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Compendium of branched broomrape research

Section 9. Control – pine oil and other soil drenches

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 5 December 2013

Disclaimer

Table of Contents

See also the following publications:

Matthews J., Miegel D., Hayton D. (2006) Seed bank and seed bank reduction of *Orobanche ramosa* in South Australia. 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. 626-628.

Matthews J. and Miegel D. (2004) Destruction of *Orobanche ramosa* seeds with a new soil drench and control of emergence by herbicides. Sixth European Weed Research Society Workshop on Physical and Cultural Weed Control, European Weed Research Society, Lillehammer, Norway, pp. 197-199.

1. The effect of pine oil applications on *Orobanche ramosa* seed numbers, native vegetation and soil residues

Dr John Matthews and Darryl Miegel

University of Adelaide

January 2005

Introduction and Methods

Interceptor Seed Inhibitor (pine oil) was applied to an experimental field site within the Branched Broomrape Quarantine area at Mannum, South Australia. The soil type is typical calcareous sandy loam with a substantial infestation of *Orobanche ramosa* (branched broomrape) seed. Loss of seed viability was established by sampling plots pre and post treatment and seed numbers evaluated by DNA association. Soil residues were established for Interceptor Seed Inhibitor by removing soil cores at various intervals following application. The soil cores were chilled in the field and frozen until mixing and shipping in a frozen condition to the analytical laboratory. The results of both aspects of the trial are presented.

Interceptor Seed Inhibitor was also applied over a small area of regrowth native vegetation on a private property and visual assessments of damage to native vegetation and of *Orobanche ramosa* emergence were made.

Two similar experiments were conducted in June and July of 2004 and the separate and combined results are shown in Table 1. The rate of water applied was 19,000 L per ha containing 1000 L per ha Interceptor Seed Inhibitor. Both trials were applied with modified spray equipment on soil with 5-10% coverage of trash. The treatments were applied to three replicate plots measuring 20 by 6 m. Twenty 50 mm x 90 mm cores samples were taken prior and post treatment from each plot and were bulked, dried and mixed and seed separated by sieving and gravity partitioning for enumeration by DNA linked association.

Residues of Interceptor applications were sampled by taking four 50 mm x 30 mm cores samples from 3 replicates treatments at 3 depths (0-30 mm, 30-60 mm and 60-90 mm) and immediately placing on ice with further storage at –20 ^{o}C. Samples were mixed and sub-sampled in a cool-room and immediately refrozen and shipped on dry ice to the analytical laboratory (Virolab, Coburg Victoria).

Results

Applications of Interceptor Seed Inhibitor on *Orobanche ramosa* seed in the soil

At our most preferred application rate 20,000 L with 5% Interceptor per ha there was 25% seed survival in these trials with 23% and 21% survival for other application rates (Table 1). In general there have been reductions of broomrape seed of about 75%-80% depending on the application rate. At best Interceptor Seed Inhibitor reduced the seedbank between 93.6% and 89%. Other application rates (15,000 per ha) should be considered if further data supports these results.

The variation is a problem for eradication and consideration is given to what caused it and about reducing variation for a more consistent result. There can be difficulty sampling as the seed is aggregated around the site of the plant and coring may not give a true reflection of the number. Also there may be a different outcome from sampling after more time has elapsed due to further degrading of seed and plant tissues.

However the major effort should be directed towards improving the kill rate if the eradication is to be successful. The "Floodjet" style of application should have better penetration and more even distribution down the profile.

The treatments including Aquaboost were disappointing and discussions with BioCentral Laboratories have been initiated to see if it has a role. Aquaboost is a product reputed to reduce evaporation and percolation, it was anticipated that better kill rates would occur with it especially in the surface layers. No improvements were seen in surface layer performance with Aquaboost compared to similar rates without Aquaboost. There was no improvement in terpineol retention at 7 and 14 days with Aquaboost, from the residue data (not shown).

There is potential to investigate some variation in application rates but this needs to be considered in the overall balance of costs between active ingredient and water applied.

Table 1. Effect of Interceptor Seed Inhibitor on the viability of *Orobanche ramosa* seed. Survival is mean percentage of seed numbers in the plot prior to treatment, *n* = 3.

	Percent % survival of seeds*			
Treatment	Expt 1	Expt 2	Mean 1&2	SD 1 & 2
20,000 L with 1000 L (5%)	11	38.6	24.8	17.79
20,000 L with 2000 L (10%)	27.5	18	22.8	21.23
15,000 L with 1500 L (10%)	7.8	34.7	21.3	3.09
15,000 L with 1500 L + Aquaboost	54.2	37	45.6	31.49

*(Does not include samples where sampling has not shown a decrease)

Emergence

The emergence of broomrape following the June 8th 2004 experiment is shown in Table 2. Untreated plots had high numbers of emerging broomrape. No broomrape emerged in plots that had received pine oil with a high water volume of 20,000 L ha^{-1}.

Table 2. Broomrape plants emerging in pine oil experimental plots at the Mannum Trial Site

Water volume ha^{-1} (L)	Pine oil concentration (%)	Aquaboost (L)	Wetter (L ha^{-1})	Mean plants pot^{-1}	St dev
20000	3.75			0	0
20000	5			0	0
20000	10			0	0
15000	5	1.5		0.67	0.58
15000	10	1.5		0	0
15000	5	1		2.33	2.08
10000	10		10	0	0
10000	10			1	1.73
10000	10			0.33	0.58
10000	10			0.33	0.58
10000	15	1		3	5.2
20000	0	2		17.33	9.81
20000	0			10.67	10.79
0	0			12.33	2.08

Residues of Interceptor Seed Inhibitor applications

The residues at 4 hours, 7 and 14 days post application are shown in Fig 1. Total extracted terpineol residues summed over each depth from one experiment are presented in Fig 2. The data shows that the level of active ingredient applied 20,000 litres of water mixture with 5% or 1000 litres of Interceptor Seed Inhibitor declined to 85 ppm in 14 days from an initial load of 3025 ppm 4 hours after application. Other work has established that most crop species can be planted into treated areas 7 days after treatment at 20,000 litres of water with 1000 litres of Interceptor. These crops include wheat, barley, oats, triticale and legumes such as peas, medicago species and vetch. It is probable by comparison of the residue concentration at 7 days that the threshold amount for causing loss of seed viability from agricultural species is about 500-800 ppm.

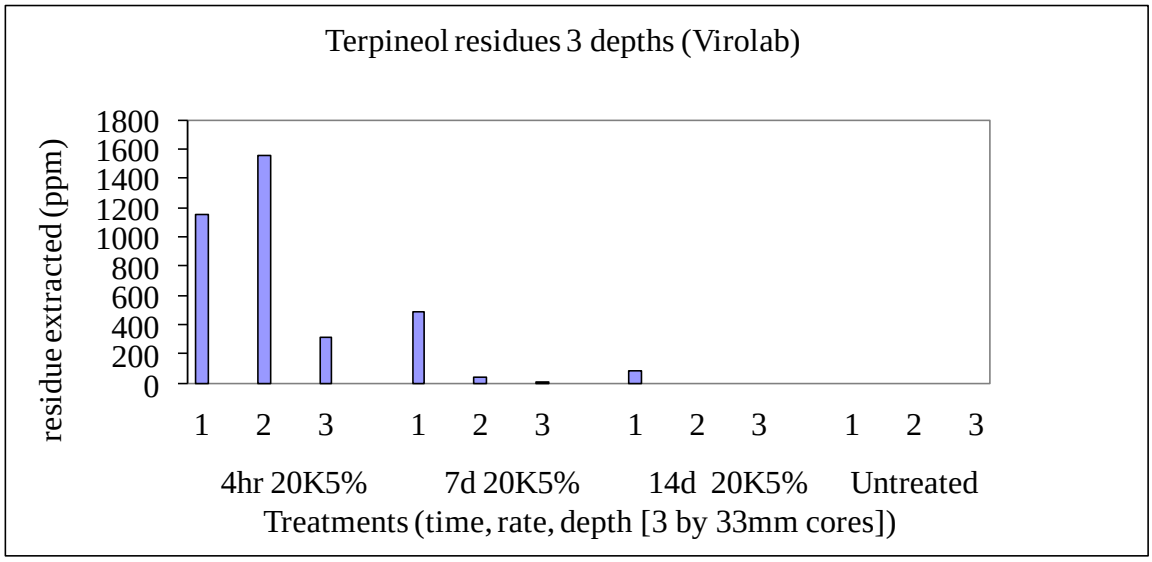


Figure 1. Residues over time of Interceptor Seed Inhibitor applied to typical Mallee soil.

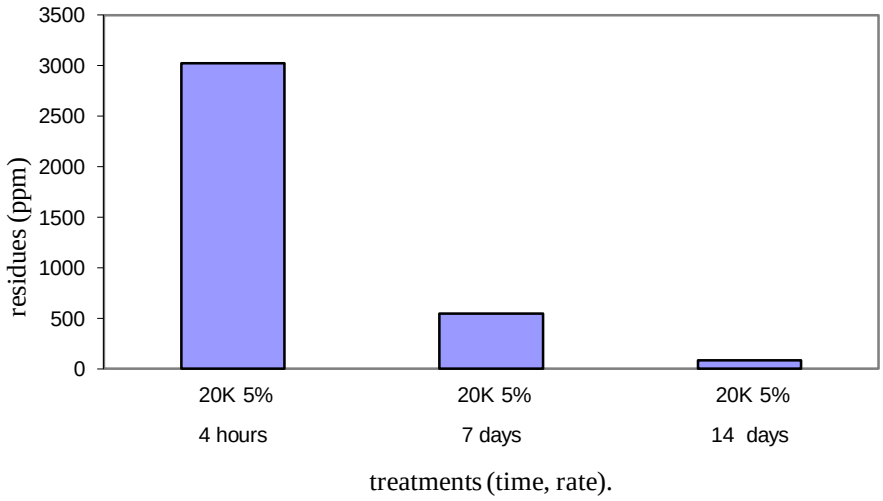


Figure 2. Extracted residue data from all depths for 2 application rates of Interceptor Seed Inhibitor.

Native Vegetation Trial

Interceptor Seed Inhibitor can also have an herbicidal mode of action and so it was also applied over a small area of native vegetation to assess damage. More than 30 native species and several exotic species, mainly annual weeds were described and located. Interceptor Seed Inhibitor was applied to the site in 4 replicates, at two concentrations, 5% and 10%, and at three rates, 5000, 100000 and 20000 litres per hectare, and compared to an untreated control. Damage to leaves, branches or stems was assessed at 7, 28 and 92 days following application. Plants were scored based on a visual assessment of damage, from 0, for no visible damage to 5 for maximum damage including death. Counts were made of plants in plots prior to spray application and twelve months following treatment to assess long-term effects of pine oil application.

In the first month following application of Interceptor Seed Inhibitor about 6 species of the 30 native species suffered serious damage to the leaves and stems (Fig 3). There were no differences between pine oil concentrations and application rates. In general, the highest application of 20,000 L ha^{-1} caused the most damage to sensitive species although this was not the highest concentration of pine oil.

Goodenia affinis and *Olearia lanuginosa* had higher condition scores 92 days following treatment, demonstrating recovery from the pine oil treatment. All monitored species showed some damage following pine oil treatment (Table 2). Large woody shrubs showed only minor visual effects of pine oil damage whilist the species showing the most visual damage were mostly herbaceous species. The annual weeds mainly *Senecio* and *Arctotheca* species were killed by the Interceptor application.

No branched broomrape emergence was recorded in the treated areas compared to 17 plants per plot on the untreated controls.

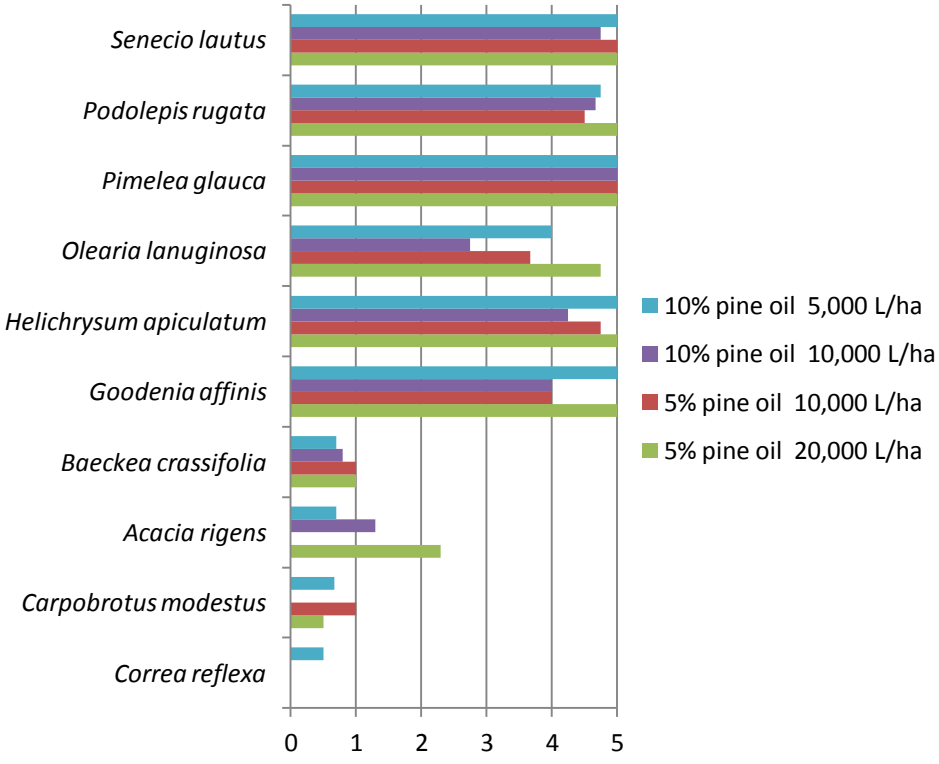


Figure 3. Visual assessment scores for the 10 most common native species 28 days after the application of pine oil. Untreated plants had a score of 0 (not shown on chart). N = 4 replicate plots for each treatment.

Table 2. List of native species and visual effect of Interceptor Seed Inhibitor on vegetation (0=no damage, 5=severe). Averaged across treatments and three sampling dates.

Species	Life form	Number of observations	Untreated	Treated
Helichrysum apiculatum	herb	58	0	4.63
Senecio lautus	herb	59	0	4.3
Goodenia affinis	herb	33	0	4.18
Pimelea glauca	herb	60	0	4.1
Spyridium eriocephalum	shrub	6	0	4
Kennedia prostrata	climber	6	0	4
Podolepis rugata	herb	57	0	3.89
Westringia eremicola	shrub	7	0	3.86
Einidia nutans	climber	6	0	3.83
Pomaderris oraria	shrub	57	0	3.67
Wurmbea dioica	herb	3	0	3.5
Enchylaena tomentosa	shrub	8	0	3.38
Olearia lanuginosa	shrub	55	0	3.14
Helichrysum leucopsideum	herb	20	0	3
Thelymitra nuda	herb	3	0	2.67
Austrodanthonia sp.	grass	22	0	2.6
Dampiera rosmarinifolia	shrub	12	0	2.5
Cassytha melantha	climber	8	0	2.14
Caladenia dilatata	herb	10	0	1.9
Acacia rigens	large shrub	35	0.1	1.04
Hibbertia riparia	shrub	8	0	1
Baeckea crassifolia	shrub	32	0	0.85
Clematis microphylla	climber	11	0	0.8
Tricoryne tenella	herb	24	0	0.78
Lasiopetalum behrii	shrub	11	0	0.78
Goodenia varia	herb	14	0	0.64
Vittadinia sp.	herb	13	0	0.57
Carpobrotus modestus	succulent herb	45	0	0.5
Melaleuca acuminata	large shrub	24	0	0.28
Correa reflexa	shrub	30	0	0.18
Melaleuca lanceolata	large shrub	14	0	0.08

Long term impacts

There were 47 species native species monitored one year following pine oil application. The species in Table 3 were present in untreated control plots and plots sprayed with a 5 % concentration of pine oil at 20,000 l ha^{-1}. Almost half of these species declined in number in the 12 months flowing pine oil application although species numbers declined in untreated plots as well. The *Helichrysum* species were the species most affected by pine oil application, with high visual damage scores (Table 2) and reductions in population size the following year (Table 3). *Goodenia affinis*, which had a high visual condition score (Table 2), also showed a large reduction in population size the following year but this reduction also occurred in untreated control plots (Table 3).

Table 3. Difference between the number of native species in plots prior to and 12 months after the application of 5 % pine oil at 20,000 L ha-1 and untreated control plots. Counts are summed across 4 replicate plots.

Species	control	Pine oil
Helichrysum apiculatum	8	-152
Goodenia affinis	-108	-89
Podolepis rugata	-10	-15
Helichrysum baxteri	17	-12
Austrodanthonia sp.	4	-8
Olearia lanuginosa	-24	-6
Lomandra effusa	-1	-3
Brachyscome ciliaris	15	-3
Baeckea crassifolia	1	-2
Acacia rigens	-13	-2
Pimelea glauca	-18	-1
Acacia pycnantha	-4	-1
Carpobrotus modestus	-5	0
Cassinia uncata	-1	0
Exocarpus sparteus	-1	0
Eucalyptus spp.	0	0
Dodonaea bursarifolia	0	0
Caladenia dilatata	-6	1
Thysanotus patersonii	-1	1
Lomandra micrantha	0	1
Ptilotus seminudus	-2	2
Gahnia lanigera	0	2
Wurmbea dioica	-17	4
Correa reflexa	-9	5
Tricoryne tenella	-15	8
Senecio lautus	64	22
Dianella revoluta	-39	23

Conclusions

It has been shown that the application of Interceptor Seed Inhibitor to agricultural land can substantially reduce the number of *Orobanche ramosa* seeds in the soil. The residue of the active ingredient alpha-terpineol declined to 20% and 3% of the original amount in 7 and 14 days respectively to a level well below the estimated damage threshold for agricultural species. Application of Interceptor Seed Inhibitor caused no long term damage to established native tree and shrub species and also controlled *Orobanche ramosa* emergence in the native vegetation during the season of application. Native herbaceous annual species may be killed or severely damaged by drenches of pine oil with some evidence of lack of recovery of *Helichrysum* species 12 months following treatment.

2. Using a helicopter to apply pine oil to eradicate broomrape seed in the soil

Nick Secomb, Project Officer

Branched Broomrape Eradication Program

2005

Abstract

In June 2004, a study was initiated into the effectiveness of Pine Oil (Interceptor Seed Eradicator Concentrate, containing 680 g L^{-1} of Pine Oil as its active constituent) on seeds of branched broomrape in the soil. Soil cores taken before and after treatment show an average of 56% of seeds were killed 6-months after treatment.

Introduction

Orobanche ramosa is a parasitic weed of a wide range of broadleaf crops in the Mediterranean, Europe, central Asia, the Middle East, South Africa and North and South America. Broomrapes are root parasites that are totally dependent on the host for all organic carbon. *Orobanche ramosa* spends most of its growing period below the ground. It is capable of setting seed within 14 days of emergence.

The only known population of *Orobanche ramosa* in Australia was discovered in 1992 in the Bowhill area. The detected plants were eradicated by fumigation. Between 1993 and 1997, plants were found at six more sites on the original property and an adjoining property. These plants were eradicated by a combination of fumigation and manual hand control. In late 1998/99 *Orobanche ramosa* was detected at a further 16 sites within 15 kilometres of the original infestation. Wide scale surveys followed these discoveries resulting in a total of 137 infestations covering 1344 ha of land.

In 2000 a containment program was introduced to prevent the spread of *Orobanche ramosa* and better define its actual distribution. The Branched Broomrape Quarantine Area was established and protocols restricting the movement of soil, machinery, livestock, conserved fodder, grain and horticultural produce were introduced to prevent the movement of *Orobanche ramosa* seed.

Measures to eradicate branched broomrape seed from the area have been implemented since broomrape was first recorded. To date, 100 paddocks have been fumigated with methyl bromide gas to eradicate viable seeds of branched broomrape in the soil. While methyl bromide has been 100% effective against broomrape seed, it is a very costly and time consuming exercise with sites having to be rotary hoed prior to treatment and then covered with plastic for at least 48-hours after treatment.

Alternatives to methyl bromide are being investigated. Pine oil shows promise in that it has been 99% effective in pot trials and is less expensive and cumbersome than methyl bromide (sites do not need to be covered with plastic). Pine Oil is also a registered organic product that has environmental benefits and which poses a lower risk of poisoning to field staff involved in the eradication program.

There is scope to use pine oil in more sensitive areas as it is an organic product. As these areas are also more likely to be non-arable there is a need to investigate alternative methods for application for sites that are not accessible by ground-based machinery. Such inaccessible areas include cliffs and rocky sites. In this trial we investigate the use of a helicopter to apply pine oil for broomrape seed bank control.

Methodology

A 30-hectare site was treated with Pine Oil that had been diluted in water at the rate of 1 part Pine Oil to 20 parts water. This mixture was then applied at 20,000 litres of mixed product per hectare treated.

The paddock was treated using a modified fire bucket attached to a helicopter. The helicopter filled the bucket (which had a capacity of approximately 750 litres) from a tank in which Pine Oil had been pre-mixed with water. The helicopter then systematically treated each part of the paddock by flying approximately ten metres above the ground and at approximately 40 km/hr. The mixed Pine Oil solution was dropped from the bucket onto a pre-marked treatment area (Fig. 1). Treatment areas were systematically moved across the paddock until all parts had been treated.

Ten samples sites were established in the treated paddock. Four soil samples were taken from each of these sites by first establishing four adjacent 5m X 5m plots at each site (see figure 1). These sites were then marked using a Differential Global Positioning System so that they could be easily and accurately re-located. In each plot, 25 soil cores, each of approximately 10 grams in weight were combined to give a single 250 gram soil sample.

The efficacy of Pine Oil was assessed by taking soil cores prior to treatment and then on a further 4 occasions at 3-month intervals after treatment. These soil cores were analysed by the South Australian Research and Development Institute and the amount of *Orobanche ramosa* DNA present was recorded using a DNA probe. After calibration, these figures were used to estimate the number of *Orobanche ramosa* seeds in each sample.

Figure 1. The helicopter drops a load of pine oil at the trial site.

Analysis

The seed number data was log- transformed prior to analysis and a repeated-measures ANOVA test was performed followed by an F-test to assess significant differences between sampling dates. The sites were included as a random blocking term in the model. Least significant difference tests were used to assess differences between sampling dates.

Results

Just prior to the application of pine oil we measured an average of approximately 200 broomrape seeds per 250 g of soil. There was a significant difference in seed numbers over time ($p < 0.001$). Seed numbers for cores collected after pine oil application had fewer seeds (Fig. 2). The lowest seed numbers were

obtained from soil cores in December 2004 and March 2005, at least 6 months after pine oil application. Seed numbers were higher in July 2005 but did not reach pre-treatment numbers.

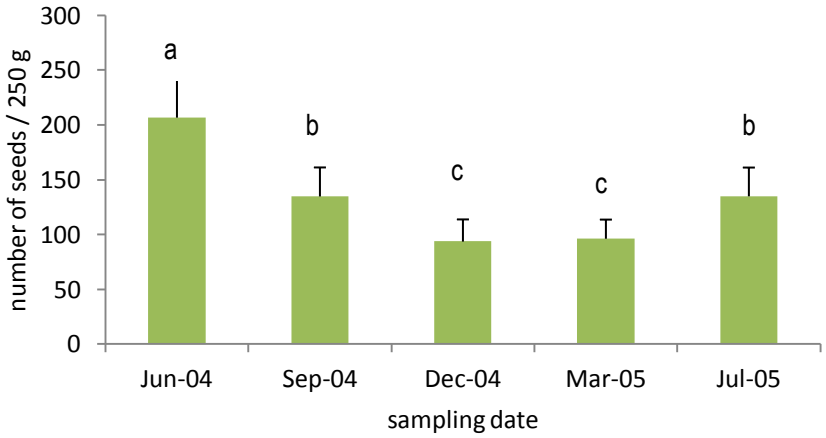


Figure 2. Estimated broomrape seed number from four pooled soil cores following treatment with pine oil in June 2004. The June 2004 samples were collected before treatment. Bars are means + 1 SE, *n* = 40. Bars labelled with different letters were significantly different at $\alpha < 0.05$ (LSD test on log transformed data).

Discussion

Results suggest that pine oil has an effect on *Orobanche ramosa* seed viability. Seed numbers measured after pine oil application were lower than numbers recorded beforehand. The difference between pre-treatment and post-treatment seed numbers persisted for 12 months after treatment. Although seed numbers were higher in July 2005 than March 2005 there would have been no further seed input. This difference may reflect the variability in seed numbers across the site.

The accuracy of the helicopter as a means of applying Pine Oil to infested sites should be questioned. Results collected at application suggest that the helicopter was, on average, capable of applying 80% of the required spray solution to each spray target. The remainder was lost to the areas immediately adjacent to each spray target. This may have had some influence on the efficacy of the treatment to date and could be addressed in the future by limiting application to a more accurate ground-based rig on all arable sites. The helicopter could still be used on sites which are inaccessible to ground equipment.

There is also some indication that the DNA from treated seeds may still be decomposing. All sites except site 7 had a substantial reduction in seed numbers from the 3-month cores to the 6-month cores. This suggests that some of the DNA recorded at the 3-month stage was in fact from seeds that had been killed by Pine Oil but which were still decomposing in the soil. By the time that 6-month samples were taken, more DNA had decomposed giving a lower seed estimate. There is a possibility that this decomposition is still occurring and that results after nine and then twelve months may give more significant results again.

3. Pot trial investigating the effect of depth on the efficacy of pine oil

John Matthews

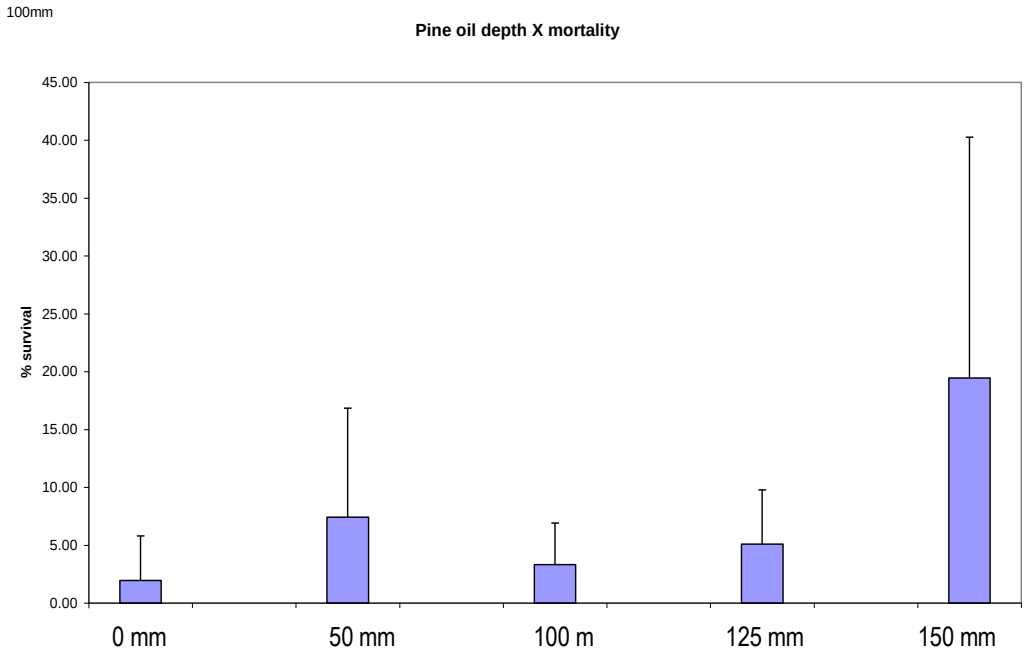
Research Fellow, University of Adelaide

January 2006

Relatively undisturbed soil from the Mannum Trial Site was captured in 200 mm columns in 150 by 150 mm steel tubing. The tube was forced into the soil and the whole column extracted intact. Broomrape seed in stainless steel packets was inserted into the soil column at 0, 50, 100, 125 and 150 mm depths via a removable side panel.

A 5% solution of pine oil was applied to the soil column at a rate equivalent to 30,000 L per hectare.

The packets were retrieved 2 weeks after treatment and seed tested for viability, the results in the graph are expressed as the % survival with standard deviation of a sample of seed from the packets. There were 3 replicate columns with the seed packets not placed vertically in line.



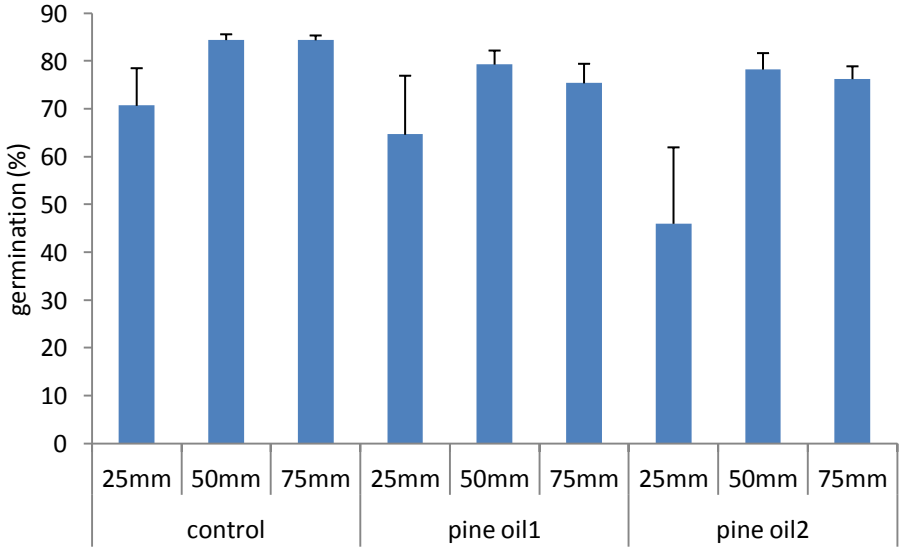
There was an effect of depth on seed mortality with a general decline of inflicted mortality as sample depth increased. There were high levels of variability at all depths and perhaps some of this can be attributed to the experimental conditions. The variability is made up of a range of mortalities from 100% - 94% at 0 mm depth and 99%- 90% at 125 mm depth and greater variability at 150 mm depth.

Loss of efficacy is not unexpected and can be attributed to the dilution effect as depth increases, and also the potential for adhesion of the active ingredient to soil particles thus reducing efficacy as depth increases. If the vapour phase of the Interceptor is responsible for seed mortality the concentration of vapour would also decrease as the depth increases.

The potential of this method of investigation seems to be to compare efficacy on various soil types (sand v's loam and loam v's the hard compact layer) or perhaps various additives to improve penetration.

Trial 2

This trial included a control treatment and a comparison of two formulations of pine oil, the formulation currently in use (pine oil 1) and a new formulation (pine oil 2). The chart shows means and standard errors.



4. Broomrape seed production after post-emergent treatment with Interceptor pine oil

John Matthews, Darryl Miegel, Dorothee Hayton

University of Adelaide

July 2005

Three application rates of pine oil were applied to emerged broomrape plants in the spring of 2004. 10,000 L, 15,000 L and 20,000 L per hectare of a 5% mixture were used. The pine oil was applied for a timed period to simulate previously calibrated application rates. Groups of plants were chosen at random and marked prior to treatment. Timing of pine oil application occurred at weekly intervals after the first emerged plants were observed (Table 1).

Emergence in 2004 was a dry period and plants were stressed and matured rapidly upon emergence. The latest treatments were applied to plants that were almost dried and mature. No attempt was made to discriminate between relative germination time of plants for allocating treatments.

Table 1. General assessment of maturity at time of treatment

Date of treatment	Maturity assessment
29-Sep	most immature
6-Oct	75% flowering 25% immature
13-Oct	40% flowering 60% dry flowers
20-Oct	20% flowering 80% dry flowers
27-Oct	dry flowers drying spikes
3-Nov	dry spikes

Not all plants produced seeds in the difficult season of 2004. However, a decreasing proportion of broomrape plants produced seed as the pine oil rate increased (Table 2). Also a decreasing percentage of seeds were viable as the rate increased. The percentage viability for the untreated was about 44% and at the 20 K rate 14%.

Table 2. Percentage of plants producing capsules with potentially viable seeds at each spray date.

	Mean % producing seed	Percentage of plants				
treatment		6-October	13-October	20 October	27 October	3 Nov.
Control	66	60	70	90	20	90
10K	62	50	30	60	90	80
15K	52	80	30	70	70	10
20K	48	50	40	50	50	50

The mean number of viable seeds produced per plant across all timings was 317 for the untreated and 52 for the 20 K rate (Fig. 1). If the last and driest 2 treatment times are not included then the untreated mean is 285 and the 20 K rate was 10.

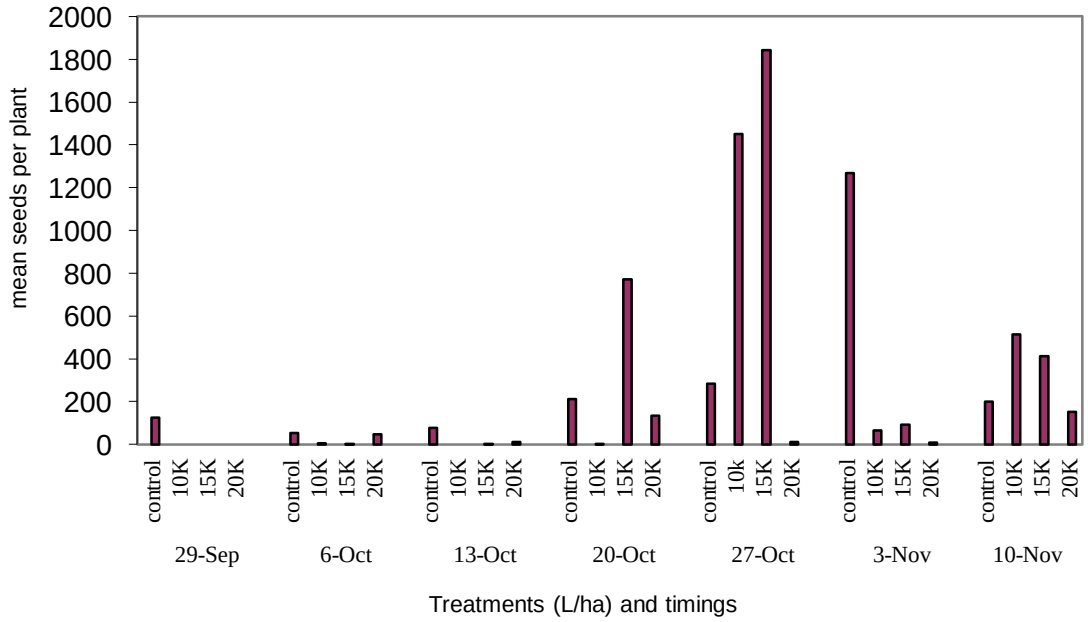


Figure 1. Estimated number of seeds produced per plant after spraying with pine oil after emergence, Bars are means, n = 10.

5. Pine oil for seed destruction post-emergence

Nick Secomb

Branched Broomrape Eradication Program

March 2006

Aim

To assess how effective Interceptor Pine Oil is at destroying viable seed on mature broomrape plants.

Methodology

Pine Oil will was applied in 2005 as a 10% solution at 10,000 litres of mixed product per hectare (ie. 100 litres of neat Interceptor for every 900 litres of water added) via the boomspray of the calibrated trailer unit to ensure accurate application.

At each site, ten mature (finished flowering) broomrape plants were marked with pink tags and then sprayed with Pine Oil at the agreed rate. Each of these plants were collected 3-days after application by cutting plants at just above the soil surface so that all of the plant but no soil was included in the sample. Plants were collected in paper bags and the date, owners name, hundred, section number and GPS location of each collection site was recorded on each bag.

At each site, ten mature broomrape plants were marked with pink tags and then not sprayed with Interceptor as a control treatment. To make sure that moisture alone is not affecting seed viability, each of the control plants was sprayed with a hand-sprayer containing plain water. Each plant was treated with 8 mls of water (equivalent of 10,000 litres / Ha). The handspray was calibrated by measuring the amount of water given off after 50 'squirts'. This was used to calculate the amount of water per pump and the number of pumps required to get 8 mls of water.

Each of the control plants was collected 3-days after water application using the same protocol as treated plants.

After collection, treated and untreated samples were placed in the Broomrape Centre oven for drying. The oven was set at the lowest possible temperature and the oven door left open. Samples were dried for 4-hours.

In the lab, some stems were divided into three sections so that separate collections of seeds were made from the bottom, middle and upper thirds of the stem. The remainder of stems were treated entire. Stems or stem portions were processed to remove all foreign material and leave as pure a sample of broomrape seed as possible. A standard amount of seeds was randomly selected from each sample and subjected to a tetrazolium solution seed viability test.

Results

Seed samples were collected from six sites. Seed from two of the sites had a high proportion of fungal infection so those seeds are not included in this summary of the results.

There were no obvious differences between the viability of seeds sampled from the bottom, middle or upper thirds of stems. Seed viability was also similar across the four sites. Seed viability was lower in treated plants than untreated plants.

Table 1 Percentage of viable seeds treated with pine oil or water after seed maturity in the field. Seeds have been sampled from different portions of the stem and from entire stems

	Untreated stem portion				Treated stem portion			
Site	entire	bottom 1/3	middle 1/3	top 1/3	entire	bottom 1/3	middle 1/3	top 1/3
1	70.8%	64.1%	34.5%	45.6%	41.3%	44.5%	29.5%	13.4%
2	24.3%	38.5%	61.0%	0.8%	32.3%	4.0%	58.2%	50.1%
3	86.2%	88.5%	90.1%	81.9%	38.8%	34.2%	23.6%	22.8%
4	71.1%	30.2%	80.1%	73.9%	35.9%	67.8%	45.5%	42.7%
average across sites	63.1%	55.3%	66.4%	50.6%	37.1%	37.6%	39.2%	32.3%

6. Testing different concentrations of pine oil and timings for post-emergent broomrape control

John Matthews, Darryl Miegel and Dorothee Hayton

University of Adelaide

November 2005

Aim

The aim of this trial was to determine which concentration of pine oil was the most effective in killing broomrape seeds on emerged plants. The trial also examined the timing of the post-emergence pine oil applications.

Methods

The experiment was conducted at the Mannum Trial Site in November 2005. The concentrations of pine oil tested were 0, 5%, 10% and 15%, diluted in water. There were four applications times: 2/11/2005, 7/11/2005, 14/11/2005 and 22/11/2005. The plants were treated for each concentration and timing. The application was made by a hand sprayer at about 40 psi and applying the equivalent of about 20, 000 L ha^{-1} equivalent.

The plants were harvested when mature and the seeds removed from plants. A subsample of plants was tested for germination.

Results

The earliest treatments were more effective than later treatments, with most seeds not germinating after treatment with pine oil at rates between 5% and 15% (Fig. 1). The 5% and 15% treatments on 7/11/2005 were unsuccessful and do not fit with the trends for the remaining data. For later treatments a higher concentration of pine oil is recommended. Viability was not tested, so although seeds did not germinate they may still remain viable (see Section 9.4).

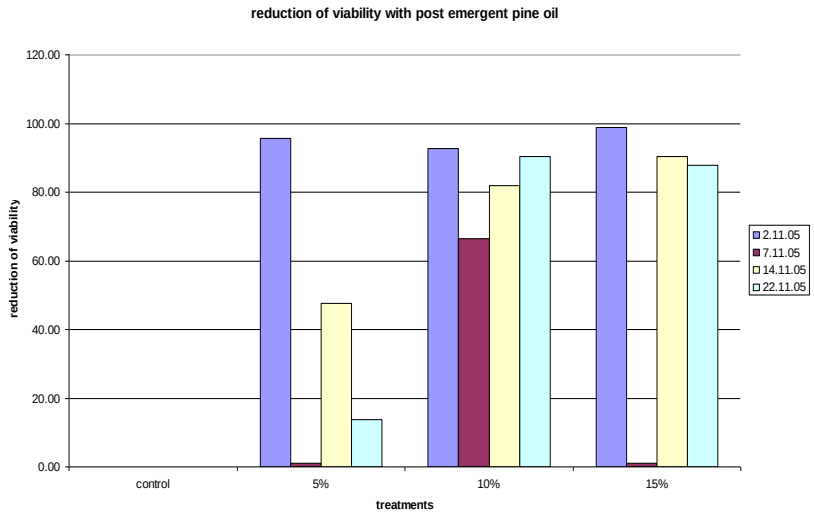


Figure 1. Germination of pine oil treated seeds as a percentage of germination of control seeds sprayed with water. Each bar is the mean, n = 10.

7. Pine oil: time of exposure

Anna Williams

Branched Broomrape Eradication Program

2007

Aim

To determine the seed viability dose-response curve after different exposure times to application of the soil drench Interceptor Weedkiller ® (Certified Organics).

Methods

Exposure prior to conditioning

O. ramosa seeds were bleached in a 1% NaClO solution, rinsed and then soaked in a 5% solution of Interceptor pine oil in small vials. Exposure times were 0, 11.25, 22.5, 45, 105, 180, and 540 minutes. Seeds were rinsed after exposure. 100 seeds were then added to filter paper discs in 5 cm petri dishes. They were moistened with 150 µl of RO water and the dish sealed with parafilm and placed in an incubator for two weeks at 20 °C for conditioning.150 µl of 1 ppm GR24 was added and dishes resealed and incubated for a further 14 days at 20 °C before germination was scored. Ungerminated seeds were assessed for viability by immersing in a 1% tetrazolium solution and incubating for 14 days at 30 °C. Seeds stained were considered still viable. There were five replicates for each treatment.

The experiment was repeated.

Exposure after conditioning

The experiment was repeated but the conditioning step occurred before exposure to pine oil treatments. GR 24 was added to seeds transferred to filter papers after pine oil treatments.

The experiment was repeated. Pine oil treatments were applied to filter papers as there were problems with the mixture of pine oil and water in the vials.

Results

Exposure prior to conditioning

Germination was poor in the first trial so results are only presented for the second trial. Germination of *O. ramosa* seed was affected by the duration of exposure to pine oil (ANOVA, $p < 0.001$). A decrease in germination was found after exposure to pine oil for longer than 105 minutes (Fig. 1).

Overall viability was higher in the first trial than the second trial although the difference was not significant (ANOVA, $p = 0.054$) so the results for the two trials have been pooled. *Orobanche ramosa* seed viability was only moderately affected by exposure to pine oil (ANOVA, $p = 0.004$). Some seed treatments soaked in pine oil for periods of time greater than 22.5 minutes had significantly lower viability than controls (Fig. 1).

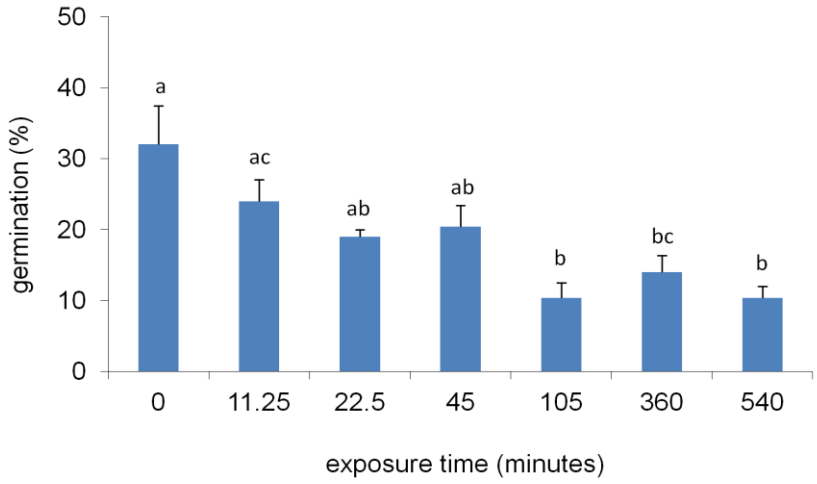


Figure 1. Germination of *O. ramosa* after soaking in pine oil prior to conditioning in second trial. Bars are means + 1 SE, n = 5. Bars labeled with different letters were significantly different at α < 0.05, Tukey HSD tests.

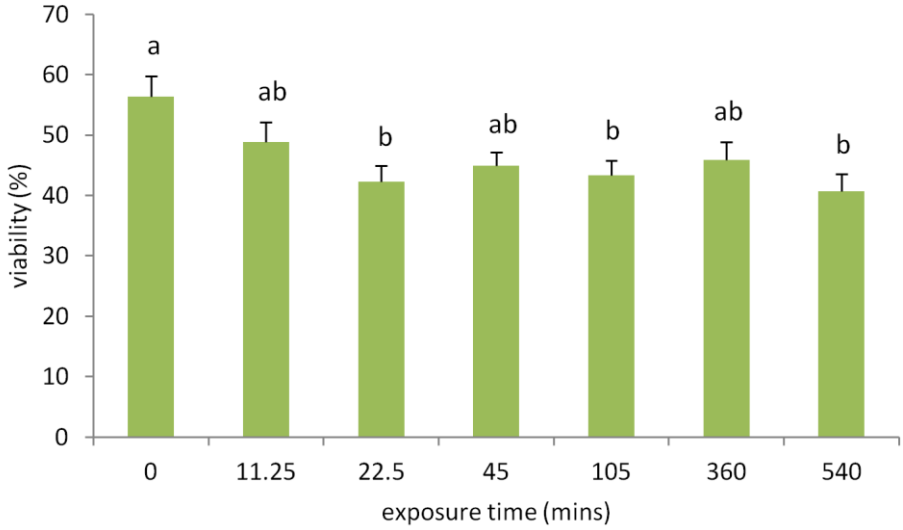


Figure 2. Viability of *O. ramosa* after soaking in pine oil prior to conditioning for both trials combined. Bars are means + 1 SE, n = 10. Bars labeled with different letters were significantly different at α < 0.05, Tukey HSD tests.

Exposure after conditioning

Germination was poor in the second trail so results are only presented for the first trial. Germination of *O. ramosa* seed was affected by the duration of exposure to pine oil (ANOVA, p = 0.003). A decrease in germination with reference to untreated controls was found after exposure to pine oil for 540 minutes (Fig. 3).

Viability results were only analysed for Trial 1 due to poor germination and inconsistent results in Trial 2. There was a significant effect of exposure time on viability. Seed viability after 540 minutes exposure was 64% compared to controls (89%) (quasibinomial GLM $p = 0.008$). Viability in treatments exposed to pine oil for only 11.25 minutes was also 64% (quasibinomial GLM $p = 0.012$) but as longer exposure times were not significantly different to controls the reliability of this result is questionable.

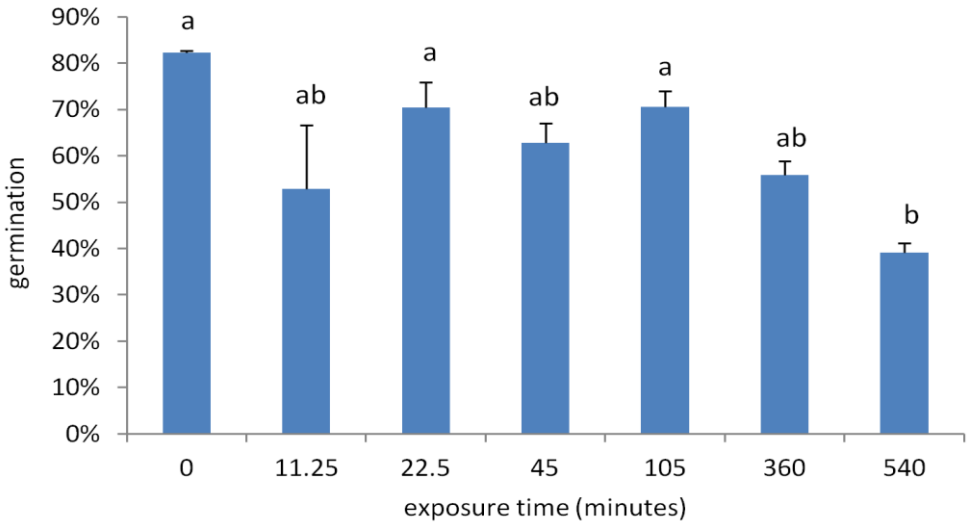


Figure 3. Germination of *O. ramosa* after soaking in pine oil after conditioning in first trial. Bars are means + 1 SE, n = 5 (control *n* = 3). Bars labeled with different letters were significantly different at $\alpha < 0.05$, Tukey HSD tests.

Discussion

Large differences in germination and viability counts in control treatments for both experiments limits the conclusions that can be drawn from this trial. As a result it is not possible to determine whether pre-conditioned seed is more vulnerable to the negative effects of pine oil than unconditioned seed.

The results show that the duration of exposure to pine oil has lethal effects on *O. ramosa* seed, Minor declines in viability were detected after 22.5 minutes exposure and further declines were detected after 540 minutes. Pine oil affects germination and survival of *O. ramosa* seed.

8. Testing new formulations of pine oil

Anna Williams

Branched Broomrape Eradication Program

March 2007

Introduction

Aim

To determine whether alternative products competitive to the currently used Interceptor Weedkiller® produce a comparable dose response curve in *Orobanche ramosa* seeds

Justification

Interceptor Weedkiller® is used in the Branched broomrape eradication Program as a soil drench for treatment of branched broomrape seeds in soil in arable and non-arable areas. The cost of this product is currently one of the factors limiting its use in the eradication program. Alternative sources of similar products have been identified which could be supplied at a considerably lower cost. However, in order to justify the cost savings of the alternative products it must be proven that they cause the same death rate (or better) as the current product.

Method

Pine oil from two sources was tested:

1. Interceptor Weedkiller® pine oil (Certified Organics) that was used in operations for the destruction of *O. ramosa* seed in the seed bank
2. Pine oil sourced from New Zealand

All trials also included a control treatment of RO water. We used seed collected from the Mannum Trial Site in 2006.

In vitro trial

Unbleached *O. ramosa* seed was placed on filter paper and soaked in 5% solutions of pine oil or water for 15 or 30 mins. Seeds were removed and then placed on a new filter paper. 200 µl of RO water was added and seeds were conditioned for two weeks. 200 µl of GR24 was added and germination assessed after incubation for two weeks. Ungerminated seeds were tested for viability using a 1% solution of tetrazolium solution. There were three replicates of each treatment.

Soil containers

Sachets were prepared from filter paper and 100 unbleached *O. ramosa* seeds were spread on the paper and the sachets secured. The sachets were moistened with RO water and kept at 20°C in the dark for two weeks to condition. The sachets were buried in tubs of Burdett sand. Solutions of the pine oil types were added at concentrations of 1, 2.5, 5, 10 and 20% to the tubs of sand (volume added not known, J. Prider). There were three replicates for each treatment including a control treatment with water. The sachets were removed, left to dry and the seeds transferred to filter paper discs. Germination and viability tests were conducted as above.

Results

In vitro trial

There was a lot of variability in the results for the treatments that had been soaked in pine oil. Some replicates had no germinations and very few viable seeds whereas other treatments were not different to controls. As a result, no statistically significant difference was found between the pine oil treatments and controls at either of the exposure times (Table 1).

Table 1. Germination and viability of *O. ramosa* after 15 or 30 mins exposure to pine oil from different sources. Values ±1 SE.

	Untreated control	NZ pine oil	Interceptor
Germination %	85 ± 2	43 ± 13	39 ± 18
Viability%	88 ± 2	56 ± 11	54 ± 14

Soil containers

Pine applications to soil gave more consistent results. There was no *O. ramosa* germination in treatments with pine oil from either source at concentrations greater than 5%. Germination of untreated controls was 31%. The dose response curves for the two types of pine oil were very similar (Fig. 1). Viability for untreated seeds was low at 48%. Less than 1% of seeds survived applications of 10 or 20% pine oil. Form the model it is estimated that 90% of seeds would be killed at concentrations of 3.7 ± 1.4 % NZ pine oil or 4.8 ± 1.8 % Interceptor (SA) pine oil.

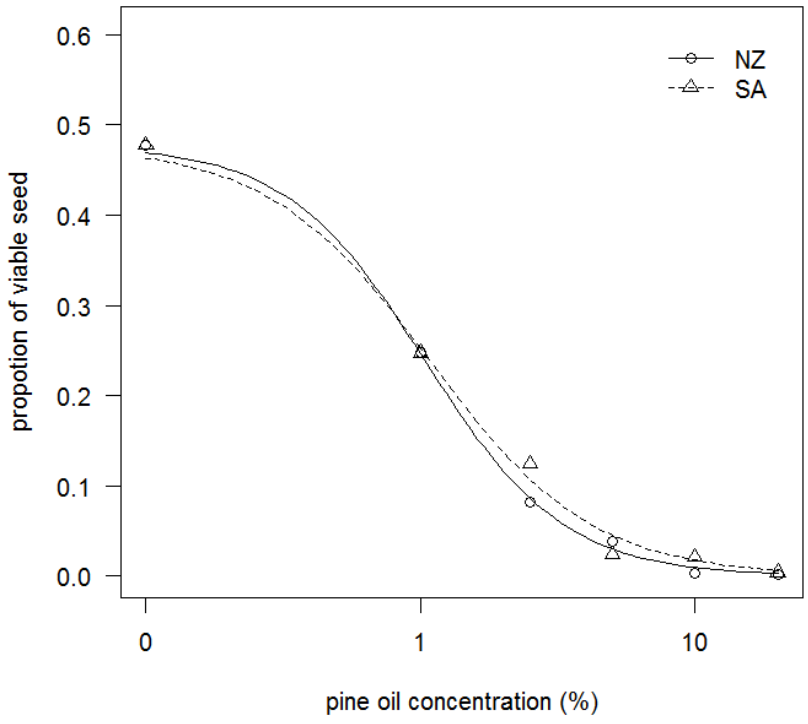


Figure 1. Dose response curves for viability of *O. ramosa* seed after the application of two types of pine oil (SA and NZ). A three parameter logistic model has been fitted to the data.

Discussion

Poor results with untreated controls in the second trial and inconsistent results between replicates in the same treatments in the second trial limited the conclusions that can be made from these experiments. Both trials showed that pine oil has an effect on the viability of *O. ramosa* seed. It was surprising that better results occurred in soils than in the in vitro trial.

Pine oil from both sources was equally effective at the same concentrations. There was no evidence that one formulation was superior to the other.

9. Smoke induced germination of *Orobanche ramosa* seeds

Anna Williams

Branched broomrape eradication Program

December 2006

Aims

- To determine if *O. ramosa* can be stimulated to germinate in the presence of smoke water
- To determine if smoke water has an effect on *O. ramosa* seed viability

Methods

In vitro trial

O. ramosa seeds collected in 2005 were surface sterilised in 5% NaClO for 5 minutes and then rinsed 5 times in RO water. 100 *O. ramosa* seeds were spread onto a 21mm glass-fibre filter paper in a 5cm petri-dish. 200µl of RO water was added to each filter paper, the petri-dishes were sealed with parafilm and kept at 20^{O}C in the dark for two weeks to condition.

After two weeks the seeds were transferred to new filter papers and 200µl of the following treatments were added to them:

1. Neat smoke water (undiluted)
2. 1/10 smoke water
3. 1/100 smoke water
4. 1/1000 smoke water
5. 1/10000 smoke water
6. RO water
7. 10ppm GR24

Smoke water was supplied by Kings Park Botanic Garden.

The petri-dishes were sealed and kept at 20^{O}C in the dark. At 7 and 14 days the seeds were scored to determine percentage germination. Germination was scored based on radicle emergence to the same length as the seed. After 14 days, any ungerminated seeds were viability tested by placing them in 1%TZ solution at 30^{O}C for 7 days. Seeds stained red or pink were scored as viable.

All germination treatments were replicated 4 times. Two replicates were tested for viability.

Soil containers

Sachets were prepared from filter paper and 100 unbleached *O. ramosa* seeds were spread on the paper and the sachets secured. The sachets were moistened with RO water and kept at 20°C in the dark for two weeks to condition. The sachets were buried in tubs of Burdett sand. The treatments, with the exception of GR24, were added to the tubs of sand (volume added not known, J. Prider). There were three replicates for each treatment. The sachets were removed, left to dry and the seeds transferred to filter paper discs. Germination and viability tests were conducted as above.

Results and discussion

In vitro trial

No seeds germinated in the smoke water or RO water treatments. There was 79% germination in the treatments with GR24. There was no evidence that smoke water promotes the germination of *O. ramosa*.

The viability of the seed lot was 83% (RO and GR24 treatments). No viable seeds occurred in the neat smoke water treatment. There was no difference in the viability of the seeds to which different concentrations of smoke water had been added, including no smoke water addition (ANOVA, $p = 0.97$). Average viability across these treatments was 77%.

Smoke water had an effect on seed viability but only when applied undiluted.

Soil containers

Although there was a concentration effect of smoke water treatments on *O. ramosa* germination following the addition of GR24 this was confounded by poor germination of controls (Fig. 1). Good germination of this seed lot in petri dishes in the previous trial indicates some other factor was affecting seed germination in this trial. Germination in controls was expected to be approximately 80%. The reduction of germination following smoke water treatments cannot be attributed confidently to the effects of smoke water treatment.

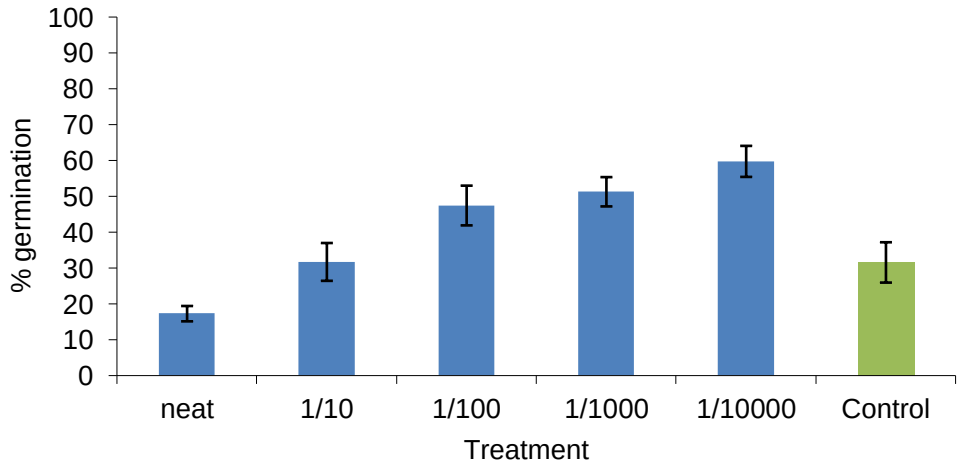


Figure 1. Germination of *O. ramosa* seed after smoke water treatments applied to sachets buried in containers of soil. The stimulant GR24 was added to all seeds before germination counts 14 days later. Bars are means ± 1SE, *n* = 3.

In soils, smoke water had an effect on *O. ramosa* seed viability when applied undiluted (Fig. 2). Some seed survived this treatment in the soil containers whereas no seed survived this treatment in petri dishes. The effectiveness of smoke water is thus reduced when added to the soil.

There are inconclusive results for a reduction in seed viability following applications of diluted smoke water. The viability of this seed lot as determined in the previous trial was 83% but the results for viability testing are confounded by poor viability results for control treatments.

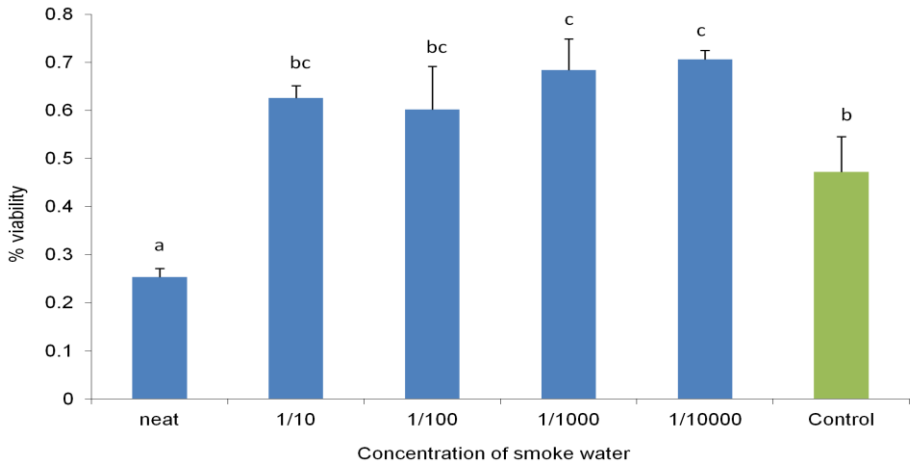


Figure 2. Viability of *O. ramosa* seed after smoke water treatments applied to sachets buried in containers of soil. Bars are means ± 1SE, *n* = 3. Bars labelled with different letters were significantly different $\alpha < 0.05$, LSD tests following ANOVA ($p < 0.001$).

10. Broomrape seed destruction with soil drench treatments

John Matthews

Branched Broomrape Eradication Program

July 2009

Background

In a weed species such as broomrape with the potential for profuse seed production and very durable seed in the soil it is important to have the ability to reduce the viability of seed in the soil. Management flexibility in response to the seed bank life; in response to potential outbreaks or reducing the risk of movement in the quarantine area means that an effective soil drench or effective fumigants are essential elements of an eradication programme.

Soil drenches

Soil drenches and fumigant work has been developing over the last three years and all the details are not yet resolved. Interactions with soils type, soil moisture and concentration of active ingredient are still being worked upon. Recent work has shown that the sampling sachets may have excluded the active ingredient and the trial results could be recalculated. Soil drench trials with pine oil products showed seed viability was reduced by an extra 23% and 10% in shallow and deep sampling respectively. Basamid treatments showed an improved efficacy of 4% at any depth. If this adjustment is reliable across all treatments the data from field trials could be adjusted to show that pine oil may reduce seed viability to about 25%, 15% or 8% depending on the application rates, depth and soil conditions. Considerations of differing responses due to soil type have not been included in pine oil trials to date.

It appears that pine oil drenches are effective in spite of dormancy status, conditioning period (but perhaps not as dry seed) and are robust against removal by washing.

Smoke Solution

Preliminary work has shown that solutions of smoke from oat straw in water reduced seed viability by 30 times, from about 75% to 2.5% *in vitro*. Smoke solutions retain potency under conditions of rewetting following drying periods, a potential useful attribute in our frequently dry soils.

Smoke solutions have been mentioned in the literature occasionally and have been trialled by our group occasionally. These recent trials used fresher and more reliable product. The potential in enormous as the product would be cheaper that existing products and may be environmentally more acceptable. There is a body of research regarding sensitivity of local species and crop species, we may not have plant back considerations but effects on soil organisms are unknown.

The role of soil drenches

The usefulness of soil drenches in this eradication programme at present is limited by the lack of sound data regarding the true efficacy. Applications to date are assumed to have killed only a proportion of seeds; and the need to protect the investment and improve eradication efficiency is proving difficult to sustain. Soil drenches and fumigants seem to be less effective than *in vitro* tests imply they could be, the work has been developing over the last three years and all the details are not yet resolved. Clearly there needs to be an assessment of the real efficacy under a variety of conditions. In conjunction with recent understanding of seed bank life the best role of seed killing drenches may be when the seed bank is

reduced by time and careful management and the soil drench may reduce the seed number to an undetectable level. These postulates could be modelled or discussed in the context of final eradication.

The need for an effective and robust seed killing soil drench to treat "emergency" or serious outbreaks is yet to be canvassed. It may well be the case that an outbreak occurs in a very sensitive or agriculturally diverse area and rapid and effective eradication is required. It is important to keep working on understanding the conditions that affect efficacy and the correct assessment of trial work.

At the request of the research group of the Broomrape Eradication Programme several products were investigated during 2007-9 in the laboratory and in the field in 2008.

Management flexibility in response to the length of time that seed may remain viable in the soil or in response to outbreaks or in the reduction of risk of movement in the quarantine area means that an effective soil drench and an effective fumigant are essential elements of an eradication programme.

General laboratory methods

Laboratory or field stored broomrape seed were surface sterilised in 2% sodium hypochlorite and washed several times in RO water, seed was then placed in between 25mm diameter glass fibre papers moistened with 200µl of RO water and placed in sealed petri dishes for 2 weeks conditioning. Treatments were applied following conditioning in most cases and seed and filter papers returned to sealed petri dishes. Following the treatment period the seeds were washed with RO water in stainless steel filter baskets, transferred to an eppendorf and tetrazolium chloride solution applied. Viability observed as red coloured seeds after 2 weeks incubation at 35^0C was expressed as a percentage of the total.

Results and Discussion

Products tested as potential seedicides

Several lipophilic substances were tested as potential seedicides, treatment *in vitro* with a 5% a.i. concentration of the product listed. Products containing pine oil, and some organosilicone based surfactants reduced the seed viability to zero (Table 1).

Table 1 Mean broomrape seed viability from seedicide trials

Product	Seed viability (%)	±SD
Bio Seed eradicator®	0.00	0.00
Bonza®	0.00	0.00
SuperCharge®	0.00	0.00
Jasol Pine oil	2.27	0.40
BioSeed Eradicator	5.35	2.03
BioSeed Eradicator A	7.38	1.12
BioSeed Eradicator	8.69	5.38
Jasol Eucalyptus Oil	12.71	11.42
Hasten®	14.47	12.63
DCTrate®	24.56	7.46
Jasol surfactant	29.94	2.52
Uptake®	30.74	8.14
Jasol Citrus oil	37.29	1.97
Control 1	57.45	17.07
Control 2	62.00	5.25
WA Smoke water	65.31	1.51

Bonza and Supercharge are organosilicone surfactants registered for use on herbage as herbicide adjuvants, Hasten, DCTrate, Uptake are also adjuvants with some capacity as lipophiles.

There was a possibility that the period of conditioning which if extended can lead to induced secondary dormancy could affect the field performance of the most likely products. A range of conditioning times was invoked to test this and the results are shown in Table 2.

Table 2. Seed viability following various conditioning periods and treated with a range of potential seedkilling products (5% conc.) unless otherwise stated. Bioseed Eradicator®, Niproquat ®, ROSW A and B are the trade names of Certified Organics Australia, Nipro Products Aust. and Rural Weed Control South Australia respectively. Rosw A is a pine oil extract and emulsifier mixture, Rosw B is a mixture of pine oil, emulsifier and Niproquat.

	Seed viability %			
	conditioning period *in vitro*			Soil pots
Product	5 weeks	7 days	4 days	2 weeks
Bioseed Eradicator	2.4	25.4	2.4	21.9
Niproquat 1%	3.5	36.5	0.5	73.1
Rosw A	16.2	49.8	0.2	60.1
Rosw B	1.2	0.0	1.3	75.1
Control	71.7	75.4	69.3	75.6

Each data set is from separate experiments and may not be exactly comparable. The average SD. was Bioseed Eradicator 3.9, Niproquat 7.6, RoswA 6.2, RoswB 11.4.

In vitro trials are done on filter paper under optimum conditions, soil pots contained 1200gms field soil.

There was the possibility that seeds stored in lab respond differently to different products, especially the lipophilic seedicides than seed from the field. Seeds stored in the lab and seeds from field storage were conditioned and treated in pots of soil, treatments, following conditioning, approximated the usual rate of 20,000L of diluent per ha. Some products currently used work better on field conditioned seeds than lab stored seeds (Figure 1). The old and fresh seeds were in the same pot so rate of product was similar.

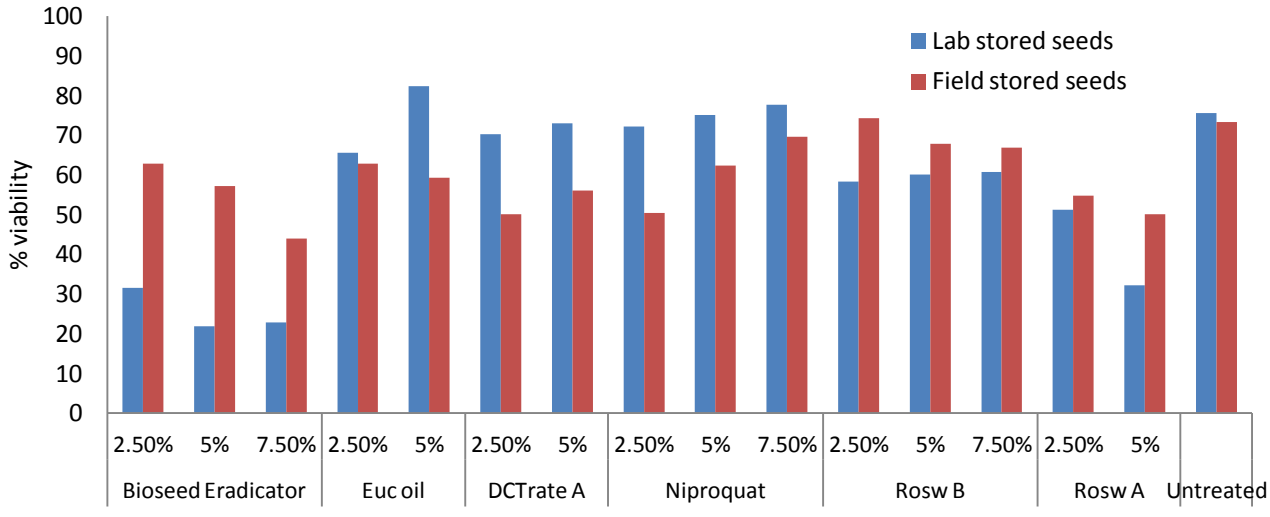


Figure 1. Comparison of effects of seedicides on lab and field stored seed treated in pots of soil.

There has been some conjecture regarding the reliability and long-term loss of viability of pine oil based products. It has been postulated that a physical barrier to prevent signal uptake or response was involved. A trial was commenced in which the pine oil was washed from the seeds at T1 5 hrs post treatment, T2 2 days, T3 1 week, T4 2 weeks Normal, as per usual. The seeds were then kept moist until the end of the

period and incubated with tetrazolium. The results of subsequent viabilities are shown. There was no positive control, but the seed source usually gives us 75% viability. Washing was done for 15 minutes in 200ml of fluid with a magnetic stirrer, fresh liquid each time. The washing fluids were RO water and 50% ethanol and each are compared. It seems that pine oil (BioSeed) is effective in spite of washing off soon after treatment (Figure 2). The results show that Pine oil products can be effective and does not cause only temporary loss of viability.

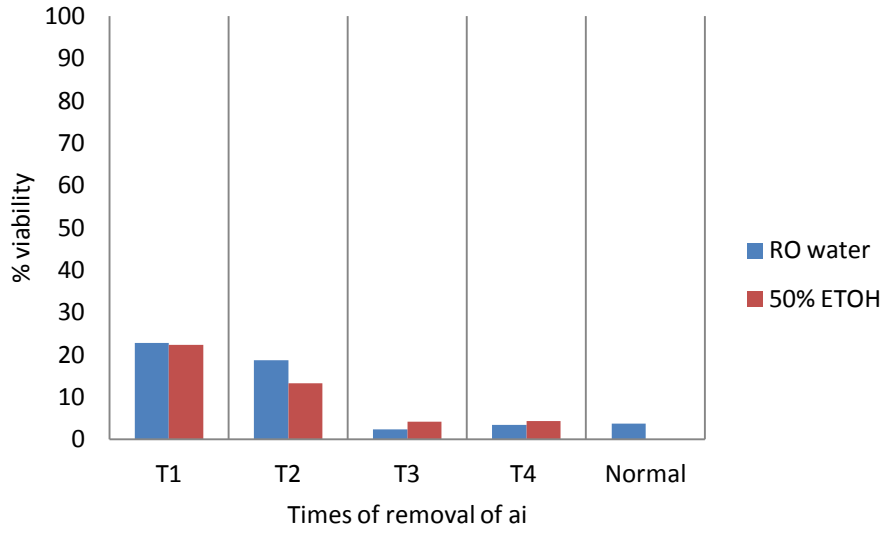


Figure 2. Viability of seeds following removal of pine oil product after treatment, T1 = 5 hours, T2 = 2 days, T3 = 1 week, T4= 2 weeks, Normal = not washed off.

A trial to assess the efficacy of various products on unimbibed seeds was established, treatment were at 10% solutions. This was designed to give us some information on late season application to emerged plants and surrounding seeds in the soil. Niproquat or niproquat mixture was the most effective on unimbibed seeds in this trial. This trial shows poor efficacy with pine oil products compared to other trials (Figure 3).

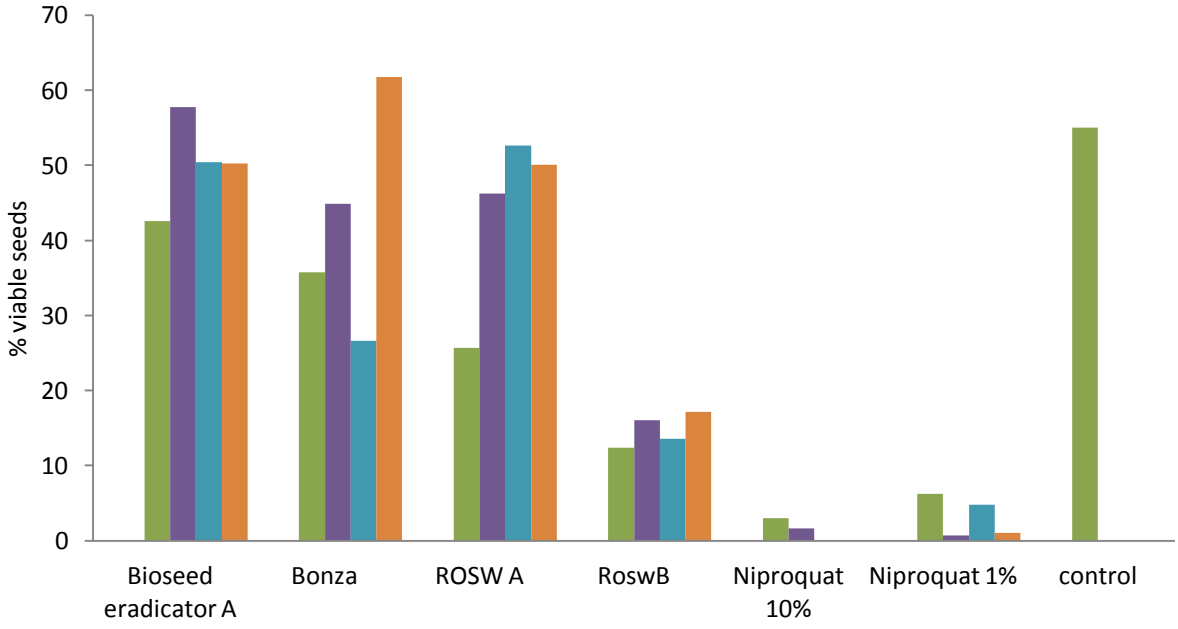


Figure 3. % viability of initially dry seeds treated with 10% solution of various seedicides

A field trial was established in the winter of 2008 to test BioSeed Eradicator, Basamid and Niproquat as the most likely seedicide treatments in the field. Basamid at 360kg/ha was the most effective treatment on the deeper seed sachets, while BioSeed eradicator at 7.5% was the most effective on shallow seed (Figure 4). Dry soils appeared to be the limiting factor as basamid powder was still present 3 months later. Added water post application on treatment Pine oil 5% wet (2mm) improved the efficacy by 15%.

A comparison of seed sachets and filter paper sachets was made in a subset of this trial to determine if seed sachets had an effect on the results. Filter paper sachets gave a 23% and 10% increase in observed seed kill at shallow and deeper depths respectively than nylon mesh sachets. (Data not shown). There was no difference in Basamid results with either type of seed sachet.

There has been conjecture as to the efficacy of smoke solutions on the viability of broomrape seed. Overseas work has shown a substantial reduction of emergence of broomrape subsequent to treatment with smoke solution. Previous work in our lab with smoke solution from WA has shown variable results. In 2009 smoke solutions were made by the author with known weights of fresh cereal stubbles burned in a controlled environment and the smoke ducted through a known amount of fresh water. The solution was stored under cool conditions until use. Several dilutions were tested in vitro.

Normal seed preparation protocols were followed by adding 200 µl of various dilutions of smoke solution as indicated, seeds were washed with RO water after 2 weeks exposure and treated with tetrazolium solution.

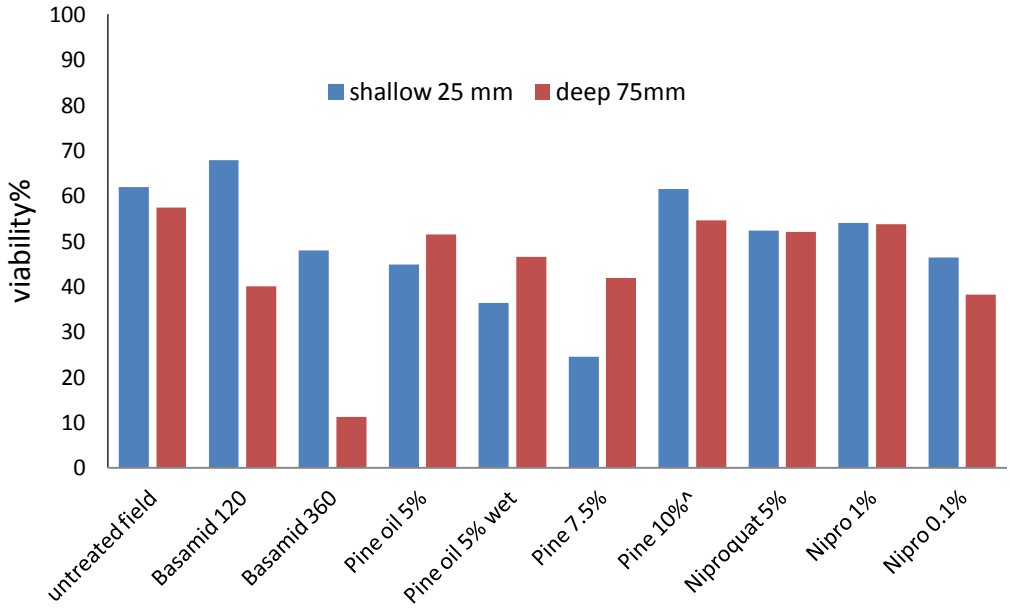


Figure 4. Field comparison of Basamid and pine oil at 2 depths

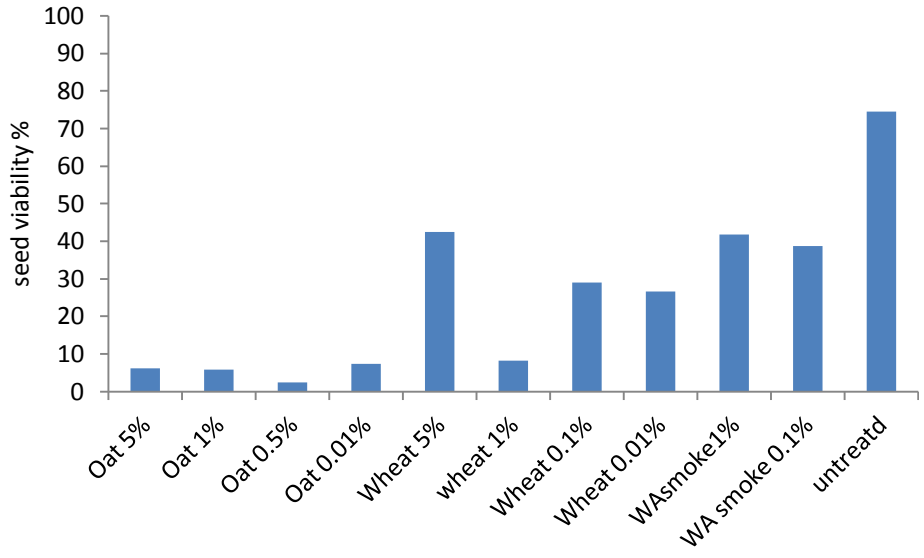


Figure 5. Effect of dilutions of smoke solutions in water on broomrape seed viability, an *in vitro* trial.

Table 3. Means relevant to the treatments shown in Figure 5. Details of treatment outcomes; mean value of seed viability, and difference from untreated, all treatments are sig ($p<0.001$) (ANOVA).

Treatment	mean value seed viability%	difference from untreated
Oat 5%	6.1	68.3
Oat 1%	5.9	68.5
Oat 0.5%	2.4	72.5
Oat 0.01%	7.3	67.1
Wheat5%	42.5	31.9
Wheat 1%	8.1	66.3
Wheat 0.01%	29	47.9
WA smoke soln 1%	38.6	32.6
WA smoke soln 0.1%	38.6	35.8
Untreated	74.4	-

The oat smoke solution was tested in 1L pots of soil in the coolroom, sachets of seed were buried at 5, 15, 25 and 35 mm depths and smoke solutions diluted at 0, 0.04, 0.2, 1 and 5% applied at a rate which approximates the field application rate. The results are shown in Figure 6.

Smoke solution has been shown to be tolerant of wetting and drying cycles. Such a characteristic would be of use in the quarantine area where the soil type and rainfall frequency makes for frequent dry periods. If smoke solution does retain activity it may have more flexibility and persistence that the usual volatile fumigants. Oat smoke solutions were applied to filter paper and to small volumes of soil (10 g), dried and rewetted and applied to conditioned seed. The viability of treated seed is shown in Figure 7.

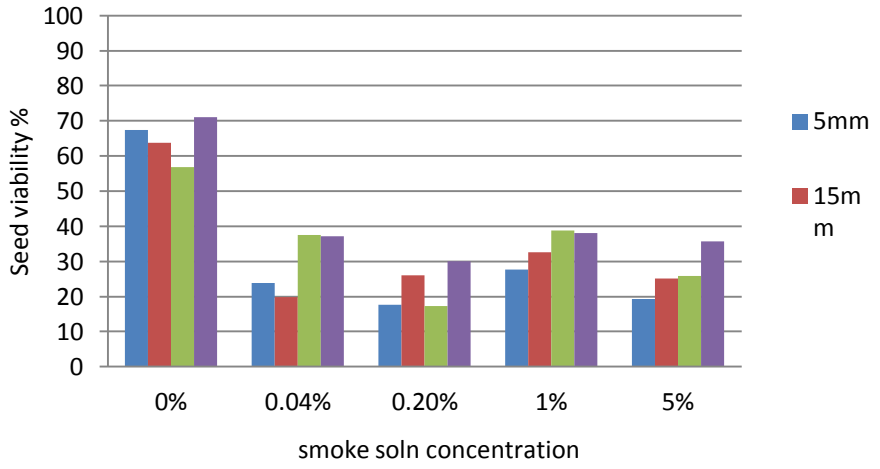


Figure 6. Seed viability after treatment with smoke solution in soil.

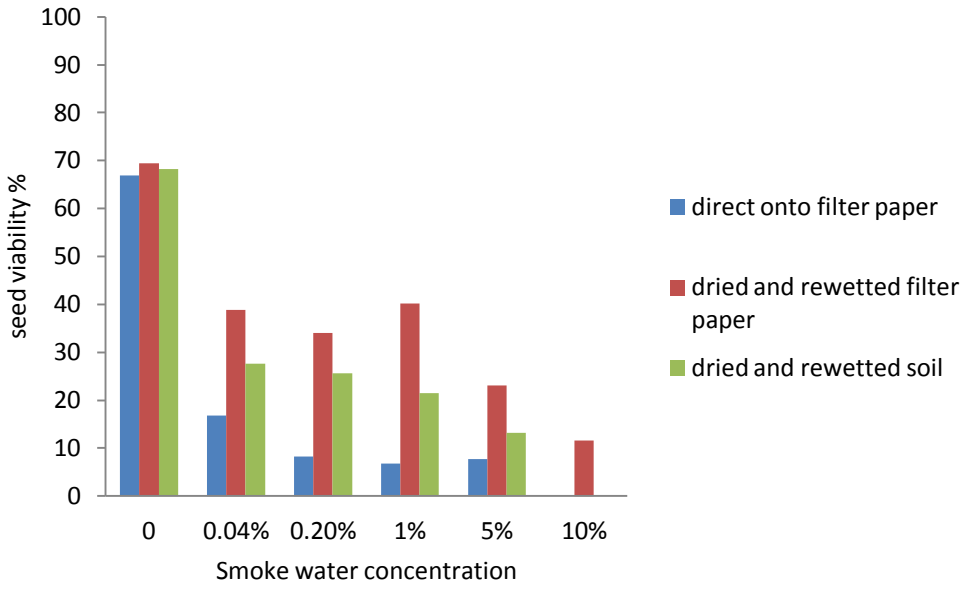


Figure 7. Effect of drying and rewetting of smoke solution on broomrape seed viability *in vitro*.

Discussion

The application of soil drenches to kill broomrape seed in the soil has potential to assist the eradication of the weed by reducing the seed burden in the soil. Products containing pine oil were the most effective from a range of products tested *in vitro*. Niproquat was also effective *in vitro*. Later testing in the field showed that Niproquat does not affect buried seeds and can be assumed to interact with the soil as it did not penetrate deeply. However mixtures of pine oil products and Niproquat may have a role when applied to broomrape plants in which seed has formed or shed to the soil surface. Data shown in Fig. 3. suggests that proving these results in the field may help reduce seed viability from emerged plants.

Pine oil products do not appear to lose efficacy when removed from the seed in *in vitro* trials. The mode of action seems to be not a short term blocking interaction but a more lethal effect. The period of imbibition is important for effective treatment in soil and seeds need to be imbibed to react with pine oil products. The efficacy on older seed retrieved from field storage also needs further investigation.

Efficacy of all products in the field was disappointing but the experiment showed the importance of adequate soil moisture and also the likelihood that we have been underestimating the lethality of soil drench treatments by using close woven nylon or stainless steel sachets to contain seeds in trials. Adjustments according to the results in Figure 4 suggest that seed viability could have been reduced to about 15% in woven fabric sachets in one treatment. Therefore the results of our trials with soil drench could be revised.

The effect of diluted oat smoke on broomrape seed *in vitro* was remarkable. A few references in the literature have trialled this product, as we have. The quality of imported smoke solutions may be degraded by storage or time and our fresh product shows potential. *In vitro* studies in figure 5, showed reduction of seed viability to about 2-5%. In soil pots the reductions were of the order of nearly 5 times, to about 15%. One useful attribute of smoke solution is that it appears to not rapidly degrade upon drying and rewetting and might maintain efficacy in the variable moisture environment of the infested area. Some pertinent *in vitro* data Figure 7 supports this. The usefulness of this in the field is yet to be proven.

Conclusions

The exact efficacy of pine oil and basamid in the field is still proving difficult to assess, it may always be so as inherent variability may preclude that. The development of sampling methods and variables affecting application has been steady but slow. The work reported here suggests that Basamid and pine oil products may be more effective than previously assumed but there are interactions with soil type and soil moisture that could be investigated. Some presumed weaknesses and unknown aspects have been identified; we can be confident that we have tested many of the presumed active products for seed destruction and identified the most useful.

The best part of the seed-life decay cycle to use these products and the best location within the geographic region is yet to be completely understood. The influence on the seed decay curve could be discussed in the future with a better understanding of real seed decay.

11. Smoke water and pine oil field trials at two sites

John Matthews

Branched Broomrape Eradication Program

October 2009

Aims of the project

To evaluate the efficacy of soil drenches applied to *Orobanche ramosa* seeds in 2 differing soil types. Soil drenches were Pine oil derived products and solution of smoke in water from burned oat straw. Soil moisture was manipulated in most treatments to test any effect on drench efficacy. Site 1 was on a dune crest on sandy soil. Site 2 was the Mannum Trial Site.

Treatments

Treatments were 5% and 7.5% dilution of BioSeed Eradicator pine oil, 5 and 1% dilution of smoke water. Product was hand applied to 1m^2 plots in the stated dilution, at 20,000L per hectare equivalent. All of the above treatments were buried at 25mm and 75mm depths in soil that was packed firmly and packed firmly around the sachets. All of the treatments were at 2 moisture levels, 1 at ambient levels and 1 wetted with 5mm of water immediately following application.

There was 1 treatment of rewetted smoke solution where fresh seed was introduced to the plot where 5% smoke solution had previously been applied and the plot rewetted.

At each of two sites standard pine oil treatments had seeds that were packaged in either mesh sachets or filter paper envelopes.

Results

Main effects are shown in Figures 1 & 2. (See Section 9.10 for a statistical analysis of this experiment).

There was a significant site effect. The differing soil types at the two sites had an effect on the loss of seed viability with Site 1 soil types showing a substantially greater loss of seed viability than Site 2 soil types under most soil drench treatments. There was about an 11% improvement in loss of seed viability in the lighter soil across all treatments.

The sites behaved similarly for the untreated control - 62% (se 1.53%) viability for Site 2 and 66% (se 1.53%) for Site 1. For pine oil there was a very significant effect of site, with Site 1 having 44% (confidence limits 57% - 34%) of the viability of Site 2. There was a site effect for the smoke treatments with the mean for Site 2 being 25.2% and for Site 1 19.4%, each with a standard error of 1.6%. The difference between sites for the smoke water treatments was significant at $\alpha < 0.05$. There were therefore differences between the sites for some treatments only; the most consistent difference was observed in the pine oil treatments.

Seed sachets of woven mesh were compared to seeds contained in glass fibre paper to evaluate differing rates of viability that I discovered last year. Seeds contained in glass fibre paper were 11% and 13% less viable than in woven mesh sachets when averaged across all treatments.

A high rate of pine oil was included to provide data on efficacy if eradication is required. Pine oil at 7.5% reduced seed viability to 3% in some treatments.

Solution of smoke in water was less dramatic with viability reduced to 20-25% depending on the soil type. Rewetting of treated plots reduced the viability of introduced fresh seed to 18-29% in this trial.

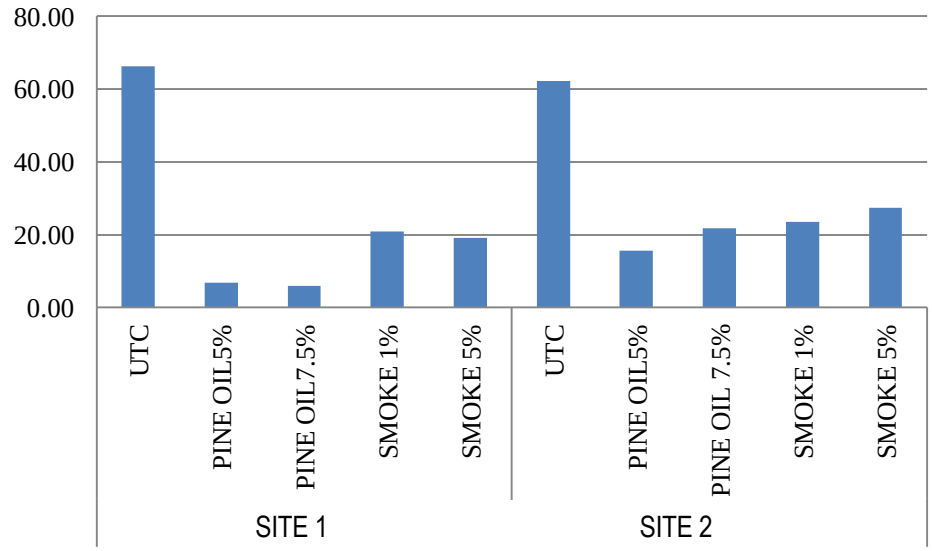


Figure 1. Effect of seed viability with site influences.

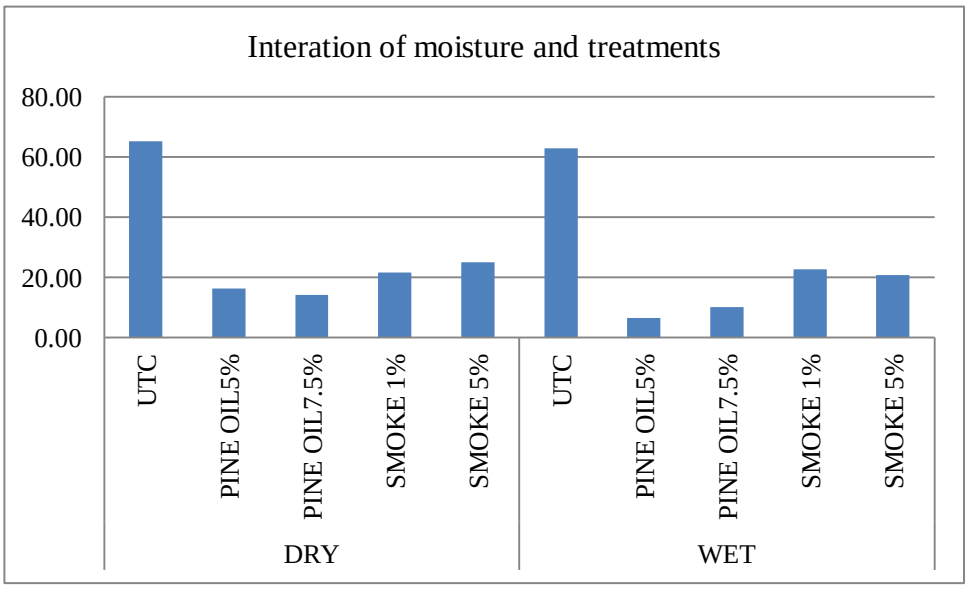


Figure 2. Influence of soil moisture on treatment efficacy across both sites.

Discussion

The differing response of all treatments to soil type is instructive and can inform a re- evaluation of application strategies for these products in these major soil types.

The difference in reduction of viability between glass fibre sachets and woven mesh bags was not dissimilar to last year's study that I undertook where a difference of about 18-20% was noted. The consistent results over last year and this year indicates a greater loss of viability than had previously been understood. (Data not shown).

The reduction of broomrape seed viability with smoke solution in this trial is remarkable, this is the first time that the product has been evaluated directly on seed samples in the soil. Application rates and dilutions should be investigated further and may be able to be refined. The product has shown stability and effectiveness over drying and rewetting cycles which means it has potential to remain effective in soils over a longer period of time as evidenced by the reduction of viability of fresh seed introduced to plots previously treated. The seed viability of a population could be reduced to 3.6% - 7.25% of the original if the outcomes are consistent. This could be a major advantage in the quarantine area which is affected by frequent wetting and drying cycles.

12. Analysis of pine oil and smoke water trial

Ray Correll

Rho Environmetrics

October 2009

Summary

An analysis of spray treatment data on the viability of branched broomrape is given.

Transformation of the data was found to be required, but standard GLMs were not applicable as the observations within a treatment group were apparently not independent.

The conclusions from the analysis were

1. There is a significant reduction in seed viability following spray by either smoke water or by pine oil;
2. The method of application affected the viability with pine oil in a consistent manner;
3. There was no consistent effect of application method on smoke water;
4. Lowest viability was obtained with either pine oil, either filtered at 5% or applied at 7.5% in conjunction with shallow wet application.

Introduction

This report considers the efficacy of two sprays (pine oil and smoke water) on the viability of branched broomrape seeds (See Section 9.9 for a description of the experiment). The sprays were applied in different rates and 4 different placement methods. The trial was conducted at two sites with three replicates at each site. An additional treatment was for 5% smoke water which was rewetted. Further details of the treatment combinations are given in Table 2.

Statistical Methods

Transformations of the data

When possible, it is better to use untransformed data because this is the most readily presented and that it gives unbiased results. Unfortunately at times the approximations made in using untransformed data are inadequate. A simple method of ascertaining this is to plot residuals from an analysis against the fitted values. Initially a full model including spray type and application was performed.

Figure 1 shows the plot for untransformed data. The residual for the lowest fitted values were close to zero, and the approximation of equal variance is not tenable for these low values. A common transformation is the logistic,

$$\lambda = ln\left(\frac{p}{1-p}\right).$$

This transformation was used and a plot of the resultant residuals is given in Figure 2. This transformation reduced the range of variability across the fitted values, but there was still a tendency for the smallest fitted values to have small residuals. An analysis of variance is robust against small departures from equal variance across treatments (Scheffe 1999).

An alternative approach is to assume the errors from the model follow a binomial distribution. This approach (a generalised linear regression) too has a problem in that it assumes the observations are independent. A modification of this approach takes into account the clustering by estimating a dispersion factor as mean deviance, and multiplying the standard errors given in a GLM by the square root of that dispersion factor.

For low probabilities the logistic transform approaches a logarithmic transformation. Use of the logarithmic transformation has the advantage that when the effects on the transformed scale are additive, they can be multiplied on the back-transformed scale. It is also noted that the back-transformed data require a bias correction, because the back-transform gives a geometric mean rather than the more usual (and usually more appropriate) arithmetic mean. The bias correction was obtained by multiplying the back-transformed data by the ratio of the arithmetic mean to the geometric mean.

Much of the variation noted was among spray types, so a comparison of application methods within a spray type is more robust than comparisons among spray types. This approach was used to generate an overall assessment of the data.

The analyses were performed in R.

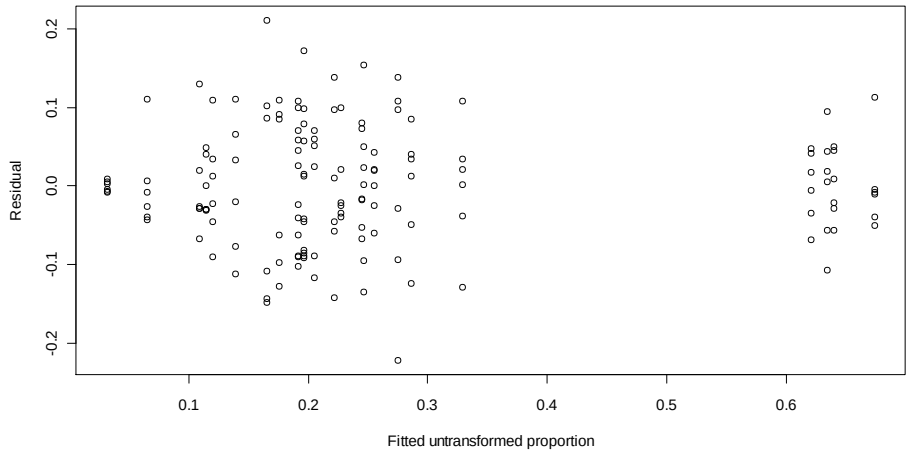


Figure 1. Plot of the residuals obtained from the analysis of untransformed data

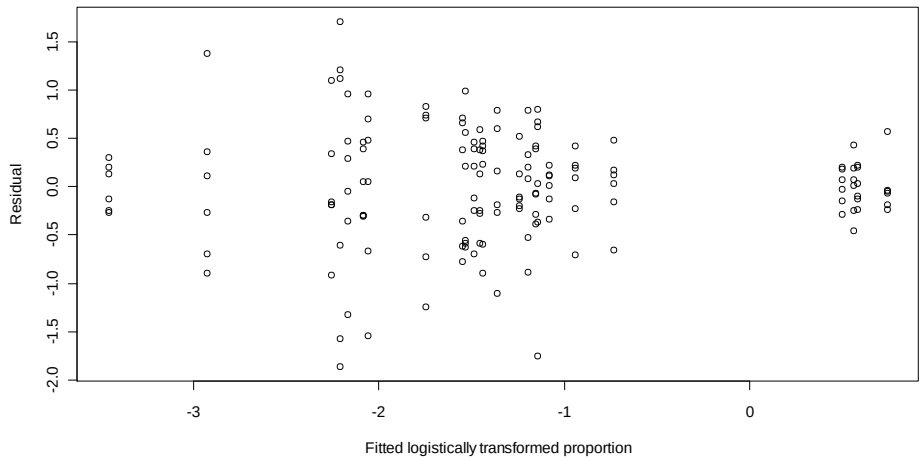


Figure 2. Plot of the residuals obtained from the analysis of logistically transformed data

Results

Effect of spray

An overall effect of spray was assessed by a one-way analysis of variance of logistically transformed data. This showed significance at the p<0.001 level. This analysis is conservative in that it did not remove the effects of the placement treatments. A summary of the overall means is given in Table 1.

Table 1. Comparison of effects of spray treatments on a logistic scale together with back-transformed estimates of proportions

Spray type	Estimate	Std. Error	Proportion
Control	0.59	0.15	64%
Pine oil 5%bags	-1.35	0.15	21%
Pine oil 5%filter	-2.25	0.15	10%
Pine oil 7.5%	-2.19	0.15	10%
Smoke 1%	-1.36	0.15	20%
Smoke 5%	-1.33	0.14	21%

Further analysis of the data indicated that there were significant effects of placement method and that there was an interaction between the spray treatments and the placement method. A full table of the means has therefore been provided (Table 2).

Table 2. Estimated proportions and standard errors based on within spray-type analyses

	deep dry	deep wet	rewetted	Shallow dry	Shallow wet
Control	0.668 + 0.022	0.633 + 0.022	NA + NA	0.612 + 0.022	0.600 + 0.022
Pine oil 5%bags	0.297 + 0.035	0.292 + 0.035	NA + NA	0.218 + 0.035	0.122 + 0.035
Pine oil 5%filter	0.186 + 0.034	0.110 + 0.034	NA + NA	0.181 + 0.034	0.031 + 0.034
Pine oil 7.5%	0.158 + 0.042	0.152 + 0.042	NA + NA	0.192 + 0.042	0.056 + 0.042
Smoke 1%	0.329 + 0.028	0.251 + 0.028	NA + NA	0.116 + 0.028	0.219 + 0.028
Smoke 5%	0.244 + 0.033	0.211 + 0.033	0.250 + 0.033	0.233 + 0.033	0.205 + 0.033

Graphical summaries of Table 2 are provided in The lack of interaction between the pine oil and placement methods enabled a simpler model to be used - namely that the spray type and placement methods were additive on the log scale. This enabled the factorial structure of that subset of treatments to be used. The effects for each combination of the subset of treatments were found as shown in Table 4.

Estimates of seed viability were obtained by exponentiating the values shown in Table 4 and allowing a bias correction. The bias correction ensured that the mean values in the estimates (Table 5) had the same mean as the original values that were in that subset of the data.

Figure 3 and

Figure 4. The lack of interaction between the pine oil and placement methods enabled a simpler model to be used - namely that the spray type and placement methods were additive on the log scale. This enabled the factorial structure of that subset of treatments to be used. The effects for each combination of the subset of treatments were found as shown in Table 4.

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Figure 3 was constructed using a GLM and

Figure 4 was formed using untranssformed data.The GLM has small standard errors at very low proportions and slightly higher levels at proportions near 0.5, but overall the two approaches have given very similar results.

Comparison of application methods

There was evidence of an effect of application method, but this was not consistent over all spray types. While it would be anticipated that the control would be similar independent of the application method, this was not expected for the Smoke 5% treatment (see Table 3).

A subset of the treatments – Pine oil spray excluding rewetting – was subject to further analysis. The analysis based on the logistically transformed data indicated that while there were large effects of both spray type and application method ($p < 0.001$), there was no interaction between the two ($p = 0.38$). A similar result was obtained on an analysis based on log transformed data; an advantage of working on a logarithmic scale is that when effects are additive.

The lack of interaction between the pine oil and placement methods enabled a simpler model to be used - namely that the spray type and placement methods were additive on the log scale. This enabled the factorial structure of that subset of treatments to be used. The effects for each combination of the subset of treatments were found as shown in Table 4.

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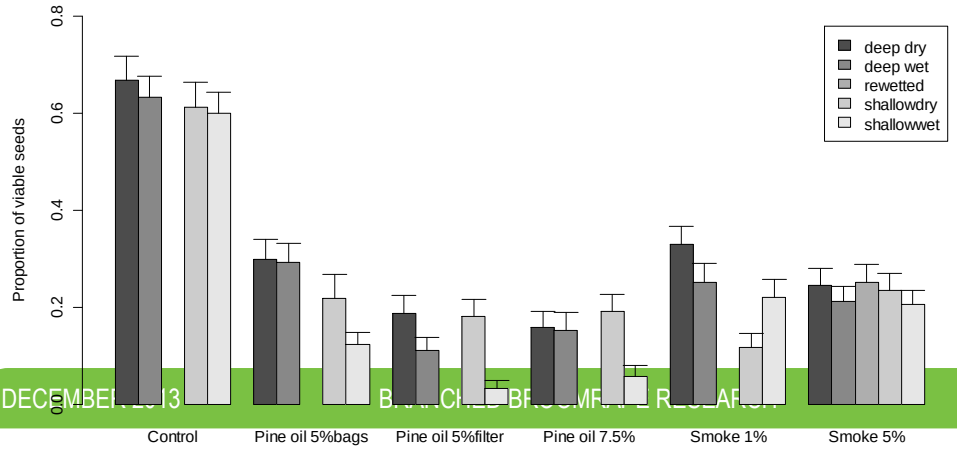

Figure 3. Proportion of seed surviving as estimated from a generalised linear model

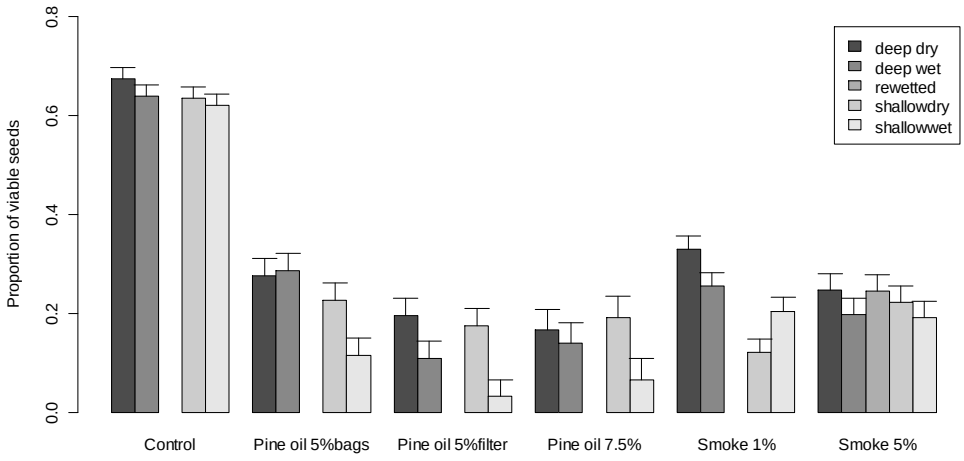

Figure 4. Proportion of seed surviving as estimated from untransformed data

Table 3. Test of significance of application methods. *

Spray type	F value	Degrees of freedom	p
Control	0.990	3,20	0.418
Pine oil 5%bags	4.964	3,20	0.010
Pine oil 5%filter	4.693	3,20	0.012
Pine oil 7.5%	1.648	3,20	0.210
Smoke 1%	9.633	3,20	0.000
Smoke 5%	0.581	4,25	0.679

Table 4. Estimators (log base e) of seed viability

	Pine oil 5%bags	Pine oil 5%filter	Pine oil 7.5%
deep dry	-1.38	-2.15	-2.11
deep wet	-1.46	-2.23	-2.18
Shallow dry	-1.24	-2.01	-1.97
Shallow wet	-2.40	-3.17	-3.12

Table 5. Estimates of seed viability

	Pine oil 5%bags	Pine oil 5%filter	Pine oil 7.5%
deep dry	0.29	0.14	0.14

deep wet	0.27	0.13	0.13
Shallow dry	0.34	0.16	0.16
Shallow wet	0.11	0.05	0.05

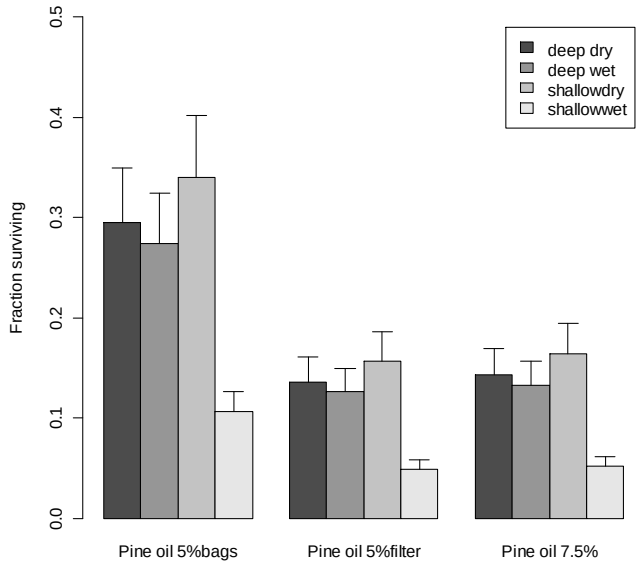

Figure 5. Survival rates of seeds treated with three types of pine oil spray applied by four different methods

Discussion

The treatments tested in this trial indicate that pine oil (and to a less extent smoke water) do effectively reduce seed viability.

A reduction of the seed viability from 60% to 5% would be expected to reduce successful establishment by a factor of perhaps 14 (if seeds behave independently). The efficacy of the treatments should be measured in terms of the fraction of seeds remaining, rather than the number of seeds killed. For example, for 5% filtered pine oil when applied dry caused a reduction to 16% (46% from the control) as compared to a reduction to 5% (55% from the control). While 46% and 55% do not seem that different, the amount remaining (16% and 5%) differ by a factor of 3.

The high efficacy of pine oil applied at 7.5% (or 5% filtered) in combination with wet shallow application, would appear to offer a useful combination.

Conclusions

- There is a significant reduction in seed viability following spray by either smoke water or by pine oil;
- The method of application affected the viability with pine oil in a consistent manner;
- There was no consistent effect of application method on smoke water;
- Lowest viability was obtained with either pine oil, either filtered at 5% or applied at 7.5% in conjunction with shallow wet application.

References

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