sand transport in field and laboratory situations (Goossens et al. 2000). It is an active, vertically integrating trap, with an opening 5 mm wide by 480 mm high (Figure 4.4a). A household vacuum cleaner is used to apply constant suction and material is caught in paper bags. It was positioned on the ground at 4.2 m down the tunnel and 0.35 m from the left hand side of the tunnel (when facing up-wind) (Figure 4.5).

Wind vane samplers

Wind vane samplers, also known as Fryrear traps (Fryrear 1986) are passive, point sampling traps with an intake area 20 mm x 50 mm (Figure 4.4b). Two wind vane samplers were positioned on the ground 4.2 m down the tunnel and 0.10 m to the left and right of the Bagnold trap (Figure 4.5).

The cross section of the tunnel had an area of 1.08 m^2. The Bagnold trap and wind vane samplers positioned at the open exhaust end accounted for 0.0044 m^2, or 0.41% of the total cross-section area.

Sticky slide traps

Sticky slide traps were used to measure the distribution of seeds from the release point along the length of the tunnel at ground level. Each sticky slide trap consisted of a glass microscope slide 25 mm wide by 76 mm long (Livingstone International Pty Ltd, Rosebery, NSW) with a strip of double sided tape 18 mm wide (Sellotape Regd. Dalton Packaging Pty Ltd Bankstown, NSW) placed down the centre. Each slide was placed on an aluminium rack 86 mm long x 30 mm wide x 10 mm high (Figure 4.4c). Thirty-five sticky slide traps were placed on the ground at seven logarithmic positions along the tunnel (0.00, 0.125, 0.05, 0.25, 0.50, 1.00, 2.00 and 4.00 m from the edge of the tunnel’s wooden board). At each of these distances, 5 slides were placed randomly across the tunnel (Figure 4.5).

Tube traps

Tube traps were used to measure the distribution of seeds from the release point at three distances along the length of the tunnel (1.0, 2.0 and 3.0 m) and at three heights above the ground (0.10, 0.30 and 0.60 m). Each tube trap consisted of 10 mm diameter aluminium tube cut in 100 mm lengths with the exhaust opening blocked off with a piece of nylon mesh (commercial fine grade pantyhose) attached to the tube with a rubber band. Three tubes were welded horizontally and in parallel onto an aluminium peg at the three specified heights, making one ‘rack’ (Figure 4.4d). Three racks were deployed across the width of the tunnel at each of the three distances (Figure 4.5), giving 27 tube traps per wind run.

<u>Soil cores</u>

Fifteen soil cores were taken from each plot after the tunnel and traps had been removed. Cores were taken using a metal cylinder (50 mm diameter by 15 mm deep) and trowel. Five samples were taken across the 1.2 m width of the wind tunnel at distances of 0.5, 1.0 and 3.0 m from the wooden board. The five samples for each distance were pooled, weighed to the nearest gram and sieved. Particles greater than 0.3 mm were discarded, and the samples were then weighed again. These samples were sub-sampled from a homogeneous mix in order to obtain samples of a suitable size for DNA testing. Testing for *O. ramosa* DNA was conducted by the South Australian Research and Development Institute (SARDI) diagnostics group. Methods for the DNA extraction and the quantitative PCR (Polymerase Chain Reaction) are the intellectual property of SARDI and the DNA analyses were conducted on a fee for service basis.

Seeds

For each wind run, forty-thousand *O. ramosa* seeds were estimated by volume and introduced into the tunnel via two aluminium tubes. The open ends of the tubes were located above the wooden board at 0.10 m height, roughly corresponding to the mean height of flower heads in the field (personal observation). Seeds were released manually once the fan reached the designated speed, which was approximately 5 s after the fan began turning. The tunnel was then operated for 60 s from the time the seeds were added, for each wind run.

Ground cover

Extent of ground cover (plant and rock material) of each plot was estimated by grid point intercept survey after the wind tunnel and seed traps had been removed. The grid consisted of 20 transects placed 0.25 m apart and sampled at 0.15 m intervals, and presence/absence point observations were made for each of 200 sample points. Data for each plot were pooled to give a total number of points (up to a maximum of 200).

Data analysis

Data collected from traps along the width of the tunnel at the same distance were pooled, as the dispersal length and height were of interest rather than variation in lateral movement within the tunnel. Univariate analyses were performed on each of the four seed trap types and the soil cores, with "number of seeds trapped" as the response variable. Data were suitably transformed (square root transformation and natural logarithmic transformation as applicable) and Analysis of Variance, Variance Ratio Tests (F-tests), and Least Significant Difference tests were performed. Data were considered significant if $P < 0.05$ ($\alpha = 0.05$).

Results

Ground cover

Ground cover estimates showed that bare plots had an average cover of plant or rock material of 9.2% (18.3 points per 200, S.E. = 1.98) which was significantly different from the stubble plots ($F_{1,23} = 81.67$, $P < 0.001$) which had an average of 61.2% cover (122.3 points per 200, S.E. = 10.30).

Wind velocities

Within each ground cover treatment, the measured wind velocities increased with increasing wind treatment (Table 4.1). At heights up to and including $z = 0.25$ m, the average wind velocities measured in stubble were less than those measured on bare ground. Conversely, wind velocities measured at $z = 0.60$ m, and derived for $z = 10$ m, were greater for stubble compared with bare ground (Table 4.1).

Friction velocity (u_*), roughness length (z_0), and z_0 ratio all increased with increasing wind velocity (although u_* was the only one showing a statistically significant difference, $F_{2,24} = 19.38$, $P < 0.0001$) and were greater on stubble plots than bare ground (u_* $F_{2,24} = 144.14$, $P < 0.0001$; z_0 $F_{2,24} = 95.6$, $P < 0.0001$; z_0 ratio $F_{2,24} = 170.41$, $P < 0.0001$) (Table 4.2).

As expected, the wind treatments (i.e. fan speed) did not yield equal wind velocities on bare ground and stubble treatments. However, the wind velocities produced by the medium (1300 rpm) and high (1600 rpm) wind treatments were consistently higher, approximately 30% and 60% , respectively, than those produced by the low (1000 rpm) treatment (Table 4.1).

Seed trapping

Table 4.3 shows P-values and significant results for number of seeds trapped and the significance of wind velocity, ground cover, distance travelled, and height of capture for each measure.

For each wind treatment, higher numbers of seeds were caught exiting the tunnel on bare ground than on stubble plots, by both the Bagnold traps and the wind vane samplers (Figure 4.6). Thus, more seeds were reaching the end of the tunnel in the bare ground treatments than in the stubble treatments.

Sticky slide traps showed that more seeds were trapped within the tunnel on stubble plots than bare ground plots, for medium and high wind velocities (Figure 4.7). Low wind velocity showed the sharpest decrease in number of seeds caught with distance from seed source (Figure 4.7) as it is assumed that at higher wind velocities more seeds are exiting the tunnel without being trapped. Overall, stubble plots trapped more seeds on sticky slide traps ($P = 0.012$).

Seeds were trapped in the tube traps at all sampled heights (0.10, 0.30, 0.60 m) with the majority of seeds trapped at 0.10 m height (Figures 4.8, 4.9 and 4.10), regardless of wind velocity.

Seed detection rates in soil cores showed no significant effects between treatments at the $\alpha = 0.05$ level (Table 4.3) although *ground cover x distance* resulted in $P = 0.06$. There was high variability in seed detection levels, and no clear spatial trends were evident from the soil cores (Figure 4.11).

On average, 530 seeds were trapped by the four seed traps on each wind run (S.E. = 83). Given approximately 40 000 seeds were released per wind run, the seed trapping rate ranged from 0.31% to 4.68% of the seeds added for each wind run.

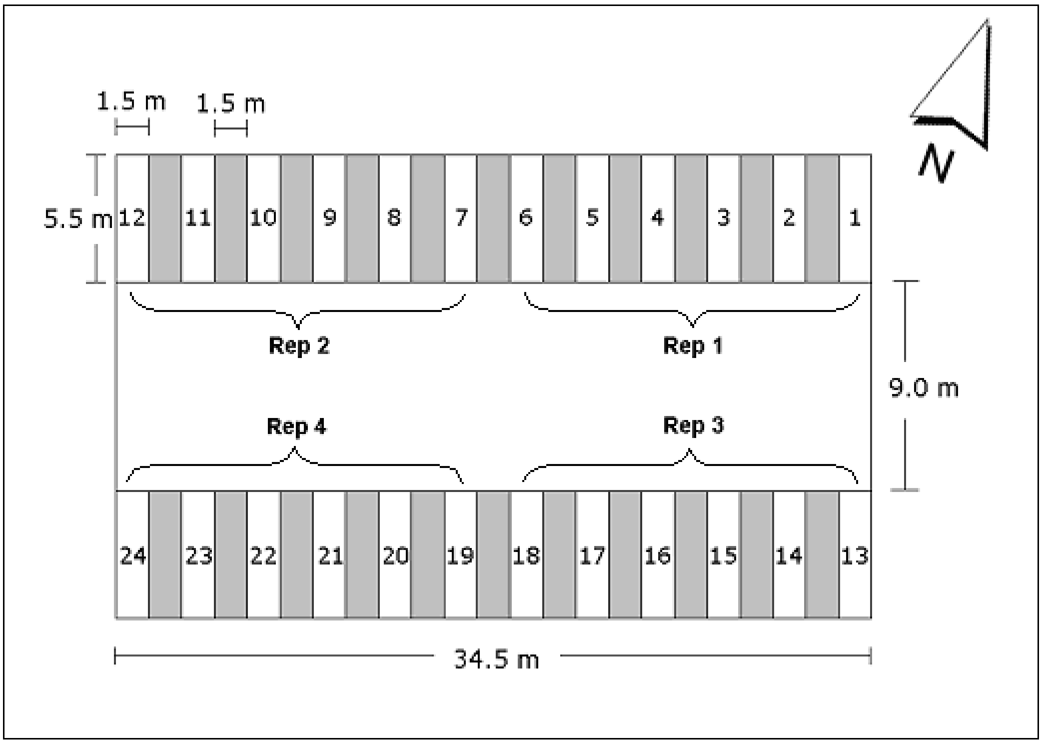


Figure 4.1. Diagram of the site layout for the wind tunnel experiments. Grey areas indicate buffer zones between the experimental plots. Experimental plots are numbered in the order in which the wind runs were performed. Three stubble and 3 bare ground plots were randomly allocated across the 6 plots within each replicate.

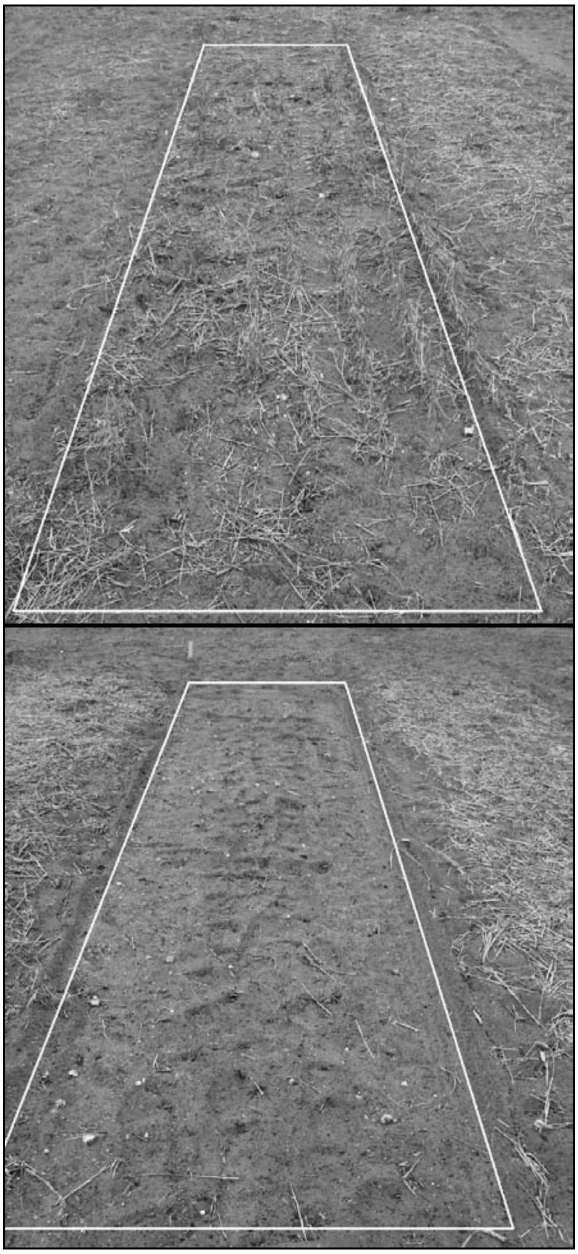


Figure 4.2. Examples of the two ground cover treatments used in the wind tunnel experiment; Stubble plot (top) and bare ground (bottom). White line indicates the approximate position of the wind tunnel on the plot.

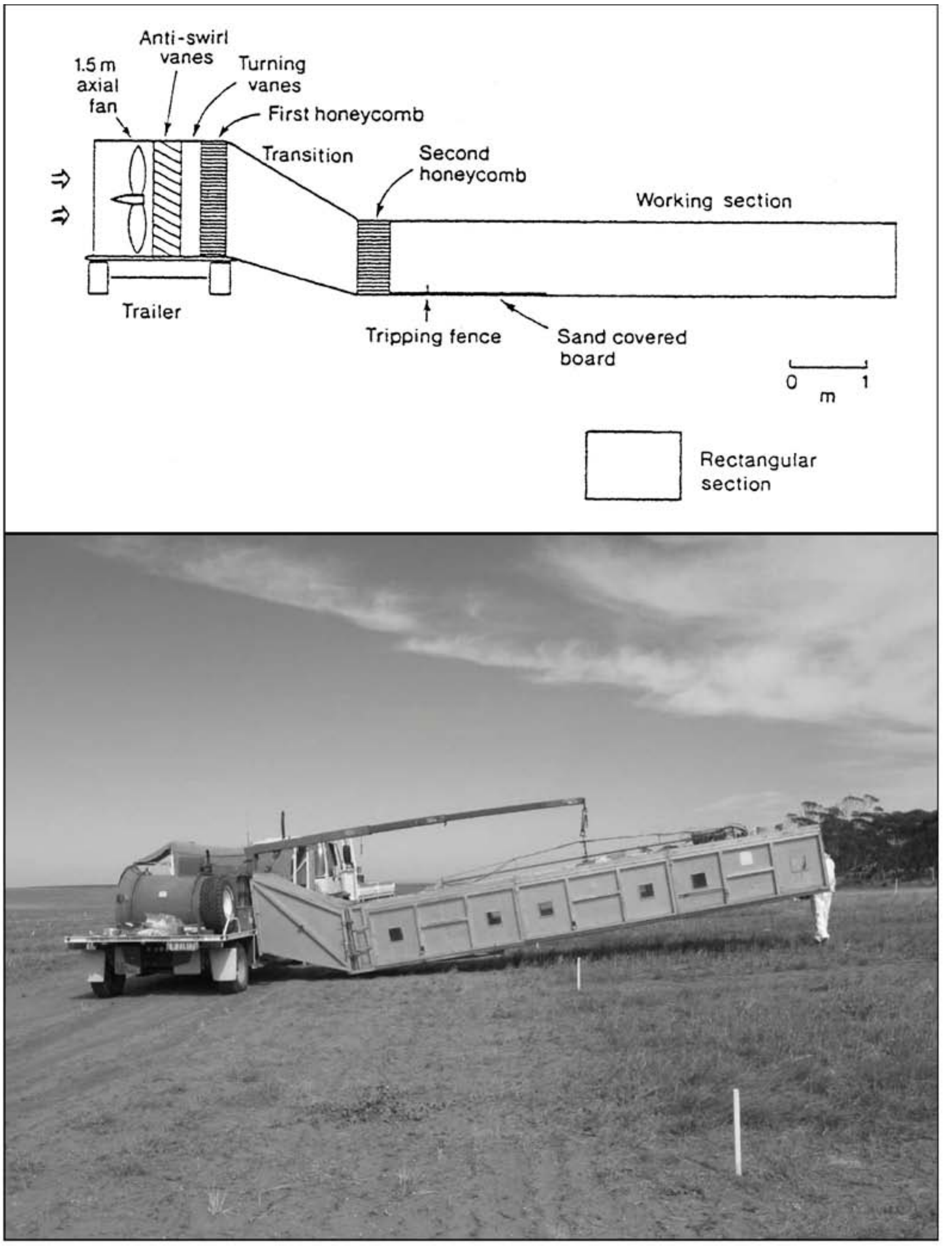


Figure 4.3. Diagram (top) and photograph (bottom) of the field-based wind tunnel used in *O. ramosa* experiments. Schematic diagram from Raupach and Leys (1990), © CSIRO Publishing, used with permission.

CSIRO Publishing - http://www.publish.csiro.au/nid/84/paper/SR9900177.htm.

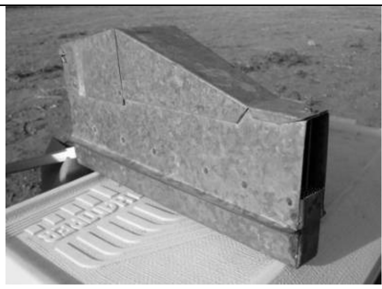
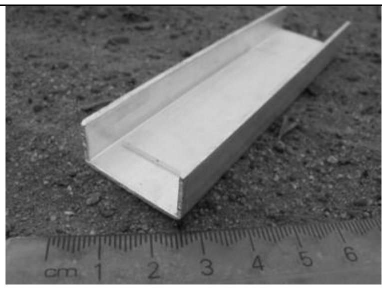
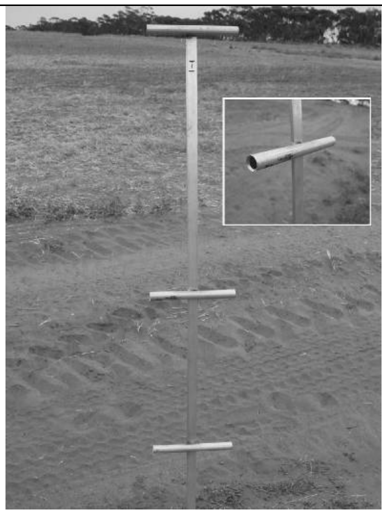
A. Bagnold trap		x 1 unit
B. Wind vane sampler		x 2 units
C. Sticky slide trap		x 35 units
D. Tube trap		x 9 racks (27 tubes)

Figure 4.4. The four seed trap designs that were used in the wind tunnel experiment. The number of each type of trap used per run is indicated beside each photograph

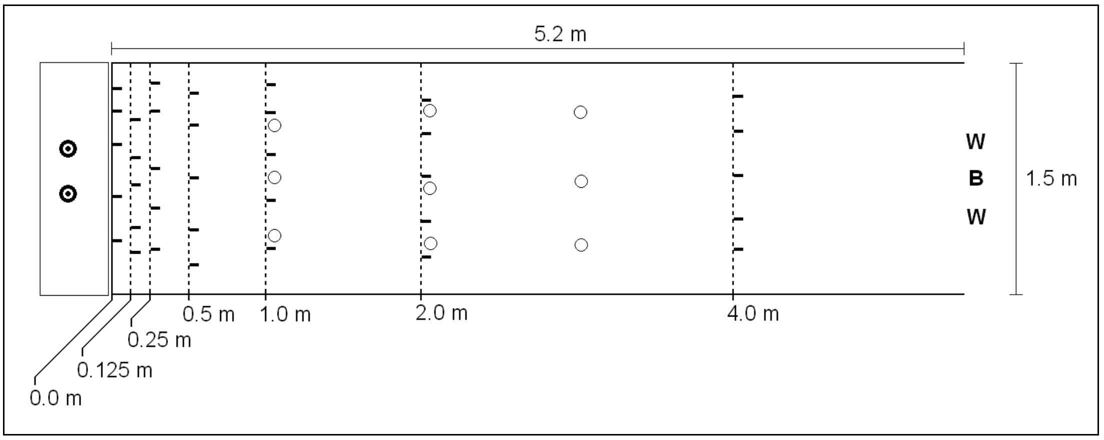


Figure 4.5. Arrangement of the four trap types in the wind tunnel, view from above looking down towards the ground. B = Bagnold trap, W = wind vane sampler, dashes = sticky slide traps, circles = rack of tube traps. The wind tunnel exhausts to the right of the diagram, the fan and wooden board are to the left of the diagram. The two bullseyes on the left of the diagram indicates the approximate location of the aluminium tubes through which seeds were released into the wind stream.

Table 4.1. Velocity profile produced in the wind tunnel experiments. Mean wind velocities ($\bar{u}$) measured by Pitot tubes placed at heights = 0.05, 0.10. 0.15, 0.20, 0.25 and 0.60 m, n = 4. Wind velocities at 10 m ($\bar{u}_{10}$) have been calculated according to Leys et al. (1996). % indicate the percent increase in wind velocity compared to the low wind velocity treatment, for each groundcover treatment.

Treatment	$\bar{u}_{0.05}$ (m s^{-1})	$\bar{u}_{0.10}$ (m s^{-1})	$\bar{u}_{0.15}$ (m s^{-1})	$\bar{u}_{0.20}$ (m s^{-1})	$\bar{u}_{0.25}$ (m s^{-1})	$\bar{u}_{0.60}$ (m s^{-1})	$\bar{u}_{10}$ (m s^{-1})
Bare-low	5.08 (0%)	5.86 (0%)	6.24 (0%)	6.56 (0%)	6.82 (0%)	7.27 (0%)	10.69 (0%)
Bare-medium	6.48 (27%)	7.65 (31%)	8.18 (31%)	8.61 (31%)	9.00 (32%)	9.81 (35%)	14.61 (47%)
Bare-high	7.98 (57%)	9.42 (61%)	10.02 (61%)	10.52 (60%)	10.97 (61%)	12.04 (66%)	17.19 (61%)
Stubble-low	3.03 (0%)	4.01 (0%)	4.72 (0%)	5.74 (0%)	6.51 (0%)	7.88 (0%)	14.50 (0%)
Stubble-medium	3.78 (25%)	5.08 (27%)	6.15 (30%)	7.56 (32%)	8.56 (31%)	10.34 (31%)	19.57 (39%)
Stubble-high	4.86 (57%)	6.51 (56%)	7.82 (58%)	9.11 (53%)	10.19 (53%)	12.56 (59%)	23.63 (58%)

Table 4.2. Friction velocity (u_*), surface roughness length (z_0) and z_0 ratio values derived from the velocity profile for the wind tunnel experiments.

Treatment	u_* (m s^{-1})	z_0 (mm)	z_0 ratio
Bare-low	0.42	0.46	1.63
Bare-medium	0.62	0.74	1.70
Bare-high	0.68	0.80	1.64
Stubble-low	0.93	14.09	2.55
Stubble-medium	1.31	15.67	2.59
Stubble-high	1.52	16.71	2.60

Table 4.3. Probability values (P-values) for Variance Ratio Tests (F-tests) of seed numbers caught by four trap types and in soil cores. Numbers in bold type indicate the highest order interaction for each trap type (where effects are significant). * indicates statistically significant *P*-values at α = 0.05 level.

Effect: / Trap type:	Bagnold trap	Wind vane trap	Sticky slide trap	Tube trap	Soil cores
Wind velocity x ground cover x distance x height				0.493	
Wind velocity x ground cover x distance			**0.020***	0.332	0.061
Wind velocity x ground cover x height				0.603	
Ground cover x distance x height				0.010*	
Wind velocity x ground cover	0.427	0.095	0.632	0.118	0.651
Wind velocity x distance			<0.001*	0.933	0.821
Wind velocity x height				**0.005***	
Ground cover x distance			<0.001*	0.056	0.060
Ground cover x height				0.002*	
Distance x height				<0.001*	
Wind velocity	0.016*	<0.001*	0.001*	<0.001*	0.901
Ground cover	0.009*	<0.001*	0.012*	0.795	0.491
Distance			<0.001*	<0.001*	0.081
Height				<0.001*	

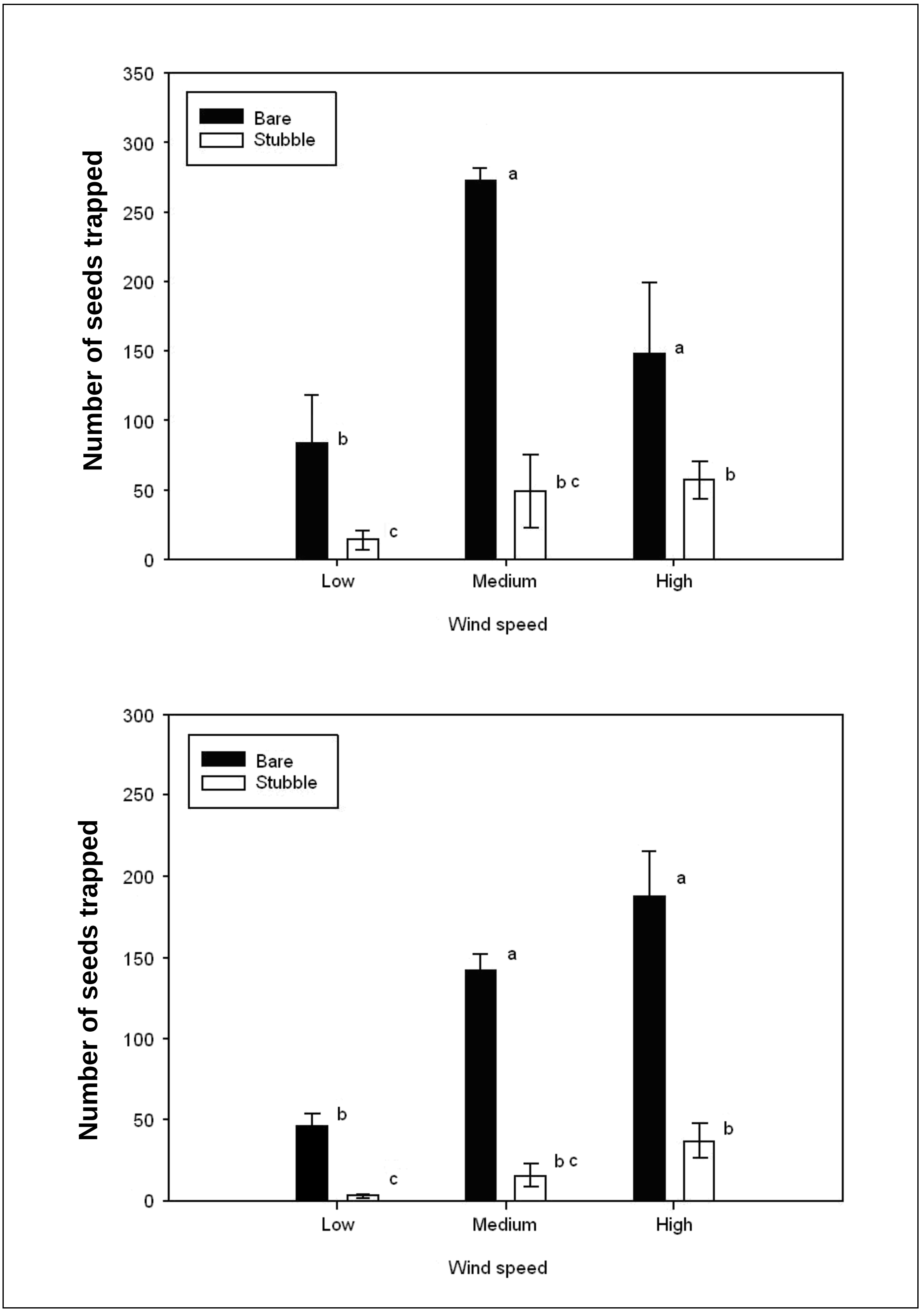


Figure 4.6. The effects of wind velocity and surface cover on the number of *O. ramosa* seeds caught in Bagnold traps (top) and wind vane samplers (bottom) at the open end of the wind tunnel. Data are means ± SE, $n = 4$. Same letters on each graph are not significantly different at $\alpha = 0.05$ level according to Tukey HSD test.

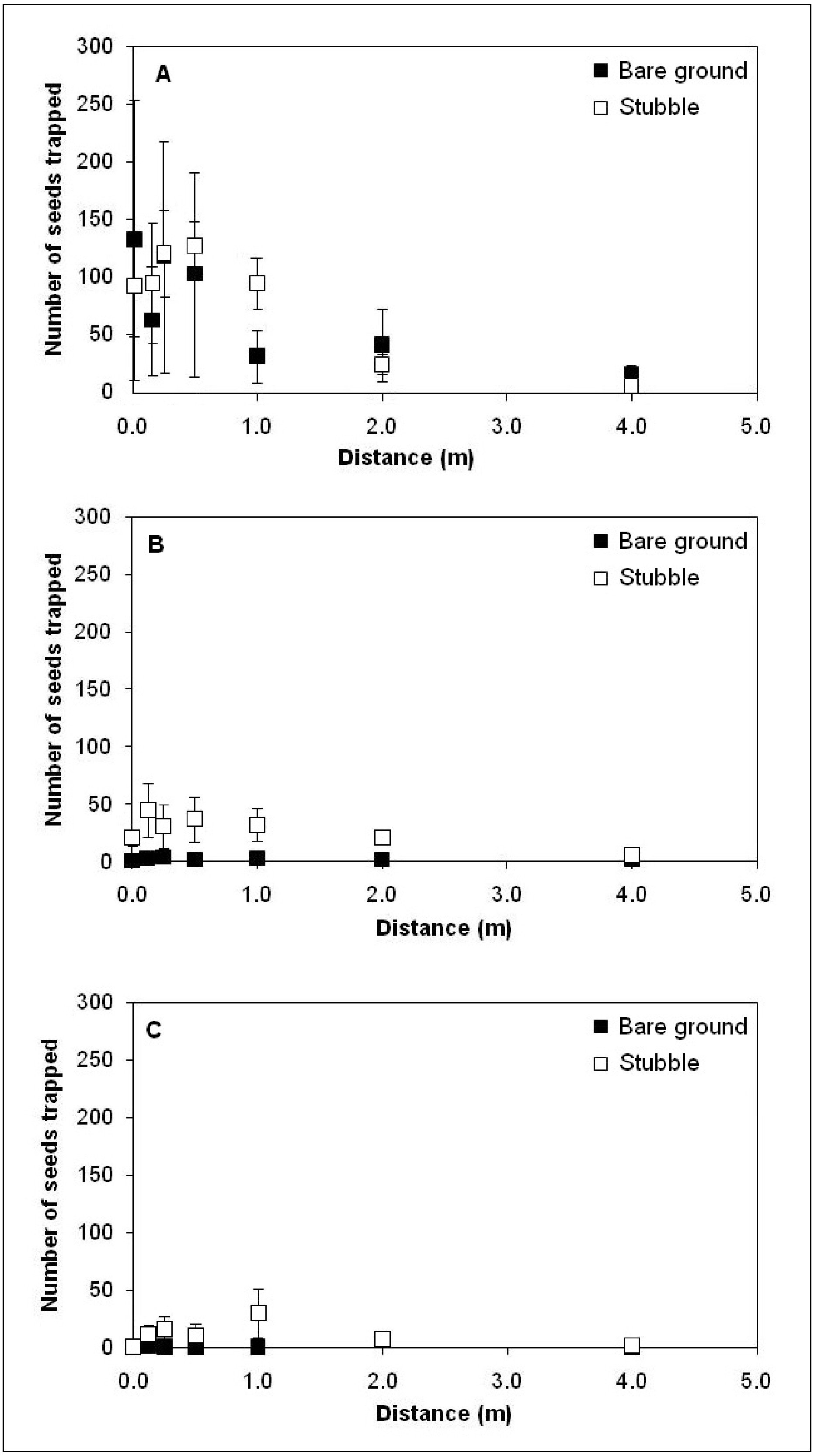


Figure 4.7. Number of *O. ramosa* seeds caught in sticky slide traps on bare ground (■) and stubble plots (□) at three wind velocities: low (A), medium (B), high (C). Numbers of seeds trapped across the width of the tunnel have been pooled for each distance point. Data are means, $n = 4$.

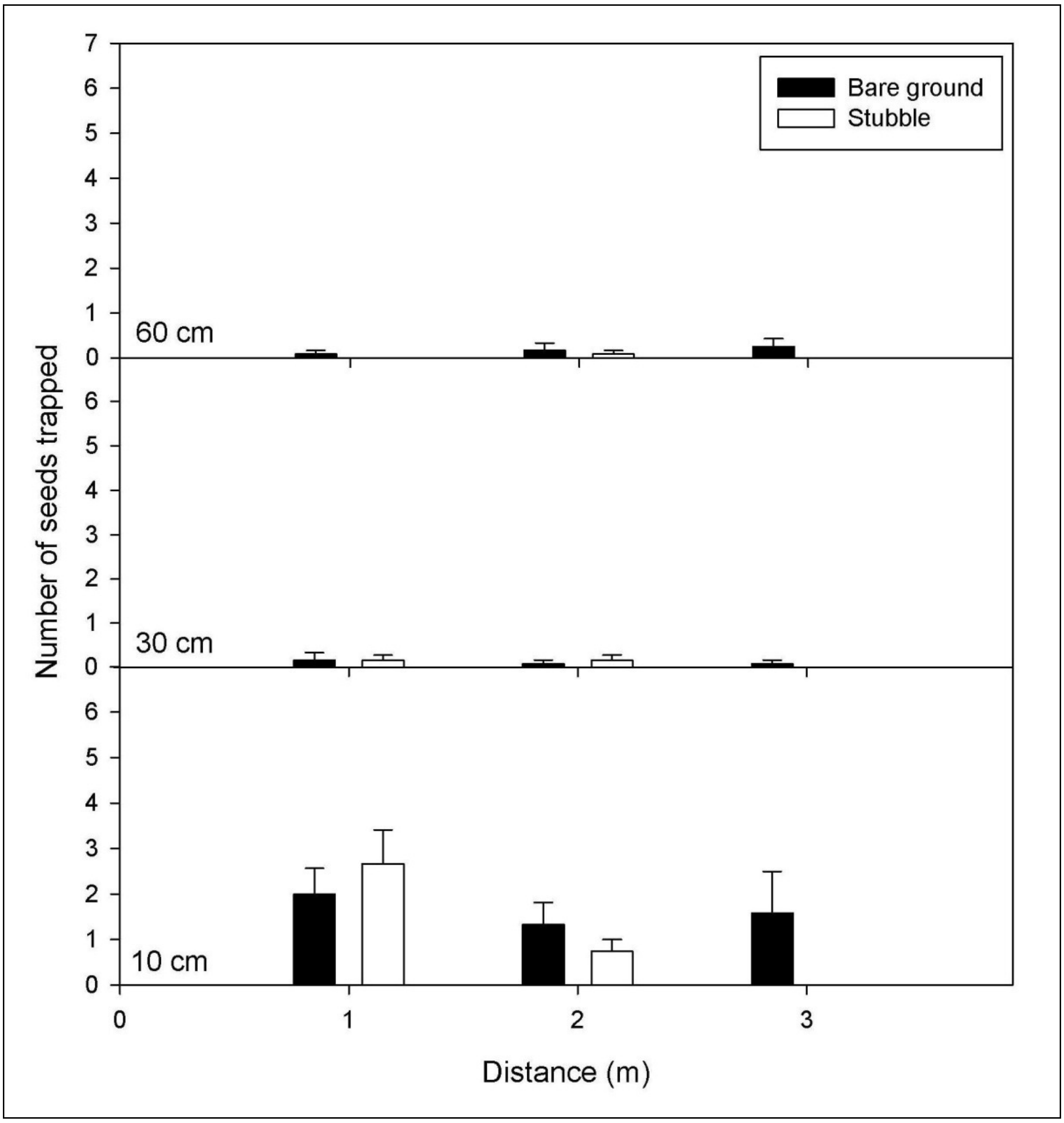


Figure 4.8. Number of *O. ramosa* seeds caught in tube traps at 0.10, 0.30 and 0.60 m above ground level, and at low wind velocity on bare ground (■) and stubble plots (□). Data are means ± SE, n = 4.

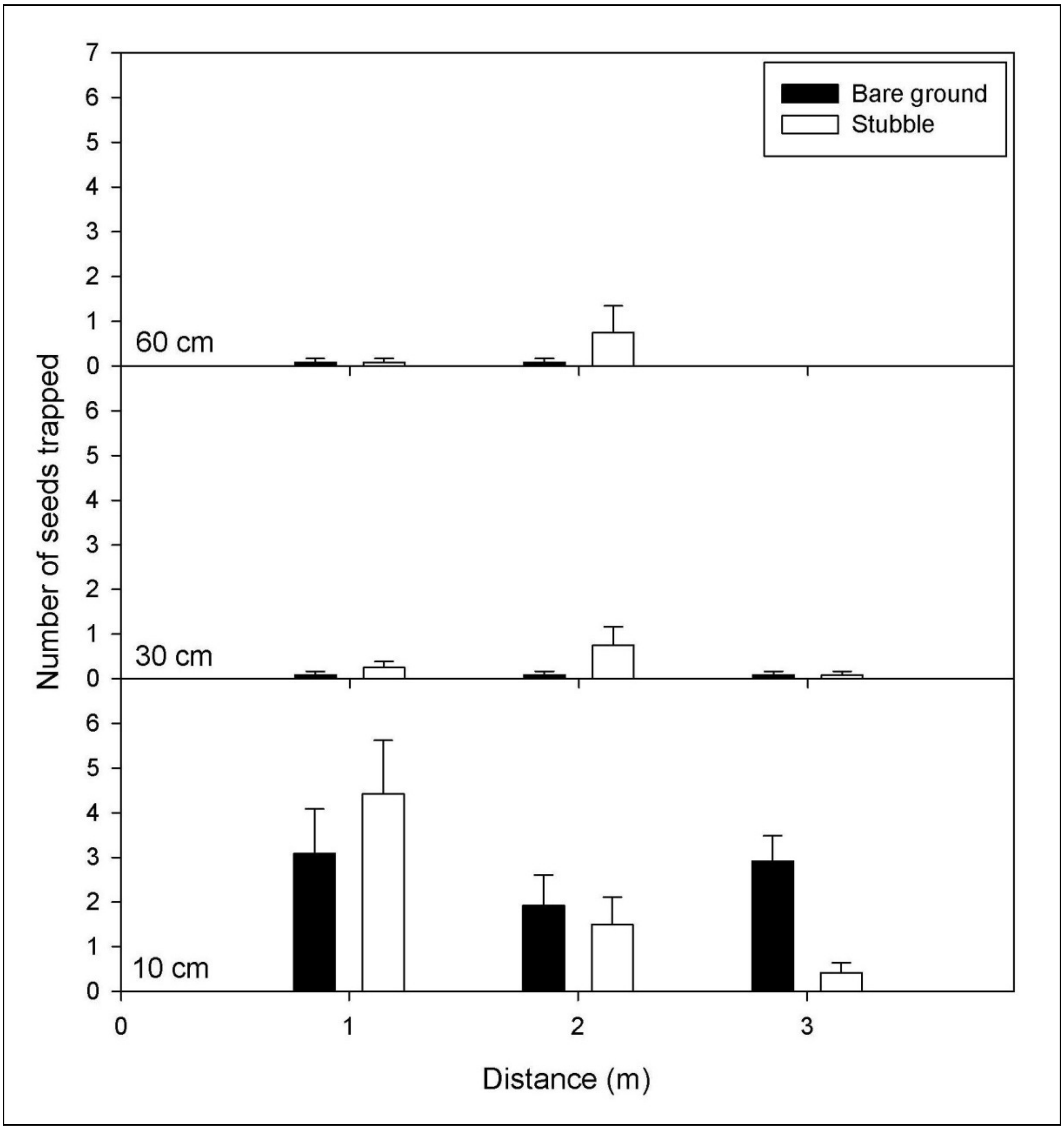


Figure 4.9. Number of *O. ramosa* seeds caught in tube traps at 0.10, 0.30 and 0.60 m above ground level, and at medium wind velocity on bare ground ■ and stubble plots □. Data are means ± SE, *n* = 4.

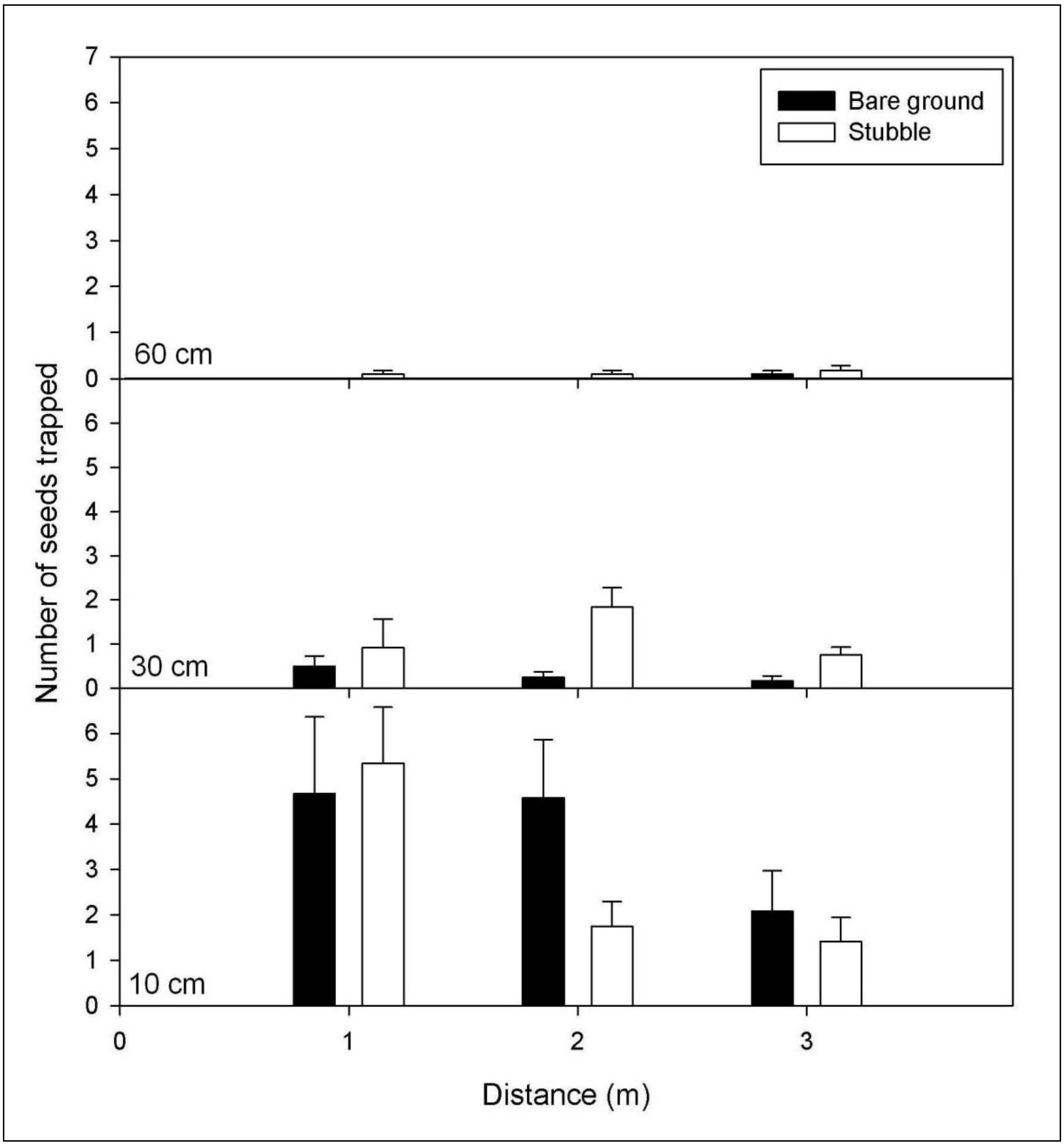


Figure 4.10. Number of *O. ramosa* seeds caught in tube traps at 0.10, 0.30 and 0.60 m above ground level, and at high wind velocity on bare ground (■) and stubble plots (□). Data are means ± SE, $n = 4$.

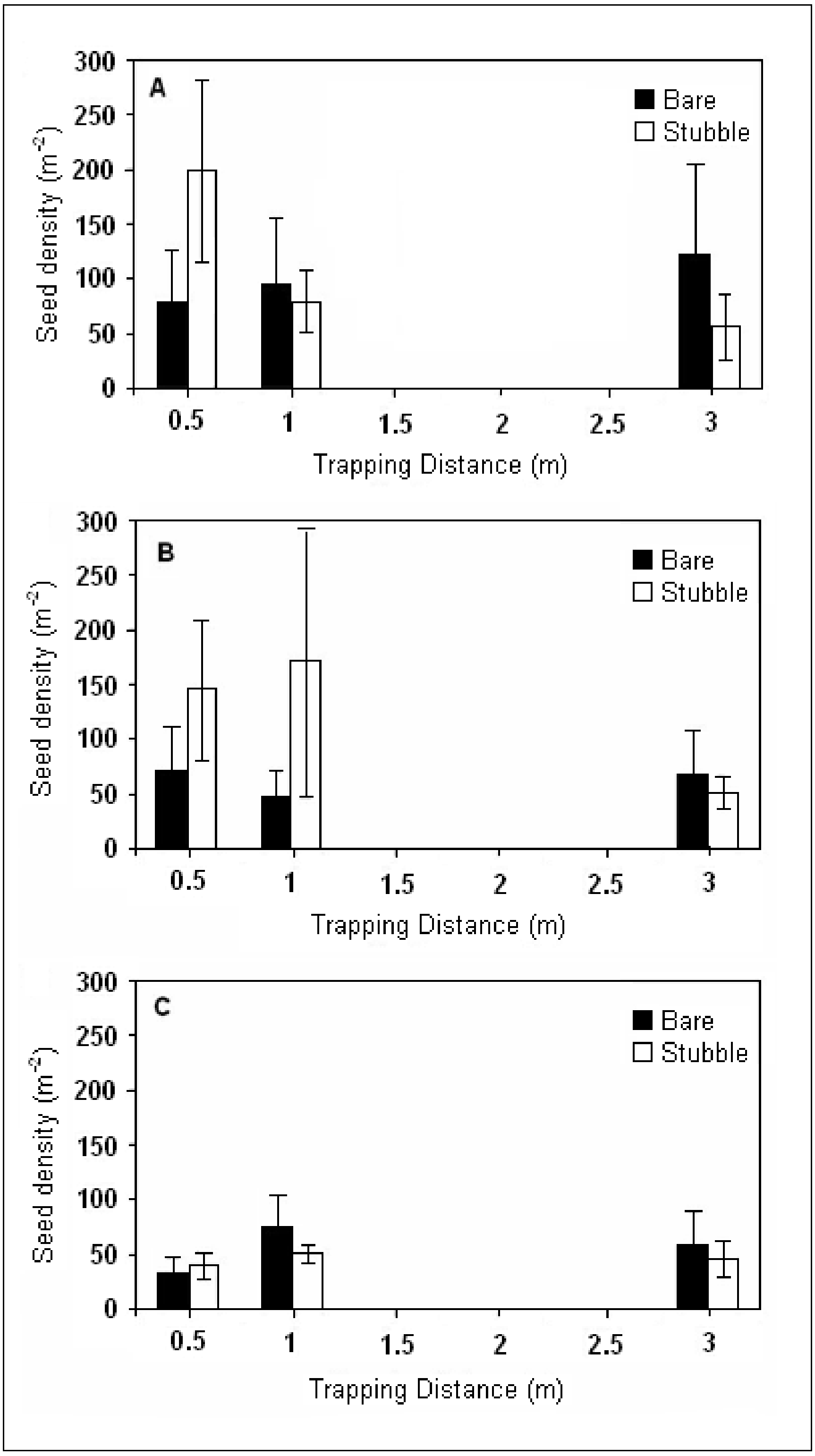


Figure 4.11. Estimated density of *O. ramosa* seeds in soil cores at three distances down the wind tunnel, (A) low wind treatment, (B) medium wind treatment, (C) high wind treatment. Data are means, error bars show SE, n = 4.

Discussion

This experiment confirms that *O. ramosa* seed can be dispersed by wind, at least 5.2 m after 60 s with a wind velocity at 10 m of 10.69 km h^{-1}. While other researchers have shown that wind can transport dust propagules (Berner et al. 1994; Murren and Ellison 1998; Bullock and Clarke 2000; Machon et al. 2003; Bullock and Moy 2004) including *Orobanche* sp. specifically (Mesa-García et al. 1986; Castejón-Muñoz et al. 1991), the current study shows the importance of paddock ground cover in seed dispersal. This study showed that plant and rock material impeded the movement of seeds by wind which has important implications for paddock management within the quarantine zone.

The cereal stubble used in this study was drought affected and even when combined with rock material afforded only 61% ground coverage. It was considered to be poorer than other cereal in the district that season, and much poorer than would be expected in years with higher rainfall (D. Miegel pers. comm.). Despite this, the poor cereal stubble still had a substantial affect on the dispersal of *O. ramosa* seeds.

Within the tunnel, it was assumed that wind velocities up to 0.25 m in height were likely to be influenced by the boundary layer (the layer of air affected by the surface roughness), whereas $\bar{u}_{0.6}$ were considered to represent the free stream velocities, the velocity of air which is unaffected by the boundary layer. This boundary layer effect was demonstrated in the wind tunnel where it can be seen that the wind velocities were lower where a boundary layer was created by the stubble (Table 4.1).

The wind treatments applied in the experiment did not yield the same resultant wind velocities, with stubble plots yielding lower $\bar{u}$ than bare ground plots for heights up to z = 0.25 m. At z = 0.6 m the stubble and bare ground plots were both operating at free stream velocity. A residual effect of the wind tunnel is that the presence of stubble reduces the total volume of the tunnel, resulting in increased air pressure above the boundary layer (z = 0.6 m). This is demonstrated by the stubble plots yielding greater $\bar{u}$ at heights above the boundary layer (Table 4.1).

Regardless, the resultant wind velocities were considered to be comparable, as $\bar{u}$ produced by the medium (1300 rpm) and high (1600 rpm) wind treatments were approximately 30% and 60% higher, respectively, than those produced by the low (1000 rpm) treatment for both bare ground and stubble treatments (Table 4.1).

The Bureau of Meteorology data indicate that the average wind velocity in nearby Murray Bridge at 0900 h is 2.5 m s^{-1}, and at 1500 h is 4.0 m s^{-1}. Thus, the resultant wind velocities created in the wind tunnel (ranging from 10 to 23 m s^{-1}) were much higher than the average for the area at those times of day. However, such a measure of average wind velocity at a given clock time would not account for wind gusts and strong wind events, which as discussed earlier would be important in triggering seed release and transport.

Constant wind velocities such as those produced by the wind tunnel are not typical in a field situation: gusts and stochastic wind events are likely to influence the way in which seeds are released from capsules and subsequently travel through the air, and this would require further research.

A range of trap types were used, both existing and new designs. This allowed the comparison of traps that had known trapping efficiencies against new designs thought to be more suitable for characterising seed dispersal.

The Bagnold trap and wind vane samplers are long standing, proven trap designs, used for sand trapping to estimate sediment transport rate. The wind vane sampler is particularly useful for remote field monitoring over long periods of time (J. Leys pers. comm.). The sticky slide traps were specifically designed for this study and had not been tested for trapping efficiency. Sticky slides trapped fewer seeds at medium and high wind velocities, largely contradicting the results of the other traps (excluding soil cores) that showed increased numbers of seeds trapped during high wind treatment.

The tube traps were also a new design that was untested for trapping efficiency. While they did not allow for fine resolution of seed dispersal in the wind profile to be determined, the tube traps did verify that seeds were carried up into the wind profile, and were trapped at a greater height than they had been released. This could have been caused by one or more factors: seeds bouncing off the length of wooden board at the start of the tunnel, or ricocheting off other obstacles within the tunnel (traps, plant material, rocks, etc.); turbulence in the air flow; and aerodynamic lift of the seeds due to the dimples on the seed surface (Kuijt 1969). Indeed in the field situation other factors may also be acting, for example updraughts have been shown to drive long distance dispersal of winged seeds of dandelions (Tackenberg et al. 2003b)

It was assumed that seeds trapped in the wind tunnel were the seeds released manually via the aluminium tubes. However, the field locality was an infested site, and would have had some level of background seeds in the soil seedbank. The method most likely to be affected by this seedbank was the soil cores. Soil cores and DNA analysis have been used in South Australia in attempts to quantify *O. ramosa* seedbank levels, but results have been highly variable (Williams et al. 2006). Similarly van Delft et al. (1997) discovered that seeds of *S. hermonthica* were not homogeneously distributed horizontally in the soil of paddocks in South Africa, even when those paddocks had been ploughed. Williams et al. (2006) hypothesise that such variability could be due to low seedbank density, spatial variability in the seed bank, and the potential for the DNA-probe to detect dead *O. ramosa* seeds or vegetative material in the sample. While not contributing clear results to the current experiment, soil cores and DNA extraction have a role in determining *O. ramosa* seedbank decline for known infestations (Correll and Marvanek 2006). In contrast, the trapping methods used here (Bagnold trap, wind vane sampler, sticky slide traps, and to a lesser extent, tube traps) are more sensitive and are able to elucidate the patterns of seed distribution throughout the wind tunnel.

While the seed trap designs utilised by some researchers attempt to focus on primary dispersal (Bullock and Clarke 2000; Bullock and Moy 2004), this study did not attempt to separate primary (Phase I) and secondary (Phase II) dispersal events. Indeed all trap types used would have been capable of trapping seeds during primary and secondary dispersal.

As seeds were released from two point sources (aluminium tubes over the wooden board at 0.10 m) the resultant lateral distribution of the seeds would not have been uniform, especially at shorter distances. Instead, there are likely to have been concentrations of seeds relating to each of the aluminium tubes, and a lateral ‘spreading’ of seeds with increasing distance; somewhat like a Gaussian plume (Katul et al. 2005). However, the intent of this study was to investigate dispersal distance and height, rather than lateral distribution of seeds within the tunnel, so the seed trap data at a given distance were pooled. More detailed investigations of seed distribution within the wind tunnel would be required to analyse this lateral distribution more thoroughly.

Conclusion

The current study shows that the presence of cereal stubble, even sparse and short cereal stubble, combined with some rock material decreases the distance *O. ramosa* seed can travel by wind. Surface vegetation can trap small seeds moving on wind, through physical interaction with the seed, and by slowing down wind velocity near the ground surface (Findlater et al. 1990; Bullock and Clarke 2000; Bullock and Moy 2004). Land managers with emerged *O. ramosa* should maintain ground cover in infested paddocks in order to reduce the distance a seed can travel on wind.

Dispersal of *O. ramosa* seeds in the soil seedbank could also be reduced by paddock vegetation cover. It is likely that soil erosion caused by wind would also transport seeds of *O. ramosa* present in that soil. Ground cover, such as pasture or cereal stubble, can substantially reduce soil erosion. Even 30% cover can reduce soil erosion by four-fold (Findlater et al. 1990). Land managers in paddocks known to have an *O. ramosa* seedbank should adopt strategies conducive to reducing soil erosion, such as maintenance of ground cover and no-till cultivation methods.

Chapter 5. Discussion

Seed dispersal is a central process in the ecology of plant population dynamics (Cousens et al. 2008), and knowledge of seed dispersal vectors is critical in the management of invasive plant species. Managers of weed infestations need to understand the specific dispersal mechanisms occurring, in order to limit the spread of the population and aid eradication. For the South Australian population of *O. ramosa*, efforts are focussed around delimitation, containment and extirpation (Warren 2006; Panetta and Lawes 2007). Containment is achieved by preventing seed dispersal to new areas outside the quarantine zone, and the prescribed quarantine procedures can be refined through a better understanding of the dispersal vectors operating in the South Australian population.

Seed dispersal mechanisms are rarely reported in the literature for *Orobanche* spp. (only Mesa-García et al. 1986; Castejón-Muñoz et al. 1991) or other small seeded parasitic weeds (e.g. *S. hermonthica*; Berner et al. 1994; van Delft et al. 1997). The results of investigations into dispersal of comparable 'dust propagule' plants such as orchids show that wind is a vector, but that most propagules remain close to the source (Murren and Ellison 1998; Machon et al. 2003). Population genetics studies also demonstrate that the majority of orchid seeds will travel fewer than 10 m (e.g. Chung et al. 2004).

While information regarding dispersal of small seeded species can be gleaned from the literature, there is little published on *Orobanche* species, and vectors will vary both spatially and temporally. Not every vector will be applicable in every population; between locations, different biotic and abiotic factors will be significant, and some vectors may be limited to certain times, seasons or micro-climate conditions. Land managers need to consider their own unique situation, and ensure that the most appropriate methods of limiting distribution are being employed, with the help of local extension officers and in adherence with the Code (DWLBC 2003).

Vectors for *O. ramosa* seeds

Sheep husbandry is one of the key activities within the branched broomrape quarantine zone, so sheep were studied as a dispersal vector for *O. ramosa*; both internal and external transport of seeds. The gut passage experiment showed the classic gut passage pattern with a seed half-life of 2 days for *O. ramosa* seed through the sheep gut. Whether seeds were viable once excreted was untested, but the literature suggests that as the seeds are small and exhibit high seedbank longevity (Parker and Riches 1993), it is likely they would survive the passage through the gut (Pakeman et al. 2002).

Experiments to determine seed transport on the wool aimed to document the two stages of attachment and retention. These experiments showed that if *O. ramosa* seeds were to come into contact with the wool, attachment is possible and retention is likely to last at least seven days, probably longer. The probability of seeds coming into contact with wool depends on the behaviour of the sheep (i.e. sitting, lying or wallowing on the ground), density of seeds in the soil and/or density of seed bearing plants in the paddock (Fischer et al. 1996).

The Code dictates procedures for inspection and written approval before livestock can be moved from a paddock within the quarantine zone. Livestock are not the only animals present in the area: pest animals (including mice, rabbits, hares, cats, and foxes), domestic dogs, and native fauna (kangaroos and emus) may be present in the cropping and grazing lands in the region (Anon 2008). The ability of some of these species to carry various seeds has been reported in the literature, but given the small seed size of *O. ramosa* it is likely to be carried in almost any animal fur (Tackenberg et al. 2006). As such, the limiting factor for seed dispersal is likely to be contact between seed bearing plants and the body fur. This contact is maximised in small to medium-sized mammals whose body height will overlap with the height of the plants. Quarantine measures may need to be expanded to include control of small to medium-sized pest mammals, particularly those with larger home ranges, in order to limit the dispersal of *O. ramosa* seeds.

The initial survey of natural wind dispersal of *O. ramosa* seeds showed that most seeds fell near the source, a common pattern found by numerous authors (Berner et al. 1994; Jongejans and Schippers 1999; Bullock and Clarke 2000; Bullock and Moy 2004; Chung et al. 2004). Control over wind speed is impossible with natural field surveys, so a portable, field-based wind tunnel was used to control wind velocity and direction, but maintain authentic soil surface conditions.

The wind tunnel experiment investigated the effects of wind velocity and ground cover on the dispersal of *O. ramosa* seeds. It showed that greater wind velocities were associated with fewer seeds trapped along the length of the tunnel and more seeds trapped exiting the tunnel. In some cases the presence of stubble increased the number of seeds trapped along the length of the tunnel, suggesting that the stubble was reducing the dispersal of seeds through the air. Indeed, the role of plants as traps for small wind-dispersed seeds has been documented by Bullock and Moy (2004).

The wind tunnel used in the experiment was designed and built for the purpose of conducting soil erosion experiments (Raupach and Leys 1990). The use of laboratory-based wind tunnels to investigate seed dispersal is a relatively recent development (vanDorp et al. 1996; Jongejans and Schippers 1999; Gravuer et al. 2003; Dauer et al. 2006; Davies and Sheley 2007). Laboratory-based wind tunnels and models have been used to show that increased wind velocities give rise to greater dispersal distances (Jongejans and Schippers 1999; Dauer et al. 2006; Davies and Sheley 2007), and that seed release height is an important factor in determining dispersal distance (Murren and Ellison 1998; Dauer et al. 2006). Increased vegetation height has been associated with shorter dispersal distances (Jongejans and Schippers 1999) as has increased seed mass (Gravuer et al. 2003). To my knowledge, the experiments reported in this thesis are the first investigating seed dispersal using a field-based wind tunnel.

The Code does not include any requirements for the management of soil erosion or wind dispersed seeds (DWLBC 2003). Such absences are not unusual in weed management guidelines around the world (Davies and Sheley 2007). The wind tunnel experiment showed that ground cover had a significant effect on the dispersal distance of *O. ramosa* seeds. Davies and Sheley (2007) report that vegetation neighbouring a weed infestation can significantly reduce the dispersal of seeds and hence spread from that infestation. It would be sensible for land managers with *O. ramosa* to maximise neighbouring paddock vegetation or use vegetated buffers between paddocks to prevent seeds blowing into uninfested paddocks, and to maintain sufficient levels of ground cover within infested paddocks to prevent topsoil erosion and concurrent seed dispersal.

Humans and their mechanical farming implements are probably the most frequent and important vector for dispersal of *O. ramosa* throughout the quarantine zone (Secomb 2006). Any equipment that comes into contact with soil (e.g. tillage equipment, mowers, seeders, harvesters, tractors and transport vehicles) could transport seeds along with soil. Management of farm machinery and the wash down procedures required before they can be moved from within the quarantine zone are well covered by the Code (DWLBC 2003).

On a paddock-scale, the use of tillage equipment may assist in mixing seeds through the vertical profile in the plough layer, thus moving seeds from the soil surface into the sub-surface levels below (e.g. S. hermonthica van Delft et al. 1997; Colbach et al. 2000). In the absence of tillage, water may play a role in moving seeds from the soil surface down slopes and into cracks and hollows in the earth (Cousens et al. 2008). Larger-scale movement of seeds by flood-waters is unlikely, although summer rains and thunderstorms during the seed shedding period are possible in the region (Anon 1995).

Future research

Investigations into *O. ramosa* seed dispersal by wind, and internal and external transport via sheep has been undertaken in this thesis. Further research would contribute to a better understanding of those two vectors.

Determining the maximum wind dispersal distance (e.g. Bullock and Clarke 2000) would permit buffer zones to be correctly applied to the margins of the quarantine zone area. Alternatively, an explanatory model could be developed to describe the wind dispersal process without the difficult task of measuring seed dispersal at such long distances (Jongejans and Schippers 1999). The terminal velocity for the seed of *O. ramosa* would need to be determined for such modelling to occur (Jongejans and Schippers 1999).

Testing trap efficiency would allow more refined wind dispersal experiments to be conducted (e.g. Shao et al. 1993). Testing the ability of seeds to traverse different environments would allow further refinement of the quarantine methods. Low biomass cereal and bare ground were investigated here, but pasture, higher biomass cereal and native vegetation are all present within the quarantine zone and are likely to differently affect wind dispersal.

For sheep, determining detachment time for seeds in wool would be important for gauging the appropriateness of quarantine procedures. It would be also be necessary to determine the viability of seeds at the end of a prolonged period of animal transport to investigate potential for germination at the new location.

Apart from the two vectors studied here, research into other vectors would be valuable. The likelihood of wild animals transporting *O. ramosa* seeds should be tested, particularly for species with large home ranges, although subsequent control of such vectors would be difficult. Similarly, the role of water in *O. ramosa* seed dispersal is unknown.

As well as investigating the possibility of individual vectors acting on *O. ramosa* seeds, a useful tool would be a map of all dispersal pathways and relative probabilities of seeds passing through these vectors (e.g. Figure 5.14, p. 100 in Cousens et al. 2008).

Grenz and Sauerborn (2007) developed a simulation model to predict areas of risk of *O. crenata* infestation, based on lifecycle parameters, soil type, climate, and host crop production. Australia was identified to be at particular risk, especially the regions of South Australia and Western Australia where pulses are commonly grown. Measures to prevent infestation and spread include careful sourcing of clean crops and plants, extension and education of farmers and land managers, and implementation of quarantine measures (Eplee and Norris 1995). Should such prevention methods fail and an infestation occurs, many of the same vectors that have been discussed here for *O. ramosa* are likely to be important for *O. crenata*.

This thesis shows several pathways where seeds might be escaping the quarantine zone. Indications of failure of containment (i.e. new infestations discovered) (Panetta and Lawes 2007) are variable: the area of new infestations declined in 2003 and 2005 (Warren 2006) but increased in 2006 (Panetta and Lawes 2007). As such, continual review and appraisal of possible dispersal vectors should be part of the ongoing *O. ramosa* control program.