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Compendium of branched broomrape research

Section 8. Control - fumigation

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

MAY 2014

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 May 2014

Disclaimer

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See also the following publications:

Williams A.,Virtue J.G., DeDear C., McInerny T. (2006) Sampling challenges in detecting branched broomrape seed bank decline. 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. 622-625.

Prider J., Williams A. (2014) Using dazomet to reduce broomrape seed banks in soils with low moisture content. Crop Protection 59: 43-50.

1. Methyl bromide alternatives for branched broomrape control

John Virtue

Animal and Plant Control Commission

December 2002

Aim

To identify potential alternatives to methyl bromide for eradication of branched broomrape (*Orobanche ramosa*)

Method

The trial was located at the Mannum Trial site on Bow Hill Road, approx. 5km east of Mannum, a cropping paddock on sandy soil, with a high branched broomrape seedbank.

The experiment layout comprised fourteen 20 X 6 m plots that had previously been sown to vetch. Each plot was split into four 10 X 3 m plots. With four replicates there were 14 possible treatments set out randomly (not all plots were used):

1. Control (no fumigants applied)
2. Methyl bromide high rate + plastic
3. Methyl bromide low rate + plastic (say 200 kg/ha)
4. Metham sodium + plastic (@205 kg ai /ha = 500 L/ha)
5. Metham sodium without plastic (500 L/ha)
6. Dazomet (Basamid @600 kg/ha)
7. Dazomet + plastic (@600 kg/ha)
8. Dazomet low rate (@40 kg/ha)
9. Chloropicrin + plastic (@300 kg/ha)
10. Telone + plastic (@470 kg Rural Telone C35** /ha)
11. NiproQuat (@ 0.01% conc.)

The plots were cultivated in late June to remove broomrape hosts and again a week prior to applying the fumigants on 20 August 2002 to give a fine tilth for ease of application and dispersion of the chemicals. The treated areas within each plot measured 2 X 10 m. The treatments were applied after the plots were irrigated. Plastic covers (polyethylene sheeting) on some treatments were left in place for three weeks. Plots were maintained free of broadleaf weeds until November. This ensured that any treatment differences in the soil seed bank were due to the fumigants only, and not also to any differences in host growth.

Twenty soil samples (13 mm diameter x 100 mm depth) were collected from each plot and combined to yield a 400 g subsample. A DNA probe was used to estimate seed number per 200 g of soil. Samples were collected in late November. This lag between samples gave a long period to allow for decay of DNA of killed seeds. The sample was only taken within the central 5 m length of plot, as fumigant rates were likely to be higher at the start and end of the plot. Samples were also collected from the area adjacent to the plot as an untreated reference.

A further set of soil samples was collected in January 2003. The DNA assay was used to estimate broomrape seed numbers per 200 g soil from treated plots and adjacent reference areas. DNA assays were used to estimate broomrape seed numbers of some of these samples, six months later in July 2003.

Results

The number of broomrape seeds in plots was very variable (Fig. 1). Fewer seeds were measured in samples from treated plots in comparison to reference areas (paired t-test, $p < 0.001$ pooled across treatments, controls omitted).

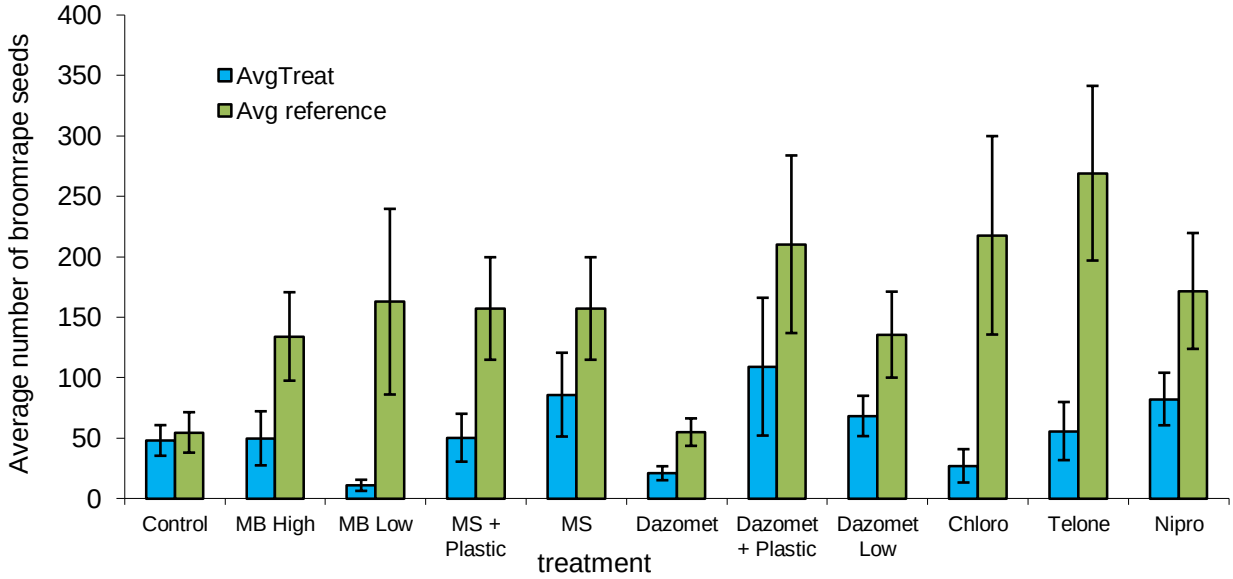


Figure 1. Number of broomrape seeds per 200 g soil collected from experimental plots 3 months after treatment (Treat) and untreated areas adjacent (reference) Bars are means ± 1 SE, n =4.

The number of seeds in treated plots in proportion to the number of seeds in adjacent reference areas did not differ significantly between treatment types (ANOVA on log-transformed data, $p = 0.122$). Given the variability in seed numbers it is not possible to determine whether one treatment was superior to another. No treatment successfully reduced the seed bank to less than 10%.

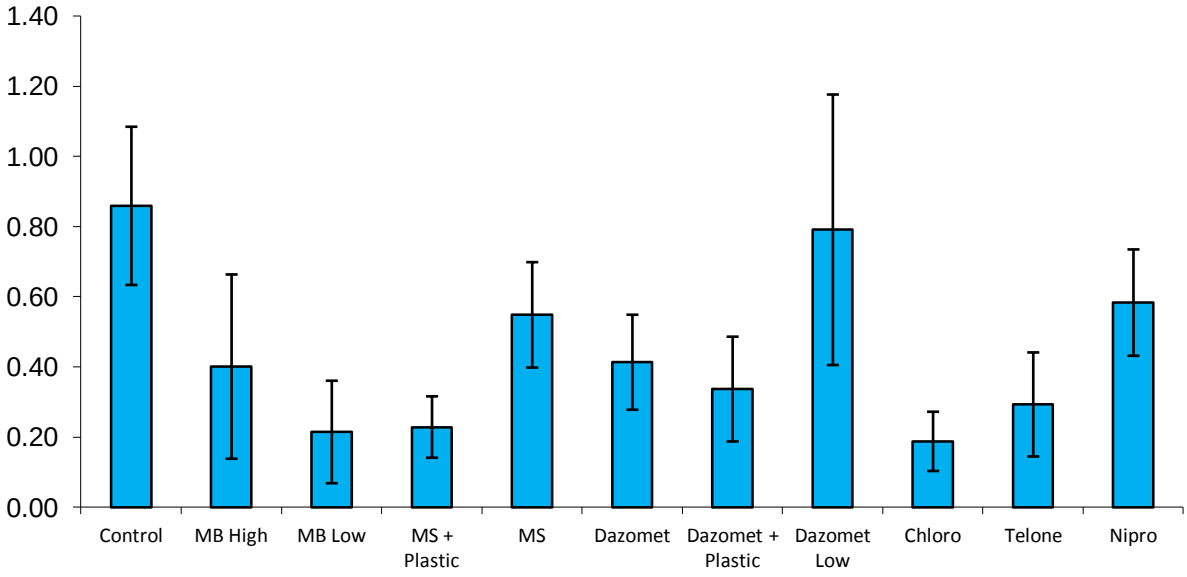


Figure 2. Difference between the proportion of broomrape seeds in reference samples and collected from treated plots. Bars are means ± 1SE, n = 4.

There were no differences in broomrape seed numbers among treatment plots, sampled in January 2003 (Fig. 3). Broomrape numbers were similar to samples collected in November demonstrating no further decay in seeds or broomrape DNA since the previous samples had been taken.

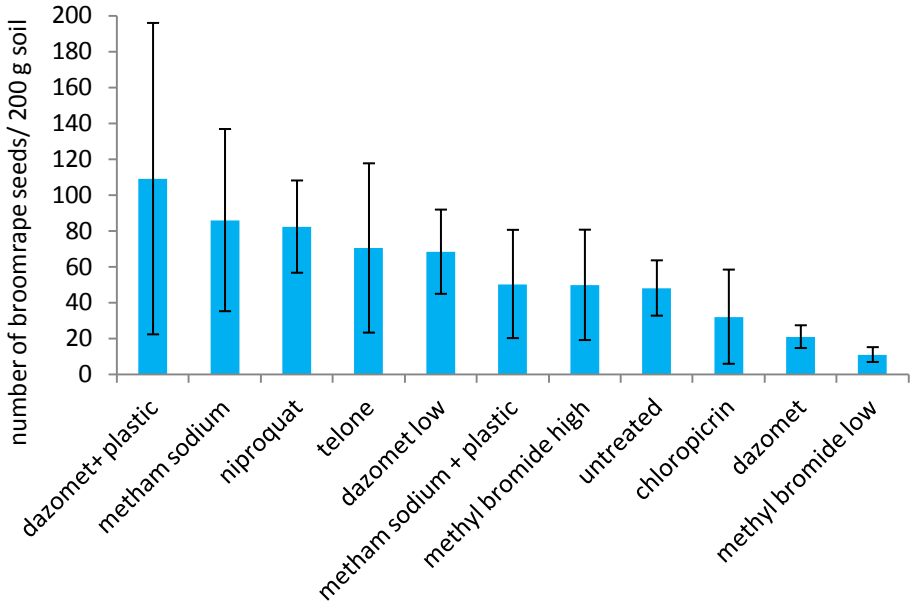


Figure 3. Number of broomrape seeds in plots treated in August 2002 and sampled in January 2003. Bars are means ±1 SE, treatment plots n = 4.

There was high variability in seed numbers between treatment plots. The sampling design included collections adjacent to treatment plots, however there were still inconsistencies in the efficacy of treatments amongst replicate plots. For example, plots treated with methyl bromide at a high rate had more seeds than adjacent untreated areas in Plot D but fewer seeds in Plots E, J and K.

DNA assays of samples collected in January 2003 but processed in July 2003 found similar numbers of broomrape seeds to samples assayed in January 2003, indicating no further DNA decline over this time period.

Discussion

The DNA assay is the most efficient means of measuring the broomrape soil seed bank. The seed bank is spatially variable and is difficult to sample adequately. This makes interpretation of the results difficult. There was large variation in estimates of seeds numbers therefore comparisons between the efficacy of each fumigant remains inconclusive.

Although soil samples were collected 3 months after treatments were applied and plots remained host-free, there is the possibility that broomrape vegetative material remained in plots. This may have over-inflated the DNA assay results. However, samples collected approximately 6 months after treatments were applied did not show any further decline in broomrape seed numbers in treated soils.

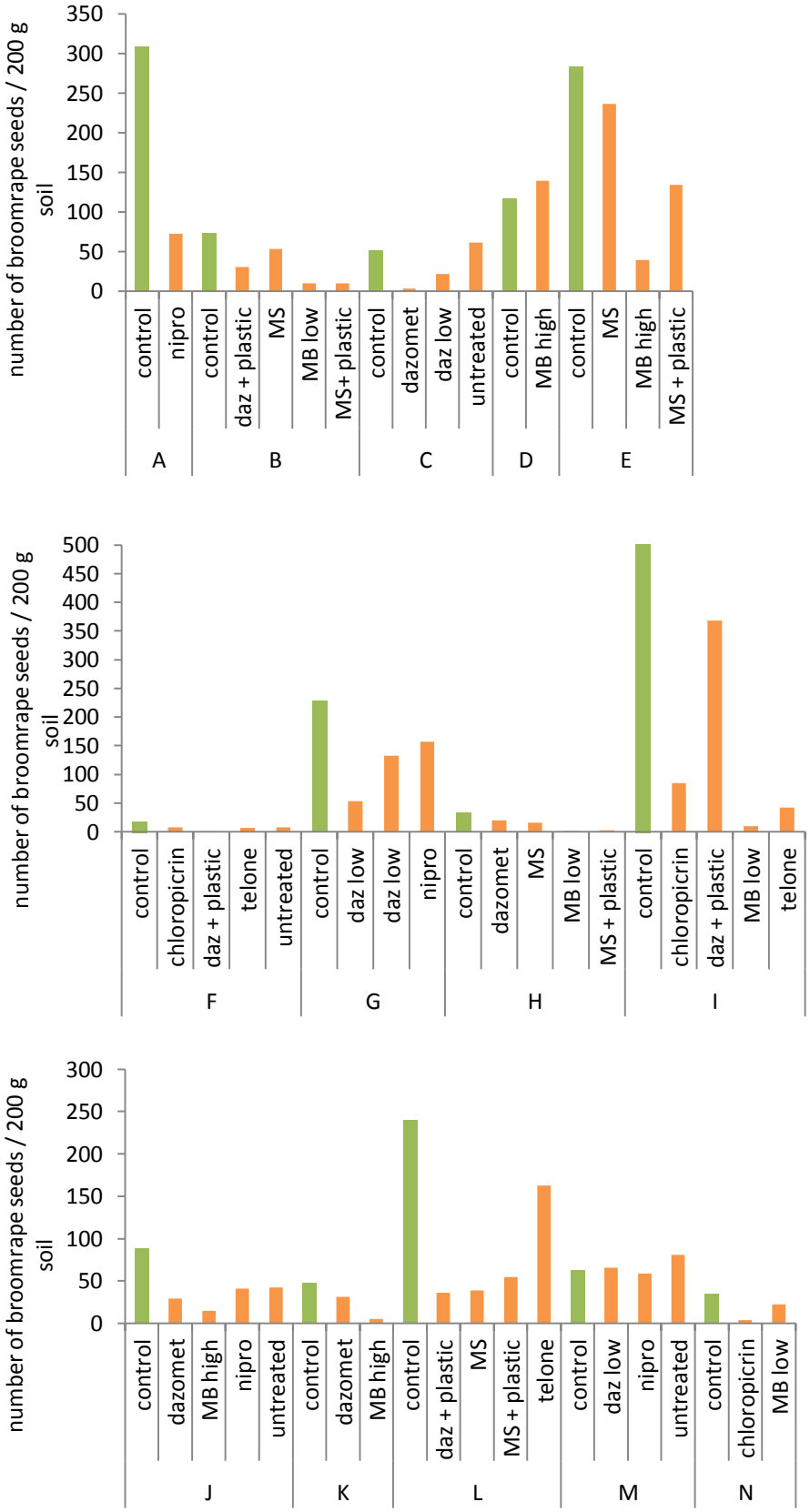


Figure 4. Number of broomrape seeds in plots sampled in January 2003. Each bar is the mean of two samples collected from each plot or control reference area, adjacent to the treated plot.

2. Seed bank reduction field trial – fumigants and chemical stimulants

John Virtue[1], Graham Fromm[2] and Tom McInenrny[2]

[1] Department of Water, Land and Biodiversity Conservation

[2] Rural Solutions South Australia

2003

Seed kill (6 treatments)

Fumigant / soil drench	Rates	Plastic	Supplier/Applicator
Methyl bromide *SA Rural Methyl Bromide 980 Fumigant (= 980g/kg MeBr + 20g/kg chloropicrin)*	1 rate: 1) 50 g/m^2	Yes	Peter Williamson, SA Rural Agencies Wet soil 1 week prior to application (if needed). 10 L H_2O/m^2.
Chloropicrin *Chloropicrin Soil Fumigant (= 985 g/kg chloropicrin)*	1 rate: 1) 25 g/m^2	Yes	Peter Williamson, SA Rural Agencies Wet soil 1 week prior to application (if needed). 10 L H_2O/m^2.
Cyanogen (carbon nitride)	3 treatments: 1) 25 g/m^3 + plastic 2) 50 g/m^3 + plastic 3) 50 g/m^3 , no plastic		Yonglin Ren, CSIRO Entomology, Canberra Wet soil 1 week prior to application (if needed). 10 L H_2O/m^2.
Pine oil	1 rate: 1) 2 L m^{-2}	No	

Chemical stimulant (21 treatments)

Stimulant	Rates	Conditioner	Supplier/Applicator
Nijmegen	3 rates: 1) 3.125 g/ha 2) 6.25 g/ha 3) 12.5 g/ha in 10 L H_2O/m^2	3 types: 1) 0.005 g/m^2 diflufenican (use Brodal @ 0.01mL/m^2) in 10 L H_2O/m^2 2) 0.1 g/m^2 norflurazon (use Solicam DF @ 0.125 g/m^2) in 10 L H_2O/m^2 3) water only (10L H_2O/m^2)	Apply conditioners 3 days prior to stimulant.
Dazomet *Basamid Granular Soil Fumigant (= 940 g/kg dazomet)*	2 rates: 1) 2 g/m^2 2) 4 g/m^2		Need to rotary hoe dazomet in after application.
Acetone	2 rates: 1) 0.1% 2) 0.01% in 10 L H_2O/m^2	3 types: 1) diflufenican 2) norflurazon 3) water only	Apply conditioners 3 days prior to stimulant.

Host crop (20 treatments)

Brassicas	Seeding Rates	Nutrition	Management
5 varieties: 1) K124* (high iTC) 2) Sapphire* (med iTC) 3) LowPE* (low iTC) 4) Clearfield 5) local best variety	2 rates: 1) 5 kg/ha 2) 10 kg/ha	2 rates: 1) standard 2) high (esp. for sulfur)	Need good weed control as other weed hosts (especially *Brassica tournefortii*) will complicate interpretation of any variety differences. Lontrel will not affect broomrape directly. Spray all varieties at 75 days after planting, using a Group B herbicide.

Controls (3 treatments)

1. Volunteer pasture/weed growth (initial cultivation only).
   This indicated normal broomrape germination % with no intervention. Group B herbicide sprayed at 75 days after initial rotary hoe.

2. Cereal with high broadleaf weed control.
   This indicated normal broomrape germination % under cropping. Standard district cropping practice followed (including early broadleaf weed control, use of Group Bs).

3. No vegetation (either frequent herbicide knockdown or white weedmat).
   This indicated broomrape seedbank levels at the end of 2003 with no germination.

General Method:

Two arable, accessible 1 hectare sites were selected with even, high density broomrape infestations: sites at Mannum Trial Site and Mypolonga. Plot size 10 x 3 m, four replicates, 224 plots per site.
A 0.5m width untreated area was allowed either side of plot (but still received the same amount of cultivation as the treated area). This provided a control for each plot to determine % broomrape seed bank decline at the end of the experiment. One rotary hoe cultivation occurred prior to pegging out, to give a more uniform broomrape seed distribution and to breakup soil for fumigants/stimulants.

All plots were kept broadleaf weed-free (barring the volunteer pasture treatment) to prevent non-treatment broomrape germination: glyphosate was used pre-treatment. No herbicides were used for volunteer pasture treatment until the Group B herbicide was applied at 75 days. Fumigants and stimulants plots were maintained host-free. Broadleaf selectives were sprayed early to prevent host development and subsequent broomrape germination.

Brassicas and cereal were sown in early May and irrigated where needed to get good establishment. Chemical fumigants and stimulants were applied in June. Plots were oversown with triticale 1 month later to provide soil stabilisation (and maintain broadleaf weed control). All non-experimental areas were treated with a Group B or glyphosate at appropriate times to prevent broomrape emergence.

Measurements:
Soil samples were collected from each plot and adjacent control buffer area for estimation of branched broomrape seed bank levels by DNA assay (SARDI soil sampler, 20 subsamples per plot to give 500g).

Samples were collected at the end of season, from July – August 2004, allowing a sufficient period for decomposition of killed seed.

Analysis

There are only results for the fumigants component of this study. Canola crops failed so no samples were collected. As low seeds were sampled from untreated plots, it was decided not to sample the stimulants plots.

The statistical analysis was done by Michelle Lorimer, Biostatistics SA. The data from the trial was analysed using analysis of covariance (ANCOVA) assuming a randomized complete block design. The number of seeds in the adjacent untreated reference area of each plot was the covariate with the number of seeds in the treated area as the response variable. These data were log-transformed. This standardized the fumigant treatments so they could be compared to the control reference plots that were presumably more closely matched in the number of original broomrape seeds.

Results

Fumigants

For the Mannum site, using the number of seeds in the untreated area as a covariate a difference was detected between treated and untreated areas (ANCOVA, p = 0.041). The highest number of seeds were sampled from the pasture plots (Fig. 1). All fumigant treatments had fewer seeds than pasture plots. Cyanogen 25 g m^{-2}- treatments had the least amount of broomrape seed and this was significantly less than the methyl bromide treatment but not the other cyanogen treatments at the higher rate (Table 1). There was no significant difference between covered and uncovered cyanogen 50 g m^{-2} treatments. Further details about the cyanogen treatments are reported in Section 8.13.

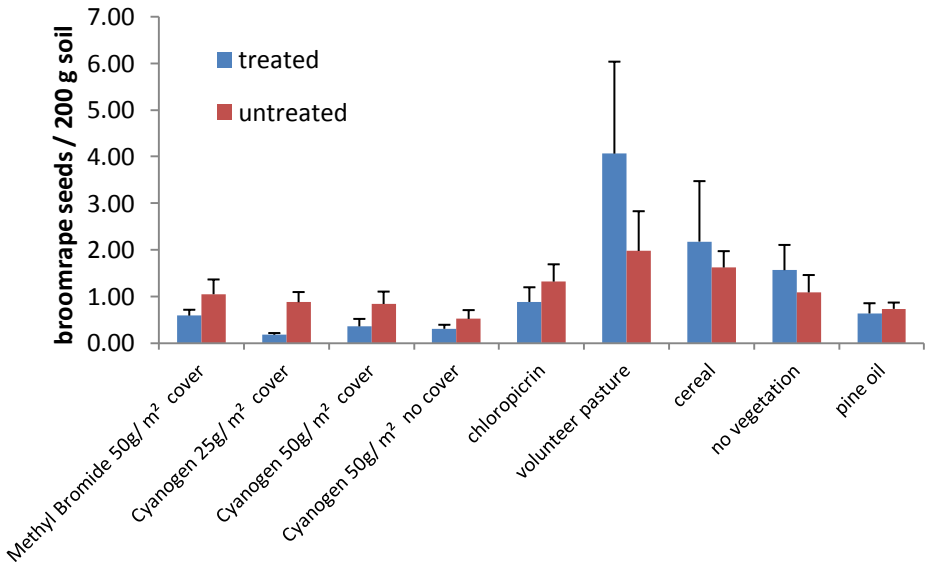


Figure 1. Number of broomrape seeds per 200 g soil collected from treated plots 3 and untreated areas adjacent at the Mannum site. Bars are means ± 1 SE, n = see Table 1.

When the control reference plots had small numbers of broomrape seed it was difficult to detect an effect of the treatments. Only the covered cyanogens treatments were successful in reducing broomrape numbers. When there is a large amount of seeds in the control reference plots, the increased impact that

the treatment has can be observed. Therefore, it can be assumed from the control reference plots that there is more seed in plots to start with, fumigants are going to have more effect (Fig. 2).

Table 1. Table of means for number of broomrape seeds in treated plots (log-transformed), at the average level of the covariate, the number of seeds in adjacent control reference areas (log transformed). Means with the same subscript are not significantly different at $\alpha < 0.05$.

Treatment	Cyanogen 25g	Cyanogen 50g	Cyanogen 50g no cover	Chloropicrin	Pine oil	Methyl bromide	cereal	No vegetation	Volunteer pasture
Mean	-0.38^{a}	0.03^{ab}	0.17^{abc}	0.35^{abc}	0.39^{abc}	0.76^{bcd}	1.43^{bcd}	1.64^{cd}	1.97^{d}
n	8	8	8	12	8	20	4	4	4

The conclusions that can be drawn from the data are limited as very few seeds were sampled from control plots. Where larger numbers of seed were sampled from these plots the effect of the treatments became evident.

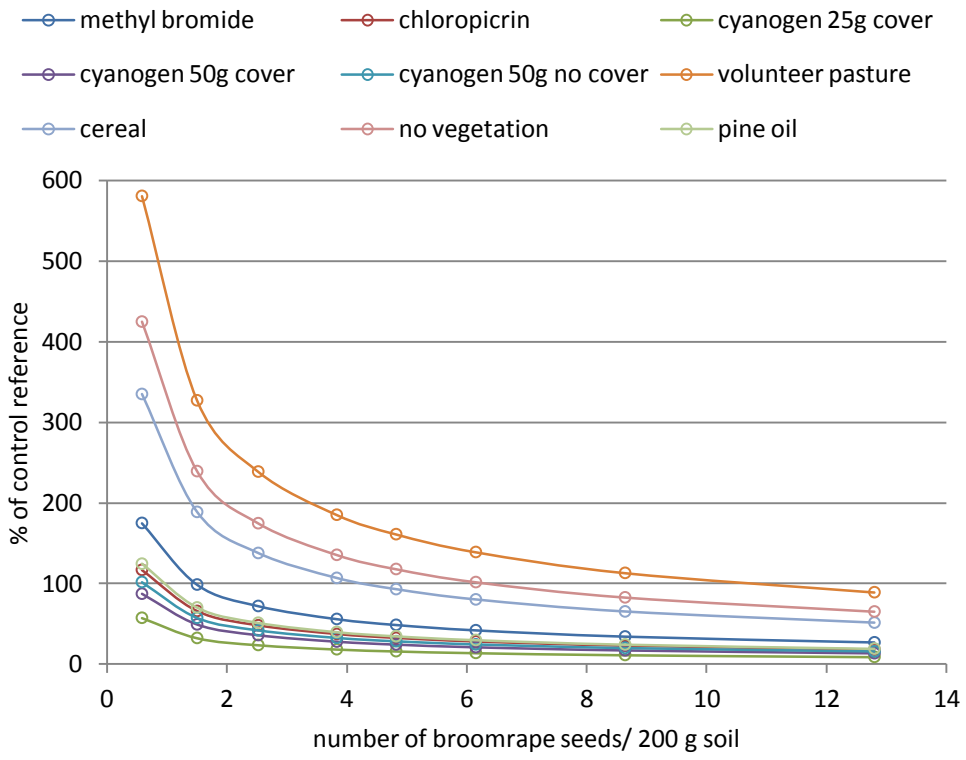


Figure 2. Fumigant response as a percentage of control reference broomrape seed number at given levels of seed density.

To further evaluate treatments, counts were made of emerged broomrape plants in treated plots and adjacent reference areas. Broomrape emergence was variable across the site reducing the conclusions that can be made about the effectiveness of fumigants. This was in part due to the patchy distribution of the favoured hosts for broomrape, cretan weed and capeweed, across the site. These plants were sometimes absent from reference plots. Some broomrape plants emerged in fumigated plots, demonstrating that fumigation did not prevent emergence (Fig. 3).

Samples were not collected from all plots at the Mypolonga site. There was also high variability in broomrape seed density at this site in control reference areas. Although fewer seeds were sampled from the treated part of the plot this did not differ significantly from the untreated part of the plot (Fig. 4).

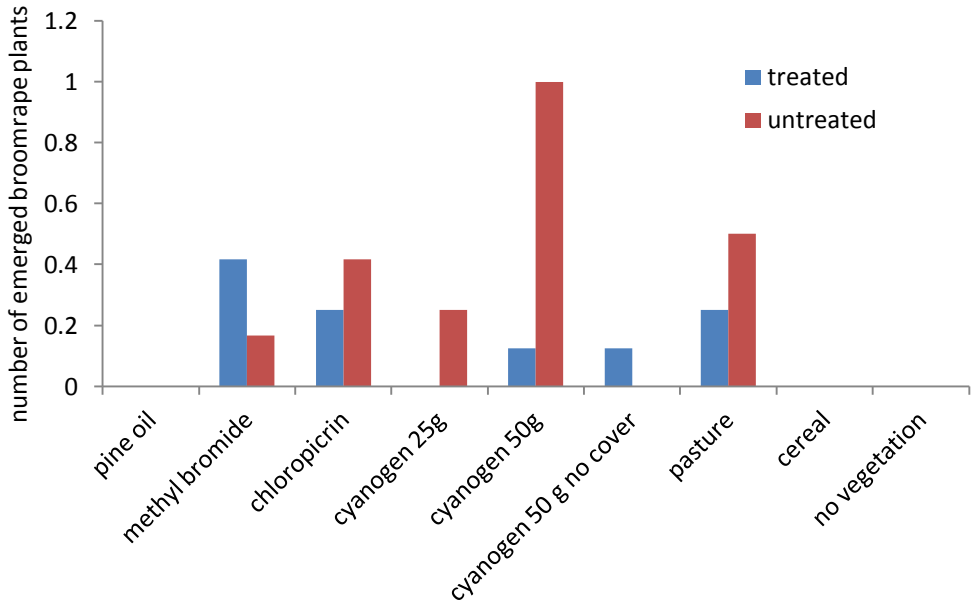


Figure 3. Number of emerged broomrape plants in treated plots and adjacent untreated parts of the plot at the Mannum site, bars are means.

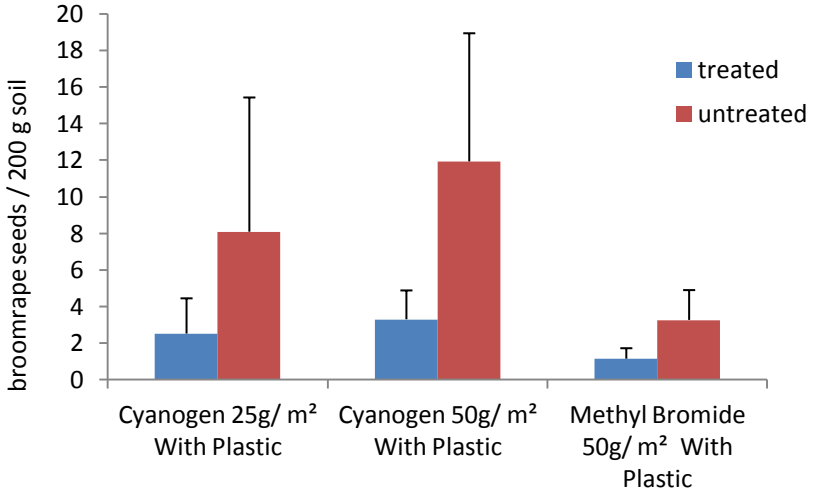


Figure 4. Number of broomrape seeds per 200 g soil collected from treated plots and adjacent untreated areas at the Mypolonga site. Bars are means ± 1 SE, n = 8 cyanogen treatments, n = 12 methyl bromide treatment.

3. Testing the efficacy of dazomet granular fumigant applied via a conventional air-seeder

Nick Secomb

Branched Broomrape Eradication Program

2006

With amendments and additions by Jane Prider (June 2011)

Aim

To determine the efficacy of Dazomet granular soil fumigant as a control agent for branched broomrape (*Orobanche ramosa*).

Methods

The trial site was located in the Hundred of Ettrick (Figure 1).

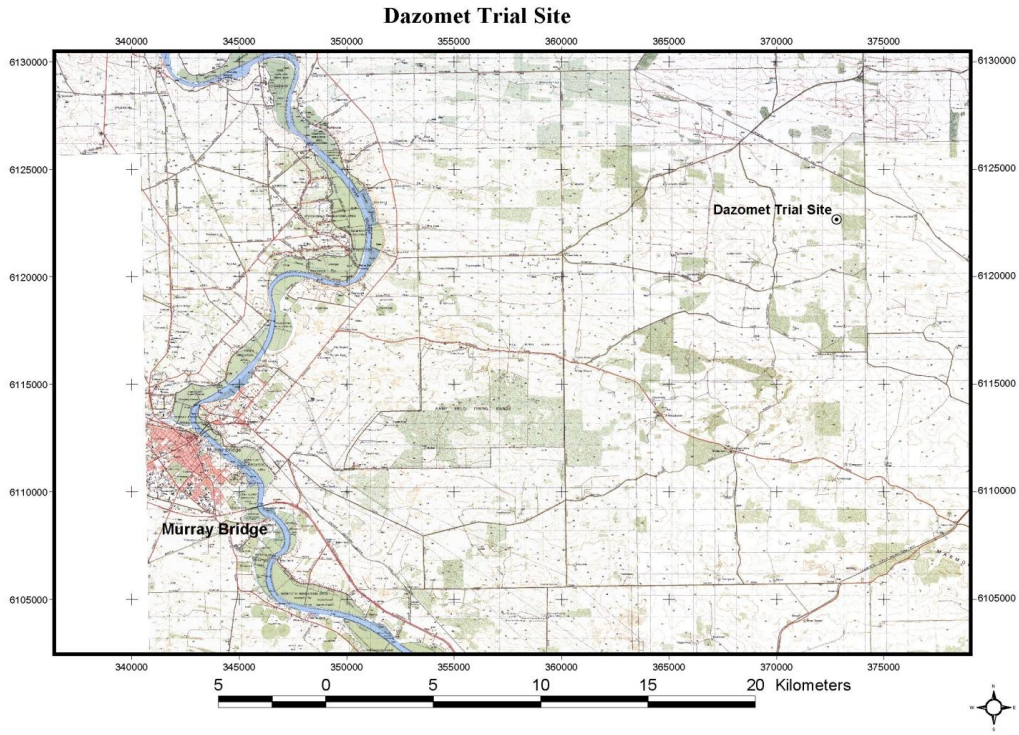


Figure 1: Location of Dazomet Trial Site

Dazomet was applied on 8th June 2004 at a rate of 120 kg of the product Basamid® per hectare. The seeder applied the dazomet at a depth of approximately 100m in rows approximately 30mm wide and 150mm apart.

Once applied, the trial site was rolled with a heavy, non-corrugated stoneroller to create a surface barrier to escaping MITC gas.

The site was not artificially irrigated after application although approximately 6mm of rain fell on the site on the night following application.

Soil samples were taken prior to application and at 3-month intervals for one year. The soil sample method is detailed below. Samples were subjected to the SARDI DNA test to obtain an estimate on the number of viable broomrape seeds present.

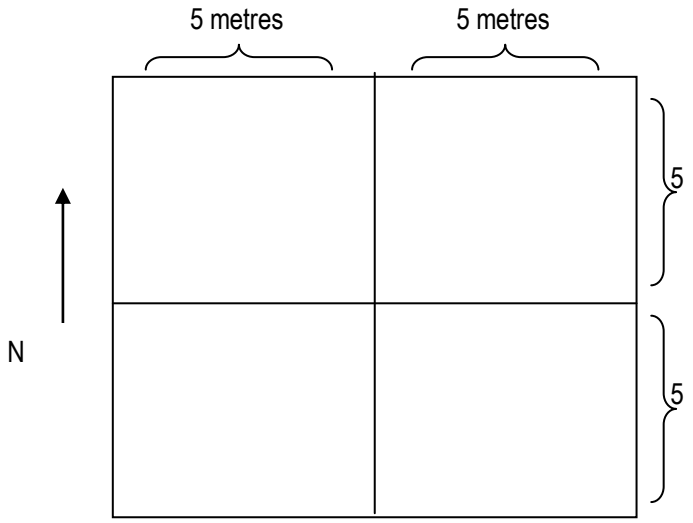
Dazomet was applied again in 2006 and 2008 (Table 1) and further soil samples collected for testing for the presence of broomrape seed.

Table 1. Site history

Year	*Treatment*	*Broomrape*
1999		Emergence
2000		No emergence
2001		No emergence
2002		No emergence
2003		No emergence
2004	dazomet part paddock 120 kg ha^{-1}	No emergence
2005		No emergence
2006	dazomet all paddock 120 kg ha^{-1}	No emergence
2007		Emergence
2008	dazomet all paddock 120 kg ha^{-1} pine oil fence lines and remnant veg	Emergence
2009		Emergence
2010		No emergence

Soil Sampling Method

Ten sites were established across a known infestation. At each of these sites, a collection plot was setup with the GPS location of the centre of each plot being recorded using a differential GPS unit for accurate location in the future. The dimensions of each collection plot were as follows:



Each 5-metre by 5-metre quadrat was then divided into five, 5m X 1m linear transects running in a north / south direction. Five soil cores were collected randomly from within each of these transects and pooled into a single sample for each quadrat. Soil was collected with a 13 mm augur to a depth of 10 cm.Four 25-core samples were collected for each site as a result.

Results

The seed estimates for each site are shown in Table 2 and in Figure 2. Total estimated seed numbers have been generated by SARDI according to the amount of identifiable broomrape DNA present in each sample.

Table 2. Estimated broomrape seed numbers, Dazomet Trial. The values are the total number of seeds from four composite soil cores in each plot.

	Sampling date					
Plot No.	June '04	Sep '04	Dec '04	March '05	July 05	May 2011
1	76	22	10	19	28	1
2	278	111	52	49	52	0
3	407	126	85	81	104	3
4	434	103	103	71	125	8
5	59	23	22	11	16	0
6	192	52	29	27	18	6
7	113	69	33	17	22	3
8	88	54	32	20	37	2
9	188	105	61	42	57	5
10	527	98	115	56	104	12

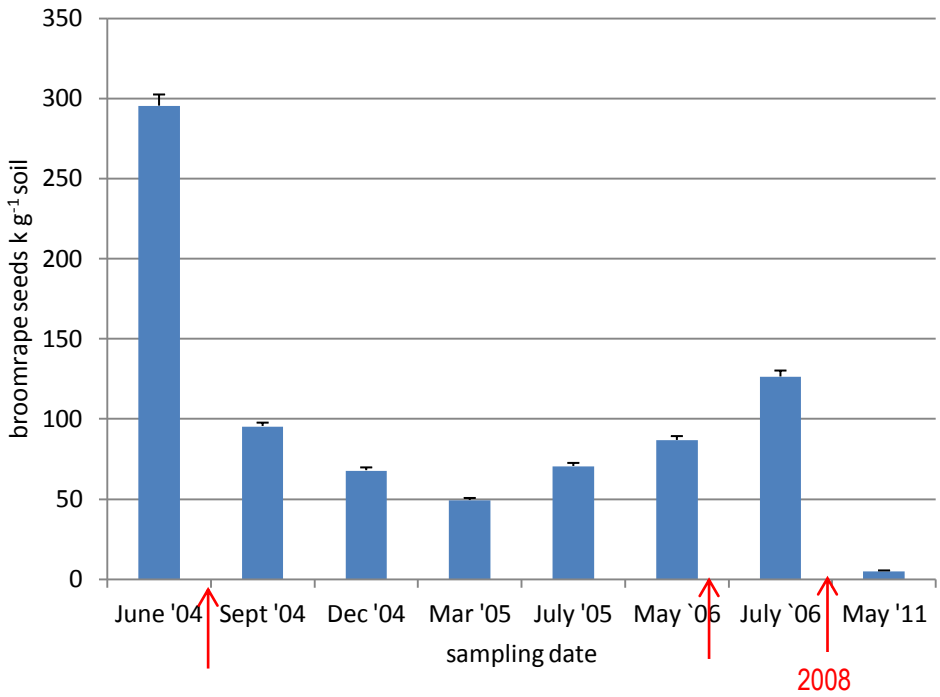


Figure 2. Number of broomrape seeds from the soil seed bank at a site treated with dazomet. The arrows indicate when dazomet was applied. Mean + 1SE, *n* = 10.

Conclusions

Dazomet has potential as a treatment for seeds of branched broomrape in soil. Sites showed a reduction in seed numbers of approximately 75% three months after a single dazomet application in 2004.

The absence of control plots means that we cannot rule out factors other than Dazomet which may be affecting the amounts of viable broomrape DNA collected.

The soil sampling method is an effective means of collecting samples for DNA analysis (estimated seed numbers reduced on a proportional scale across sites over time).

Analysis from BiometricsSA shows that there is a significant initial drop in viable DNA 3-months after application and another significant drop in the following 3-months with no significant change after this time. This suggests that a waiting period of at least 6-months is required prior to analysing results from Dazomet application.

Problems Encountered

Basamid is difficult to handle (it's a fine, flowable powder) and needed significant assistance to maintain consistent flow through the seed box of the air-seeder.

Dazomet efficacy will most likely be affected by soil moisture and temperature at the time of application and the amount of rainfall after application. This will give variable results when used on a broad acre basis.

Dazomet has low lateral mobility in soil. Results may be improved if Dazomet can be applied as an even band at depth rather than in rows 150mm apart.

DNA results can be skewed by the presence of developing broomrape tubers. If broomrape tubers are collected as part of a soil sample, this can artificially inflate results.

2011 - Has the Basamid been effective?

Due to the sampling design it is not possible to confidently attribute any decline in the broomrape seed bank to the fumigation treatment. We would need to compare the treated sites to other sites that had not been treated to see if the seed bank there had declined as well. However, the decline in seed numbers from June 2004 to May 2011 is consistent with what we would expect from losses due to fumigation (Figure 2). The trend for increasing seed numbers that occurred from March 2005 to July 2006 has not continued. It also appears that broomrape emergence post 2006 has not resulted in an increase in seed bank size so there have not been substantial seed inputs.

4. The mobility of MITC in field soils for the destruction of branched broomrape seeds

Anna Williams and Nick Secomb

Branched Broomrape Eradication Program

July 2006

Aim

To determine the mobility of methyl isothiocyanate (MITC) gas in Mallee soil & the concentration of MITC required for optimum branched broomrape seed mortality.

Methods

This trial was conducted at a field site on a sandy loam soil at Younghusband.

Basamid granular fumigant was applied at a depth of 50 - 100 mm and the rate of 2 g per linear metre (equivalent to 120 kg ha^{-1}) to an open furrow and the furrow then covered with soil. Two such furrows were made in five replicate plots. A portion of the furrow was left untreated as a control. Basamid was applied to plots 1 and 2 on 18 July 2006 at a soil temperature of 12.4°C and a soil moisture content of 14%. Basamid was applied to plots 3 – 5 on 26/7/2006. The soil temperature was 10°C and average soil moisture content was 10.4%.

The concentration of MITC was measured using gas analysis tubes (Gastec Corporation) supplied by the Victorian Department of Primary Industries. In treated plots, samples were taken at three distances from the application furrow:

- 0 mm – at depths of 25, 50, 75, 100 and 200 mm
- 50 mm – at depths of 100 mm
- 100 mm – at depths of 100 mm

Samples were collected at three time intervals: 24, 48, and 72 hours after Basamid application. Three sets of replicates were collected from each plot. Control gas samples were collected from the untreated furrow line at depths of 25, 50, 75 and 100 mm.

To assess broomrape seed viability, stainless steel seed sachets were prepared, containing approximately 200- 500 seeds. Six sachets were buried at in each plot at four depths: 25, 50, 75 and 100 mm on the treated furrow. Two sachets were buried at the same depths on the untreated control furrow. Sachets were retrieved one month after treatment. Viability was assessed using germination and tetrazolium solution tests. The average seed viability was calculated for the six sachets within each plot.

Results and Discussion

Gas

The lowest concentration of MITC as recorded at the shallowest depth, up to 48 hours after Basamid application (Fig. 1). However, there was little difference in MITC concentration at other depths in the soil profile or between control plots and Basamid treated plots after 72 hours. This indicates lateral and vertical movement of Basamid over this time period. The results indicate Basamid is resident in the soil profile for at least 72 hours after application and does not decrease over this time period.

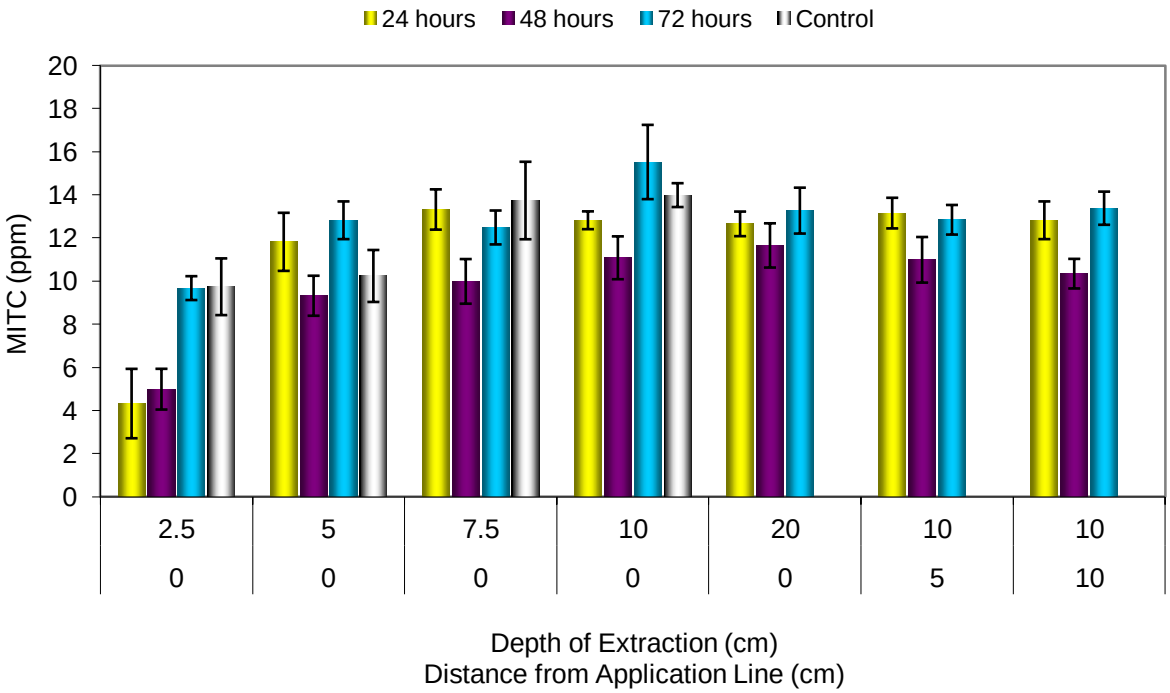


Figure 1. Concentration of MITC measured on gas extraction tubes at different depths, distances and times after Basamid application. Basamid was applied at distance zero at a depth of 10 cm at time 0 to five plots with three samples collected in each plot. Bars are means ± 1 SE, *n* = 15.

The concentrations measured in this experiment are comparable to those measured by Goldwasser et al (1994) in soils at similar depths. They measured concentrations of MITC of less than 5 ppm in the upper 10 cm of soil after the application of metham sodium by drip irrigation to a sandy soil, with concentrations up to 20 ppm in a loamy soil. Most MITC had dissipated after 6 days at this depth.

Seeds

Seeds retrieved from Basamid-treated plots had lower viability than seeds retrieved from untreated control plots (ANOVA, $p < 0.001$). Although there was a trend for broomrape seed viability to decrease with depth into the soil profile to 75 mm in Basamid plots, this was not significant (Fig. 2).

The gas results indicate that there is only a minor difference in MITC concentrations form 2.5 to 10 cm and this is correlated with no difference in the seed viability results. As MITC was detected in control plots, there may have been some loss of viability in control seed treatments. Seed viability for most broomrape seed lots is typically at least 90%.

Under the conditions during this trial, Basamid has not been effective for destroying broomrape seed banks. This may have been the result of low temperatures and soil moisture levels following Basamid application which may have reduced the release of MITC.

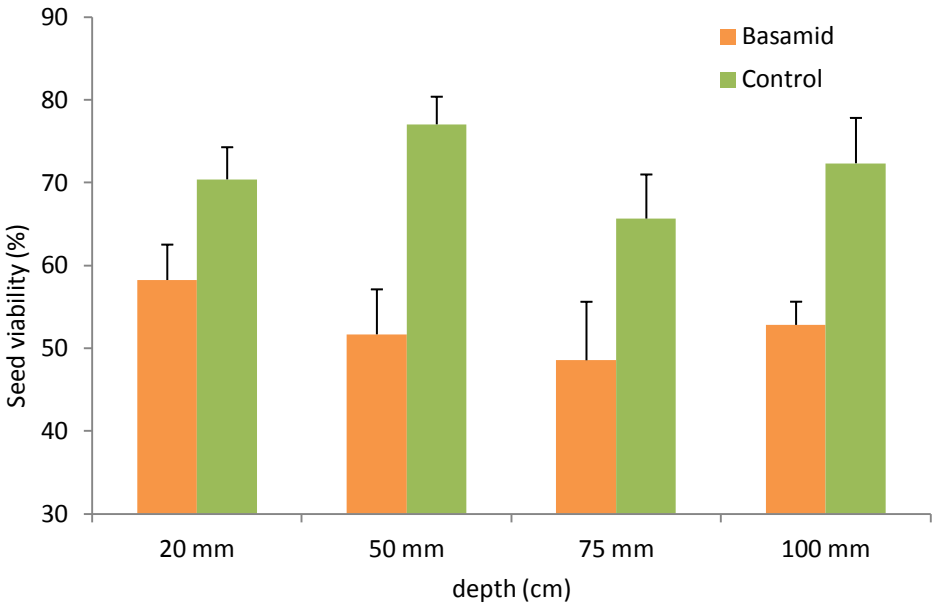


Figure 2. Viability of broomrape seeds retrieved from soil at different depths, one month after Basamid treatment. Bars are means + 1SE, *n* = 5.

Reference

Goldwasser, Y., Kleifeld, Y., Golan, S., Bargutti, A. and Rubin, B. (1994). Metham sodium's dissipation from the soil and its effect on the control of Egyptian broomrape (*Orobanche aegyptiaca* Pers.). *In* 'Biology and Management of *Orobanche*', p. 550-560. (Royal Tropical Institute, Amsterdam Netherlands).

5. MITC release in wet and dry soil at two rates of Basamid application

Anna Williams

Branched Broomrape Eradication Program

December 2006

Background

The Younghusband Basamid trial, run during July 2006 (Section 8.4) generated the following questions:

1. Were the gas tubes sensitive enough to detect the concentration of MITC being emitted from the 120 kg ha^{-1} rate used?
2. Was the soil too dry?
3. Was the temperature too cold?

To answer some of these questions the following trail was done at the Mannum Trial Site:

- 3 concentrations of Basamid: 0, 120 and 360 kg ha^{-1}
- Wet/dry conditions: dry = field conditions, wet = soil moistened to 50% holding capacity
- 3 times of extraction of MITC: 24, 48 and 72 hours after application
- 5 replications per treatment

A plot measuring 5m by 7 m was marked out and divided into the treatment subplots, with 50 cm spacing between sub-plots.

At each of the target timings a 5 cm soil core was collected from each plot and the allotted amount of Basamid added before the core was replaced and compacted by treading on it. Water was added to the wet treatments.

Gastec tubes were used to sample MITC concentrations in the soil.

Results

There was no difference in the amount of MITC detected by the Gastec tubes among the three different Basamid rates, including controls (ANOVA, $p = 0.35$). This indicates there was movement of MITC from the treated plots into the control plots. Higher concentrations of MITC were sampled from the wet plots (ANOVA, $p = 0.008$). The highest concentrations of MITC were recorded 24 hours after Basamid application (ANOVA, $p < 0.001$).

None of the interactions between treatments were significant but this was probably confounded by the movement of MITC between treatment plots and the variability in measurements that reduced the power of statistical tests to detect differences. From the chart (Fig. 1) it can be seen that after 72 hours, MITC was not detected in dry soils at the lower Basamid application rate or controls. MITC was still detected in the wet soils after 72 hours in all plots. MITC was only detected in control plots in dry soils at 24 hours.

From this trial it can be concluded that for future trials plots need to be well spaced as there is considerable lateral movement of MITC in the soil, particularly in wetter soils. The Gastec tubes appear to lack the sensitivity required to detect differences between treatments.

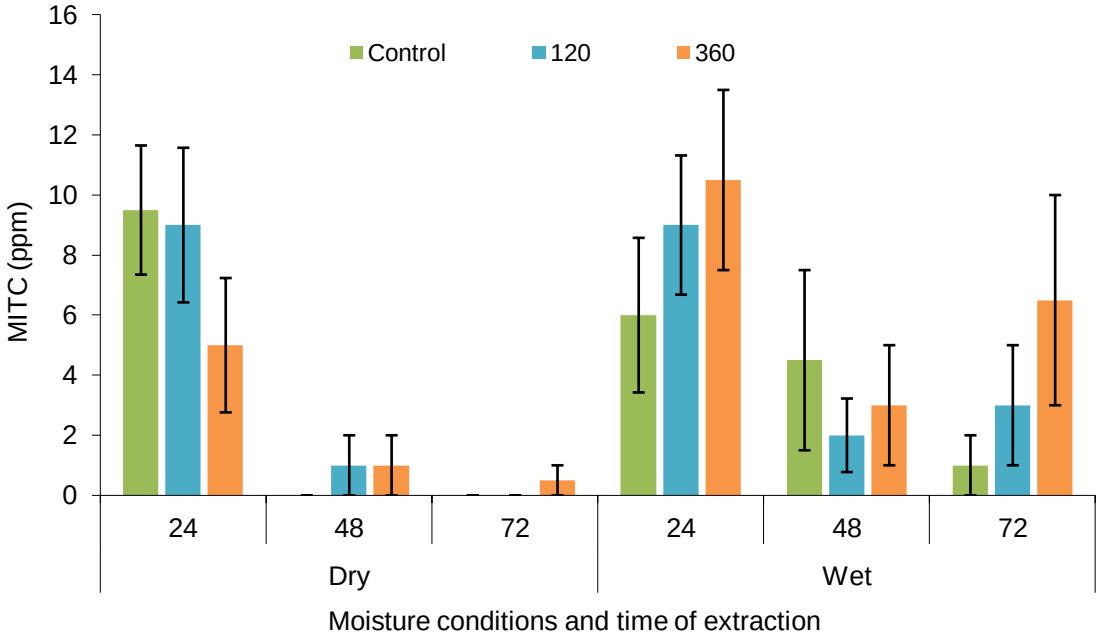


Figure 1. MITC concentration measured by Gastec tubes over time in plots treated with Basamid at different application rates. Bars are means ± 1 SE, n =3.

6. Dose-response calculations for application of Basamid and Interceptor (pine oil) to branched broomrape seed

Anna Williams

Branched Broomrape Eradication Program

November 2006

Aims

1. To determine the seed viability dose-response curve after application of the soil fumigant Basamid or the soil drench Interceptor
2. To determine the rate of stimulation of suicidal germination after application of the soil fumigant Basamid or the soil drench Interceptor
3. To determine the rate of DNA decay of Branched Broomrape seeds after treatment with Basamid or pine oil. This is to provide an indication of how long we need to wait after field application of Basamid or Interceptor before we should collect samples for DNA analysis.

Methods

Containers of broomrape infested soil were prepared by filling plastic containers to 2.5 cm deep x 8.6 cm diameter with 260 g of sterilised Tailem Bend sand (surface area of 58 cm^2 = 0.00000058 ha). Exactly 100 seeds were added to each container and mixed thoroughly through the soil. 21 ml of water was added to each container, which adjusted the soil moisture to ~60% field capacity. The lids were closed and the containers were kept at 20 OC in the dark for 10 days for the seeds to condition.

Sachets of broomrape seed were prepared by placing approximately 50 branched broomrape seeds between two glass-fibre filters in petri-dishes. 0.3 ml of water was added to moisten the filter discs without runoff. The petri-dishes were sealed with parafilm and kept at 20 ^{O}C in the dark for 10 days for the seeds to condition.

The containers of soil were placed in the fumigation chamber, which is not temperature controlled. Ambient temperature was logged throughout the experiment. 5 replicates of each of the following amounts of Basamid were added to vials: 1.08, 2.175, 8.7, 34.8, and 52.2 mg and stirred thoroughly through the soil with a fork. 30 replicates of each of the following amounts of Basamid were added to vials: 0, 4.35, 17.4 mg and stirred thoroughly through the soil. 11.6 ml of water was added to each of the Basamid treatments so that the soil moisture was ~83% field capacity.

5 replicates of each of the following rates of Interceptor were sprayed onto the surface of the soil: 0.116, 0.29, and 1.16 ml each in water so that the total liquid applied was 11.6 ml. A volume of 11.6 mL is equivalent to application at 20,000 L/ha. This is based on current rates used in the field by the BB team. This will increase the soil moisture to ~83% field capacity.

30 replicates of each of the following rates of Interceptor were sprayed onto the surface of the soil: 0, 0.58 and 2.32 ml, each in water so that the total liquid applied was 11.6 ml.

One broomrape seed sachet was placed approximately 1 cm below the soil surface in 5 replicates of every treatment. This was done by digging a shallow hole in centre of soil tub, placing a sachet in hole and

gently covering the sachet with soil. 25 replicates of the 6 treatments with 30 replicates prepared do not have a sachet inserted.

In all treatments the soil was squashed down to replicate rolling after application of a fumigant.

All containers were left in the fumigation chamber for 1 week to allow the active compounds and all the by-products to dissipate. During that week soil moisture levels were monitored to ensure that none of them dropped below 60% field capacity.

After 1 week, the broomrape seed sachets were removed from the containers and each placed in a petri-dish.

The broomrape from the seed sachets were assessed for germination. A seed was regarded as germinated when the radicle had pierced the seed coat. Any non-germinated seeds were tested for viability using the method described below.

12 g (one teaspoon) of sieved biologically active soil collected from outside the quarantine area at Murray Bridge was added to each container and thoroughly stirred in with a fork.

5 replicates of the 0, 4.35 and 17.4 mg of Basamid and 0, 0.58 and 2.32 ml of Interceptor treatments were taken for DNA testing after; 0 days, 5 days, 10 days, 20 days, 40 days and 80 days.

The other treatments were all taken for DNA testing after 40 days.

Viability testing of BB seeds

For two replicates of each treatment, the non-germinated seeds were put into a test tube and 1ml of 1% tetrazolium stain was added. For three replicates, the tetrazolium solution was placed on filter papers but some of these dried out during incubation resulting in poor staining. These results are therefore not reported. The tubes were placed at 35 ^{O}C in the dark for 9 - 10 days.

The seeds were sieved from the tetrazolium solution and immersed for 5 min in 4% NaOCl to bleach the seed coat. The seeds were then rinsed three times in water. Seeds were observed under a microscope. Embryos coloured red or pink were considered viable, those not coloured were non-viable or dead.

(method modified from Lopez-Granados & Garcia-Torres (1999))

Analysis

Ritz and Striebig's (2005) procedure used for fitting dose response models to data is described in Section 12.4.

Results

No germination was observed in any of the treatments.

There was a significant treatment effect resulting in a decline in seed viability after the application of either Basamid ANOVA, $F = 3.5$, $p < 0.001$) or pine oil (ANOVA, $F = 4.39$, $p < 0.001$). The effectiveness of pine oil increased with increasing concentration from 1% to 20% with almost complete loss of viability at the highest concentration (Fig. 1). Basamid only reduced seed viability at application rates of 240 g ha^{-1} or more (Fig. 1). Applications of 360 g ha^{-1} reduced viability to 10%.

Dose response curves were fitted to the data set for each product (Fig. 2). Logisitic models significantly fitted the data sets for both products (Table 2). These models were used to estimate doses required to reduce seed viability by 10, 50, 90 and 99% (Table 2). The dose required to achieve 99% seed kill, over

550 g ha^{-1} for Basamid and greater than 38 % concentration of pine oil, was outside the range of concentrations tested in this study and may therefore be an over estimate.

Counts of broomrape seeds using DNA bioassay found no detectable decline in seed number in samples collected up to 48 days after treatments were applied (Fig. 3).

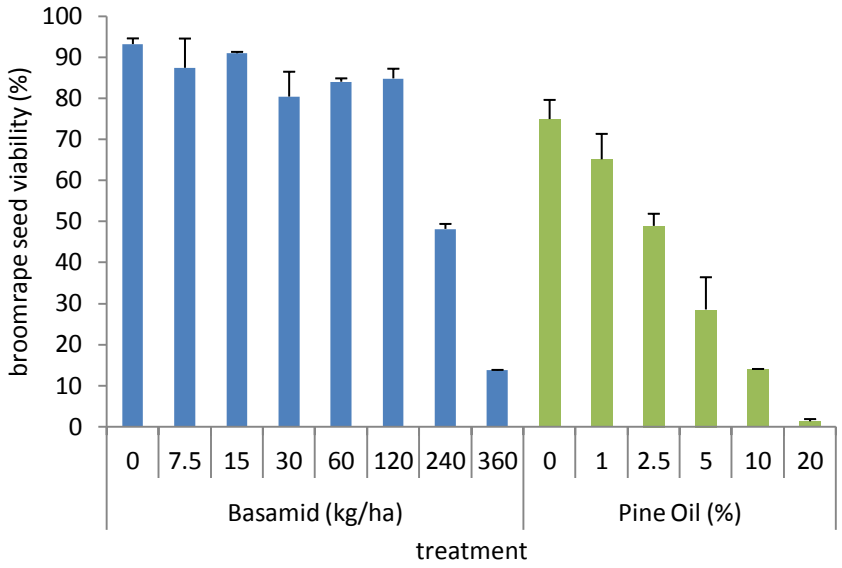


Figure 1. Viability of branched broomrape seeds after treatment with Basamid (B) or Pine Oil (PO). Each bar is mean ± 1 SE, n = 2.

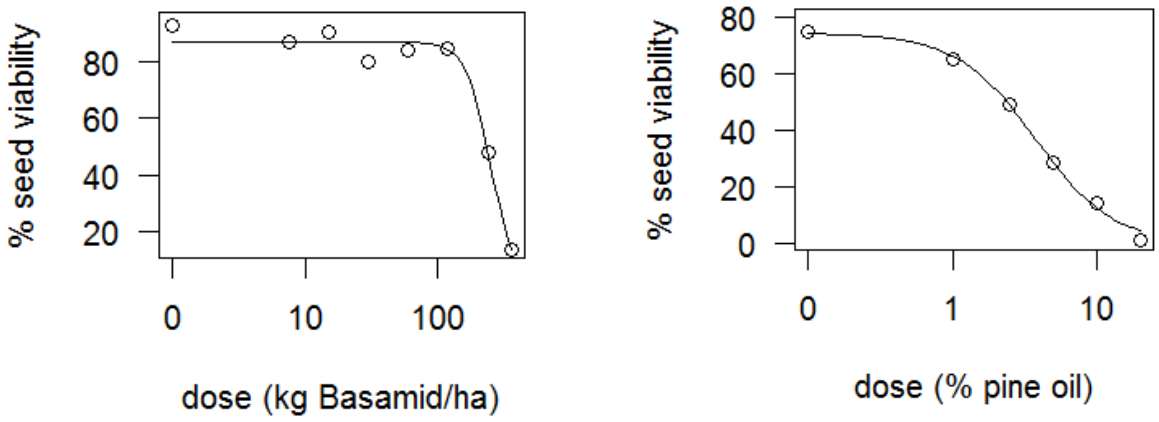


Figure 2. Dose response curves for Basamid and pine oil. Each data set has been fitted to a three-parameter logistic model. Curves were fitted using the package drc in R v 2.15.2.

Table 1. Parameter estimates and fits of three-parameter logistic models in Fig. 2. The model is described by a shape parameter (b), the maximum viability (d) and the dose where viability is reduced to 50% (e).

	parameter	estimate	Standard error	t-value	p-value
Basamid	b	4.65	0.89	5.24	<0.001
	d	87.25	1.68	51.87	<0.001
	e	251.02	9.28	27.05	<0.001
	Residual standard error (df)		5.58 (13)		
Pine Oil	b	1.61	0.26	6.29	<0.001
	d	74.17	3.76	19.73	<0.001
	e	3.72	0.47	7.93	<0.001
	Residual standard error (df)		5.70 (9)		

Table 2. Estimated effective doses of Basamid and pine oil to reduce seed viability to given levels based on fitting of the three-parameter logistic models in Fig. 2. Estimate ± Standard error is shown.

Target seed viability (%)	Product Basamid (kg/ha)	Pine oil (%)
10	156.4 ± 16.5	0.95 ± 0.28
50	251 ± 9.3	3.72 ± 0.47
90	402.8 ± 35.6	14.53 ± 2.89
99	675 ± 123.4	64.28 ± 26.68

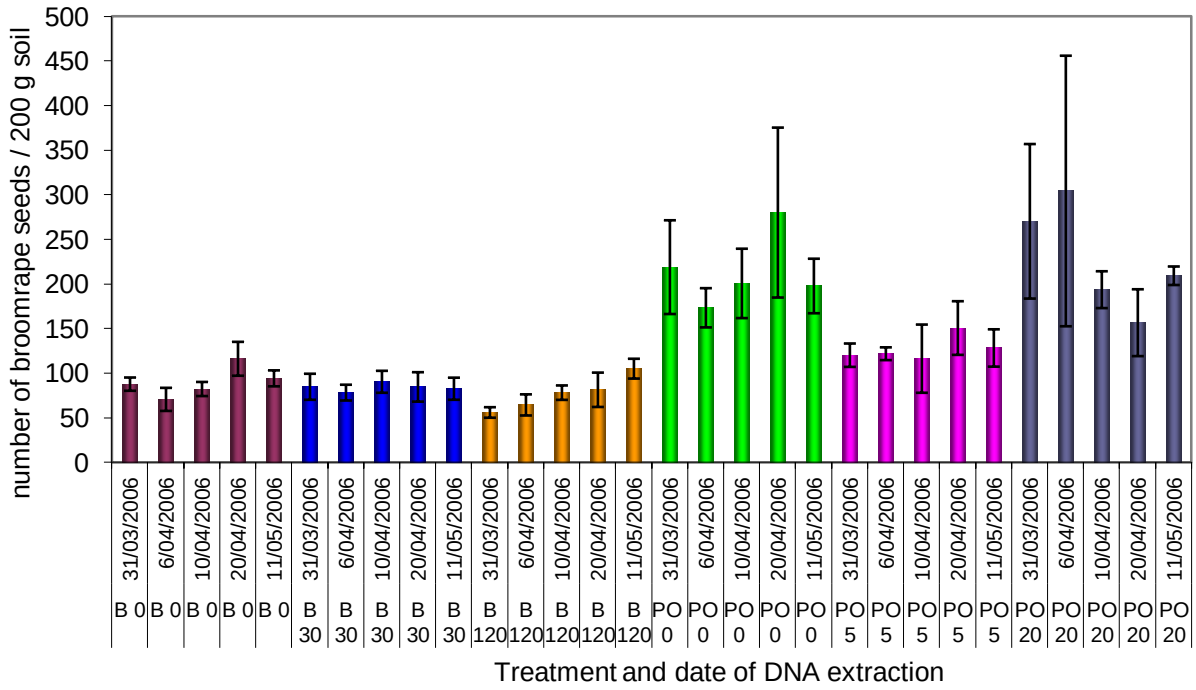


Figure 3. Degradation of broomrape DNA after treatment with basamid (B) or pine oil (PO). Bars are mean ± 1 SE, n = 5.

Discussion

The failure of Basamid to stimulate germination of broomrape at low concentrations was unexpected as *in vitro* trials of the application of methyl isosthiocyanate (MITC) promoted germination at concentrations similar to those used in this study (see Fig. 5, Section 3.7). It was calculated that soil at 80% field capacity would have 21 ppm MITC with an application of the equivalent of 120 kg ha^{-1} Basamid. There was some inhibition of germination at MITC concentrations greater than 10 ppm (Fig. 5, Section 3.7) but seed continued to germinate up to concentrations of 100 000 ppm, much greater concentrations than were used for this study. In fact, germination has never been observed in any of our soil trials with Basamid application.

The lack of germination response to applications of pine oil is consistent with other studies. Pine oil has been found to inhibit germination when application does not result in seed mortality.

Seeds were removed from tubs 7 days after treatments were applied, which may have affected the activity of MITC in promoting germination and affecting seed viability. In a field experiment it was also found that seeds had not lost viability when exposed to MITC in the soil after 7 days (see Section 8.6). Other tub experiments where seeds had been left in contact with MITC for up to 4 weeks or more achieved high levels of seed kill at Basamid application rates up to 360 kg ha^{-1} (Section 8.6). Another issue may have been the poor distribution of MITC through the soil profile as very small quantities of Basamid were added to each tub. Higher amounts of added Basamid are more likely to be distributed throughout the soil in the tub and hence seed will come into contact with released MITC. MITC can also dissipate very quickly from the soil surface. In the shallow tubs used in the study the MITC may have volatilised into the atmosphere rather than remain in the soil profile long enough to affect seed viability.

In contrast, pine oil was effective in reducing seed viability after 7 days, however high concentrations of the drench were required for adequate activity. The results of this study indicate that the 5% concentration of pine oil that is currently used could kill over 50% of seed at shallow depths in the soil profile.

The DNA bioassays show that broomrape DNA is still present in the soil up to 48 days after seeds had lost viability. If DNA tests are to be used to evaluate decline in viable seed in the seed bank, sufficient time needs to elapse to allow DNA to decay before collection of samples for testing.

References

Lopez-Granados, F. and L. Garcia-Torres (1999). Longevity of crenate broomrape (*Orobanche crenata*) seed under soil and laboratory conditions. *Weed Science* **47**(2): 161-166.

Ritz C, Streibig J (2005) Bioassay analysis using R. *Journal of Statistical Software* **12**, 1-22.

7. The behaviour of dazomet in dryland farming soils at three sites

Anna Williams

Branched Broomrape Eradication Program

May 2008

Summary

A field trial was conducted in May 2007 on three farming properties located within the Branched Broomrape Quarantine Area. These properties were chosen because they have branched broomrape infestations. Soil air monitoring showed that the concentration of methyl isothiocyanate (MITC) produced in plots treated with 112.8 or 338.4 kg ha^{-1} of dazomet peaked two days and four days after application respectively, but had similar peak levels despite the higher rate being three times the lower rate. MITC remained in the soil for greater than one week. MITC moved laterally into the control plots, a distance of 4 m from adjoining treated areas.

A small in seed viability was detected in both dazomet treatments and untreated controls in tow sites after 4 days with a further decline after 7 days. Movement of MITC into control plots may have affected seed viability in untreated controls. It is expected that the decline in seed viability would continue with longer periods of exposure to MITC. Previous monitoring, utilising a DNA test to detect branched broomrape in soil, has found a 60% decline in branched broomrape three months after dazomet treatment.

Introduction

The Branched Broomrape Eradication program utilises dazomet (Basamid ®) to destroy the branched broomrape (*Orobanche ramosa*) seedbank. Dazomet is a granular soil fumigant, which reacts with water to produce methyl isothiocyanate (MITC). Dazomet is typically used in conjunction with irrigation. However, the predominant land use targeted for fumigation by the Eradication Program is dryland farming. Reaction times and concentration of MITC produced in these conditions is poorly understood. This field trial investigated the release of MITC after application of dazomet. The trial was repeated at three sites.

Aims

To determine the lethal dose exposures for branched broomrape to MITC.

To calculate a concentration x time (ppm/hrs) measurement of MITC production

Methods

In May 2007, three field sites, each containing an infestation of branched broomrape, were selected within the Quarantine Area. Five blocks were located at each field site, with each block containing three plots. The treatments of 112.8 kg.ha^{-1} dazomet (120 kg ha^{-1} Basamid), 338.4 kg.ha^{-1} dazomet (360 kg ha^{-1} Basamid) and 0 kg.ha^{-1} dazomet (control) were randomly allocated to the plots of each block. The treatments were applied at a depth of 10 cm from the soil surface with a 24-foot airseeder equipped with Morris tynes attached at 9" spacing and 10" sweeps. Specialised seeding boots equipped with spreader plates to disperse the fumigant are also attached. The plots were then rolled with a ribbed roller to seal the soil surface.

Collection of gas samples: The volatilization of MITC from soil was captured in soil samples and soil air samples (extracted onto ORBO 32 activated charcoal tubes). These samples were collected prior to application of the treatments, immediately following application of the treatments and at 1 day, 2 days, 4 days and 7 days post-application. The soil samples were collected using a 1.5 cm x 10 cm soil corer, the lower 5cm of soil was discarded and the upper 5cm was collected into a labelled, screw top, 50ml glass jar. Sufficient soil samples were collected from within each plot to fill one jar. The jar was then capped with a screw cap lid and immediately placed on ice. The soil air samples were collected by snapping off the ends of the charcoal tubes using a glass cutter. One end of the charcoal tube was inserted into a collection needle and the other end was attached to a 50 ml syringe. The collection needle was inserted vertically into the soil to a depth of 5cm. A 50 ml sample of air was slowly and steadily drawn through the charcoal tube over a period of 2 minutes using the syringe. Immediately following collection of the sample the charcoal tube was removed and capped and placed on ice.

The samples remained on ice during transport back to the laboratory where they were transferred into a -20 ^{o}C freezer.

Seed viability

Seed sachets measuring approximately 4 cm x 7.5 cm were constructed from 100 μm nylon mesh. Branched broomrape seeds, collected in 2006 from the Mannum trial site, were surface sterilised for 5 min in NaClO and rinsed with distilled water until the runoff water was clear. Quantities of approximately 300 seeds were placed on 2.1cm GF/A filter paper discs with 150 ml RO water and photographed. The seeds were then washed into the nylon sachets and covered with one teaspoon of sand. The sand was local sand to the quarantine area that had been sterilised and sieved. Four sachets per plot were prepared at 4 weeks, 2 weeks, 1 week and 1 day prior to application of the treatments. The sachets were kept moist after construction to condition the broomrape seeds, and stored in a 20^{O}C incubator in the dark. The sachets were buried, at a depth of 5cm, immediately following application of the treatments, and prior to rolling. One sachet from each different conditioning period was exhumed after 1 day, 2 days, 4 days and 7 days after dazomet treatment.

The seeds removed from the buried sachets were assessed for germination and viability. When compared with the exposure data determined from the soil and soil air samples, this will verify the lethal dose exposures required for branched broomrape seed.

MITC analysis

The collected samples were stored on ice for transportation back to the laboratory and then stored in a freezer until the extractions were performed. To process the soil samples, the jars were removed from the freezer and the samples defrosted (approximately 1 hour). In a 50 ml capacity centrifuge tube, 30 g of soil was weighed out and 15 ml of re-distilled ethyl acetate and 15 ml MilliQ water were added. The tubes were then agitated in a shaker for 1 hour, then centrifuged for 2 mins, at 1500 RPM, break speed 9. The supernatant was pipetted off and stored in a 4°C fridge. When ready to analyse, 1.5 ml of supernatant was transferred directly into 2ml GC vials with ethyl acetate, then analyzed by mass spectrometry.

MITC was extracted from the charcoal tubes with carbon disulfate, adding 1 ml to a GC vial. The samples were agitated in a vortex for 10 seconds before storing in a freezer prior to mass spectrometry analysis.

Results and discussion

MITC in soil samples

Following Basamid application, high concentrations of MITC were detected in soil samples, with concentrations of MITC at the higher application rate much higher than the lower application rate. The gas was also detected in soil sampled from control plots two days after Basamid application. From Days 2 – 7, MITC concentrations in control and the low Basamid treatment were similar. MITC was detected in soil

samples at the high Basamid application rate at Day 7. The detection of MITC control plots was unexpected as these plots were located approximately 4 m from treated plots.

There were site differences in the concentration of MITC in soil samples. Higher concentrations were recorded at Site 2 than Sites 1 and 3 (Figure 2). At Site 1 the highest MITC concentrations were sampled from control plots, which suggests there may have been a sampling or labelling error with samples from this site. At Site 3 there was no difference in MITC measured from control plots or those with 120 kg ha^{-1} Basamid.

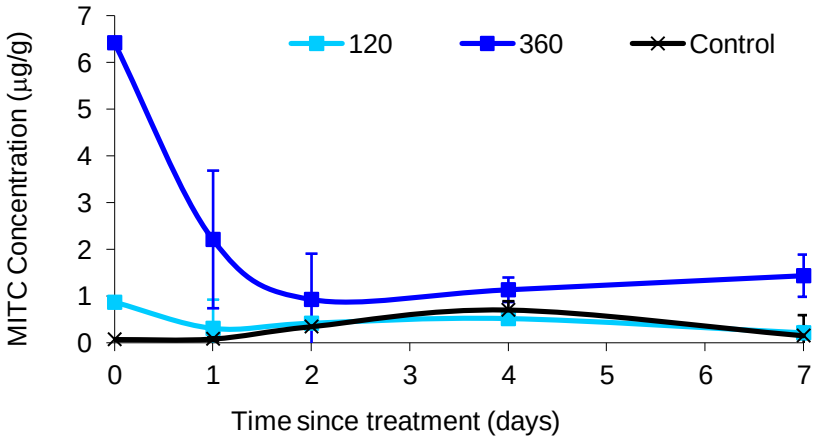


Figure 1. Concentration of MITC in soils samples, pre and post-Basamid application, combined across three sites.

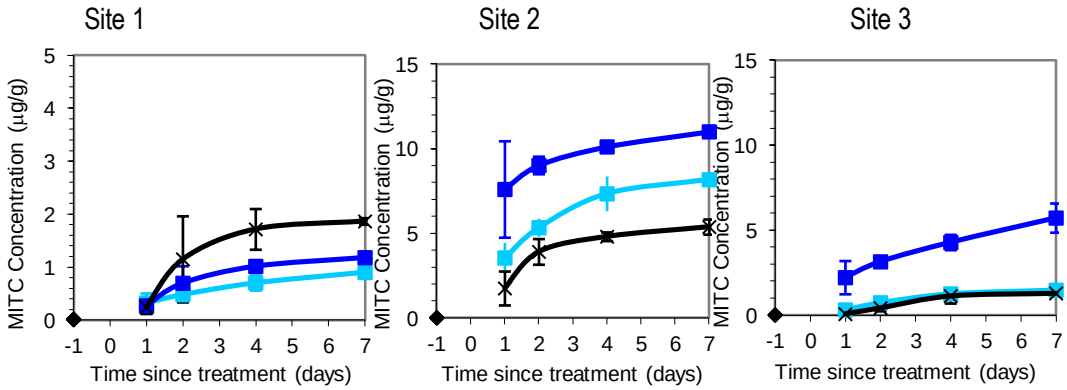


Figure 2. Cumulative concentration of MITC in soil samples collected from 3 sites. Basamid was applied at day 0. Labels as Figure 1.

MITC in soil air samples

Concentrations of MITC in the soil air spaces was much lower than in soil samples and after two days similar concentrations occurred at both application rates (Fig. 3). After 4 days concentrations were higher at the higher application rate but had decreased at the lower application rate. MITC remained detectable after 7 days. MITC was detected in control plots where it reached its highest concentration after 4 days and fell to almost zero by day 7. The peak concentration in MITC did not differ between application rates suggesting that not all the Basamid applied had reacted with soil water to release MITC.

There were site differences in MITC concentration. As for the soil samples, soil air samples in control treatments from Site 1 had the highest MITC concentration, suggesting some error in collection or labelling. In Site 2, MITC concentrations peaked in both Basamid treatments after 2 days but dropped in the 120 kg ha^{-1} treatments but remained high in the 360 kg ha^{-1} treatments. At Site 3, similar MITC concentrations were recorded for both Basamid application rates.

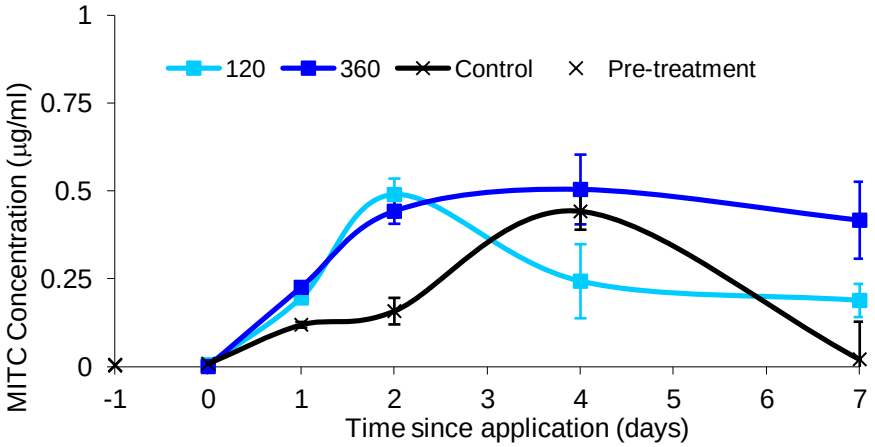


Figure 3. Concentration of MITC in soil air samples, pre and post-Basamid application, combined across three sites.

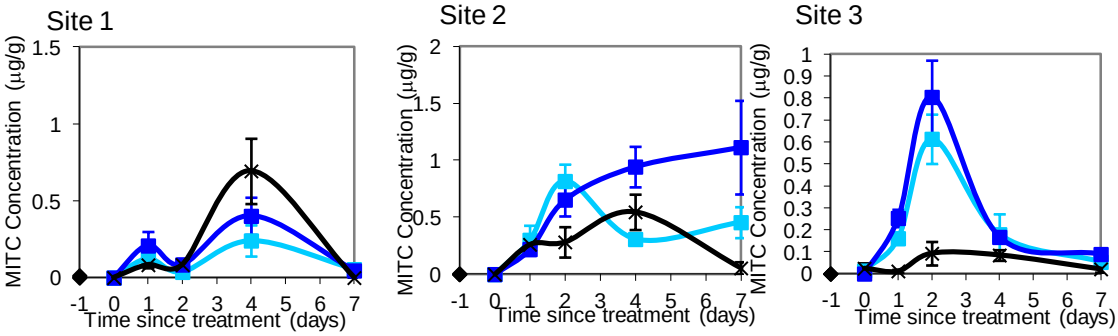


Figure 4. Cumulative concentration of MITC in soil air samples collected from 3 sites. Basamid was applied at day 0. Labels as Figure 3.

Seed viability

There were no significant differences in viability between seed from Basamid treated plots or untreated control plots (Figure 5). Seed viability loss occurred at Site 3 after 2 days and there was a further loss in viability after 7 days. At Site 1 there was some loss of viability between days 2 and 7. No germinated seeds were found in any sachets so MITC did not stimulate broomrape seed germination.

The detection of MITC in control plots affects the conclusions that can be made from this study. Lateral movement of MITC into control plots may have resulted in loss of viability of seeds buried in untreated control plots. However, the results demonstrate that loss of viability requires more than 7 days exposure to MITC, at least under the conditions at the sites used during this trial. The site where viability loss was the

highest (Site 3) had soil with the highest water content at the time of Basamid application, thus soil moisture may have important effects on the release, movement or activity of MITC.

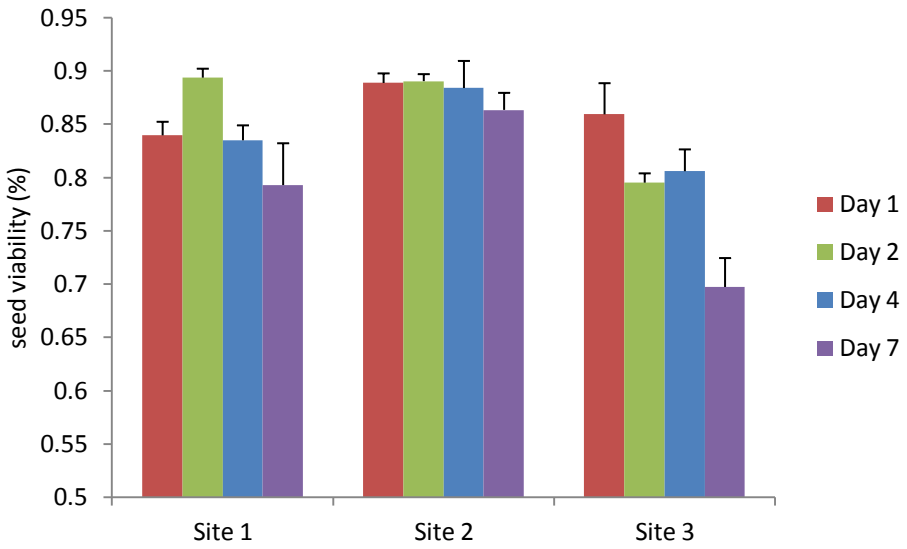


Figure 5. Viability of branched broomrape seeds at three sites collected at different times after the application of dazomet at Day 0. Dazomet treatments and controls have been combined. Bars are means + 1 SE, n = 15.

8. The influence of roller type on the retention of MITC in soil

Anna Williams and Nick Secomb

Branched Broomrape Eradication Program

August 2008

Background

Basamid is currently being used by the Branched Broomrape Eradication Program as a fumigant, which can be applied to arable areas for killing branched broomrape seeds in the soil.

Presently, farmers roll the paddock after application of Basamid with whatever rolling equipment they own or have access to. The type of rollers ranged from heavy (e.g. cement rollers or water filled tanks) to light tyre rollers.

Because the use of irrigation for sealing the soil surface is not available in the situations where we apply Basamid, good sealing of the soil surface must be achieved in another way to maximize the time that MITC is trapped in the soil before it dissipates. Therefore the ability of the different rollers at sealing the soil is an important part of the Basamid application process and could influence the concentration X exposure of broomrape seeds to MITC considerably.

Aim: to calculate concentration X time (ppm h^{-1}) measurements of MITC production when Basamid is applied at 120 kg ha^{-1} followed by heavy or light rolling.

Method

The trial was done at a site that was scheduled to be treated with Basamid as part of the Fumigation Program. The trial comprised 5 replicate blocks, with five roller treatments and an unrolled control, all replicated once in each block in random order. The roller treatments were a light ribbed roller, a light flat roller, a heavy ribbed roller, a heavy flat roller and a rubber-tyred roller.

Basamid was applied to the entire area at the rate of 120 kg ha^{-1}. The soil moisture at the time of Basamid application on 28th May 2007 was 6% and the air temperature was 16.8 °C.

Two nylon seed sachets containing 200-500 broomrape seeds were then buried in each treatment plot followed by the application of roller treatments. Soil samples and soil air samples for MITC analysis were collected before dazomet application, after rolling, and 1, 2, 4 and 7 days later. Seeds sachets were collected after 9 days. Seed viability testing, MITC sample collection and processing were as described in Section 8.5.

Analysis

The proportion of viability seed was calculated as:

$$\frac{\text{Number germinated + (number not germinated X proportion stained red)}}{\text{Total number of seeds in germination test – seeds affected by fungal infection}}$$

These data were arc-sine transformed. An ANOVA tested for roller treatment effects and for any difference in seed viability between the two sachets buried in the same treatment. Sachets were nested in treatments, which were nested in plots for the analysis.

Results and discussion

Rainfall

Soils were relatively dry at the time of Basamid application (6% soil moisture) but rain was recorded the evening after application. There were three rainfall events of 8 mm or more in the week following Basamid application and smaller rainfall events over the following three weeks (Fig. 1).

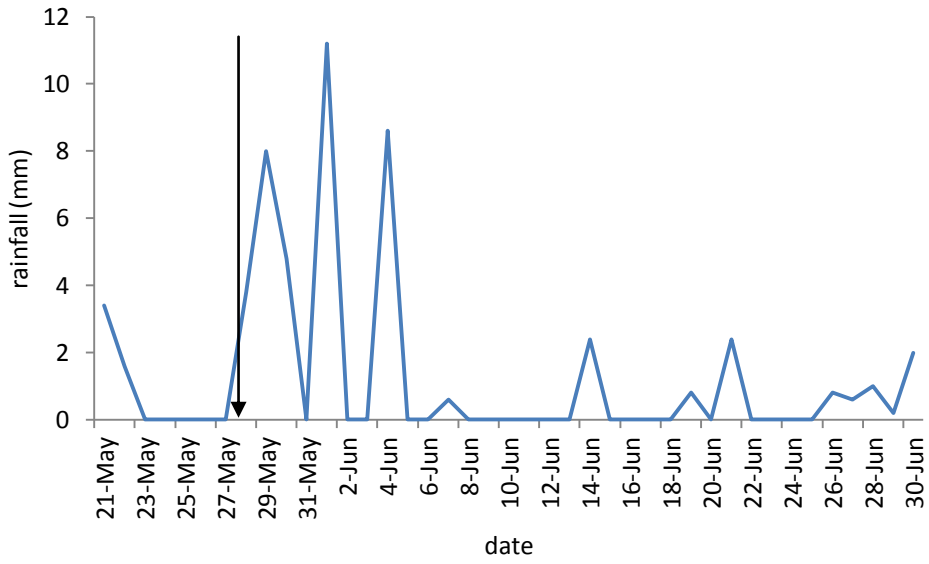


Figure 1. Daily rainfall from nearby Tailem Bend during the trial (data from Bureau of Meteorology). Basamid application is indicated by the arrow.

MITC concentration

MITC concentrations in the roller trial were variable across treatments. For most treatments there was a peak in MITC concentration on the day following Basamid application with a rapid decline and then another increase in concentration at Day 7 when sampling stopped (Figs 2 and 3). As sufficient rainfall occurred to release MITC within the 7 days following Basamid application, the second peak in release may have been the result of increased soil temperatures.

There were no differences in the concentration of MITC in soil or air samples under the different roller treatments. The highest soil MITC concentrations were recorded from the tyre roller treatment (Fig. 2) but the highest soil air MITC concentration was recorded in the light ribbed roller treatment (Fig. 3). Variability in these measurements was high. The lowest soil MITC concentrations were recorded from the heavy and light flat roller treatments (Fig. 2).

There was no evidence that roller treatment had any effect on the retention of MITC in the soil at 5 cm depth. The dry conditions during Basamid application and post-application rolling may have diminished the effects of the roller treatments. On the sandy soils at the trial site, roller treatments may be ineffectual unless the soil is very moist. The rainfall that occurred post-application may have produced a sufficient seal to retain MITC in the soil profile regardless of roller treatment.

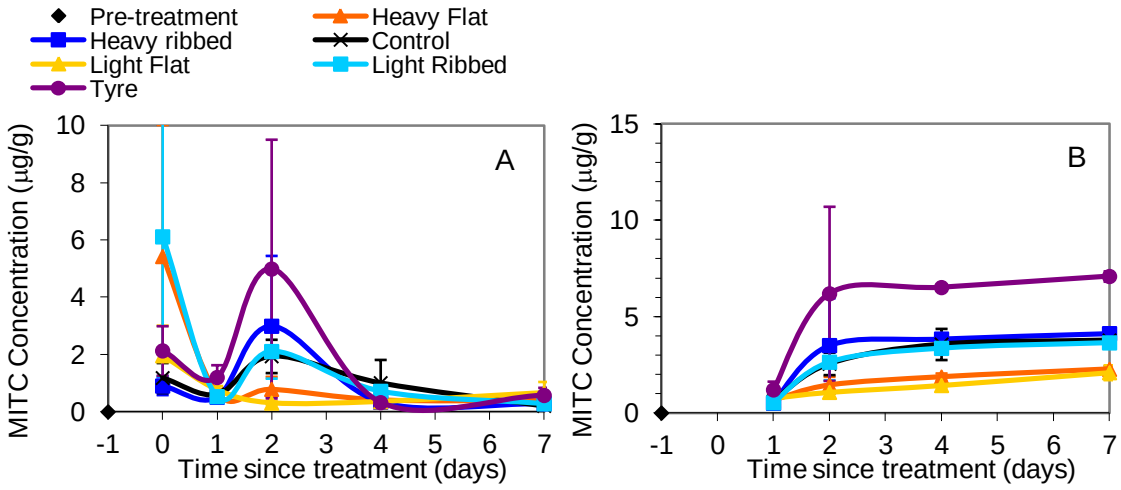


Figure 2. MITC concentration in soil samples collected from Basamid application A) measured values, B) accumulated values. Points are means ± 1SE, n = 5.

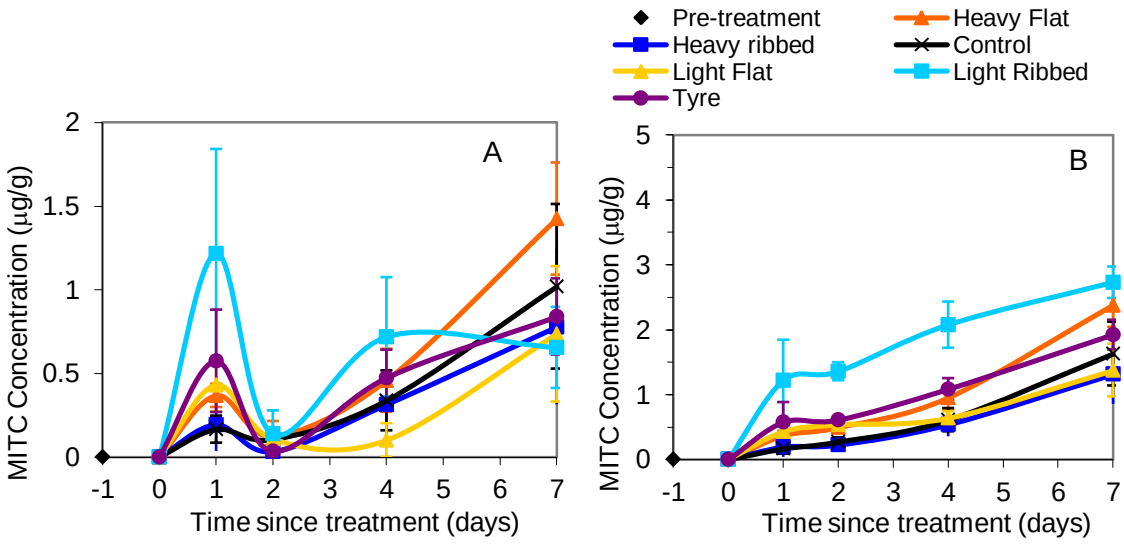


Figure 3. MITC concentration in soil air samples collected from prior to Basamid application up to 7 days later A) measured values, B) accumulated values. Points are means ± 1SE, n = 5.

Seed viability

There were differences in seed viability between the two sachets within the same treatment plot ($p < 0.001$). There were also differences in seed viability between roller treatments ($p = 0.012$) but this was consistent across the two sachets within a plot. Seed viability was high across all treatments but as there were no controls without added Basamid it is not known whether all treatments resulted in a reduction in seed viability. The highest viability loss occurred in the light ribbed roller treatment, which had lower viability than the unrolled and heavy ribbed treatments (Fig. 4). This treatment also had the highest measured soil air MITC concentration (Fig. 3B).

Results from other Basamid field trials found that sachets need to be in place for longer than 7 days for MITC to accumulate to sufficient concentration to kill broomrape seeds. The sachets in this experiment were in place for 9 days, which also was not sufficient time to achieve seed kill.

The MITC concentration appears to be very variable within the seed profile, hence the large amount of variability associated with MITC measurements and the difference in seed viability loss in sachets within the same treatment plot.

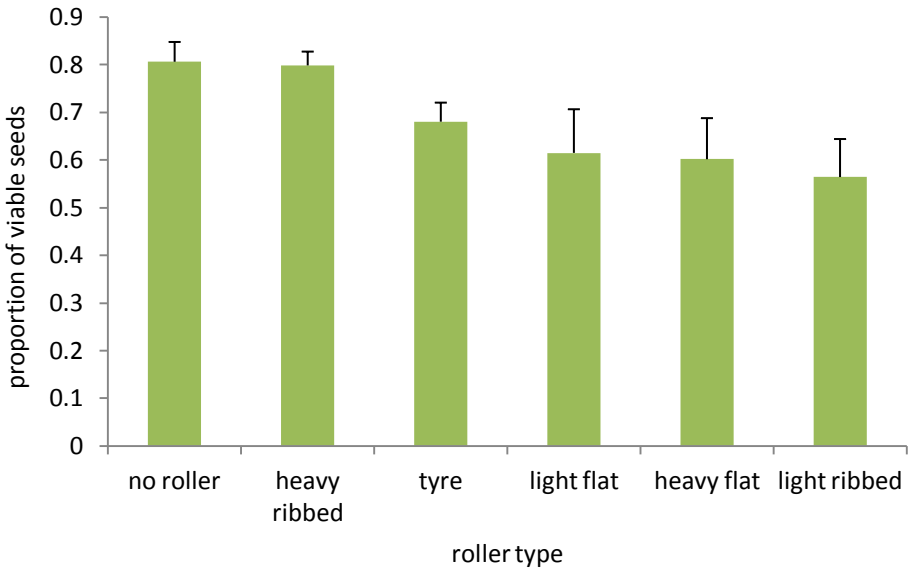


Figure 4. Branched broomrape seed viability following 9 days exposure to MITC in plots with different roller treatments after Basamid application. Bars are means + 1SE, n = 10.

9. Basamid container experiment: effect of soil moisture on MITC release

Anna Williams, Andrew Craig and Darryl Miegel

Branched Broomrape Eradication Program

2008

Aim

The aim of this experiment was to investigate the timing of Basamid conversion to MITC in different moisture regimes. We knew from a field trial in 2007 (Section 8.5) that broomrape seeds required more than one week exposure to MITC to reach their lethal dose. We also knew that Basamid took longer than one week to fully react and release the full dose of MITC available in field conditions. Based on Veronica Ward's lettuce seed tests we expected that this may take three weeks. How is this related to soil moisture levels?

Methods

As it is difficult to vary soil moisture levels in the field an experiment was designed to test the moisture conditions in smaller containers which could be stored in sheltered conditions and monitored for moisture loss for the duration of the experiment.

Pilot study

This study trialed the equipment that was to be used for the main study. Soil was collected from the Mannum Trial Site and 37 ml of water was mixed into every 1 kg of soil to yield a soil water content of 0.15 g water g^{-1} soil (15% soil moisture).

Six, 13.5L containers (Menzel plastic MH0250; internal measurements 39.4 mm long x 28.5 mm wide x 10.8mm deep), were prepared for gas sampling by drilling 8 X 4.5 mm holes around the container, 5 cm above the base. The hole was re-filled with silicone until time of sample collection. At each time of collection a soil air collection "needle" of 15 cm length was inserted into one of the holes to collect the sample. That hole was then re-filled and marked to indicate that the location was used.

Containers were set up by placing a 2 cm layer of the prepared soil in the container and then sprinkling over 1.347 g of Basamid (equivalent to 120 kg ha^{-1}). One container had no added Basamid. This layer was covered with 3 cm of soil, a sachet of branched broomrape seeds between two filter papers placed on top (omitted for the pilot study), and then covered with a further 5 cm of soil. The soil was compacted and then left unlidded. The container was weighed and the weight recorded so that water could be added by spraying the surface with water to maintain the 0.15 g g^{-1} soil water content.

The containers were stored in a shed at the Mannum Trial Site.

Soil air samples were collected the following day (day 2), and at days 3, 5 and 8. The sampling "needle" was inserted horizontally into a randomly selected hole in the container to a depth to 13 cm. A 50 ml sample of air was drawn slowly, taking 2 minutes, into a charcoal tube attached to the needle using a disposable syringe. After collection the ends of the charcoal tube were immediately capped and stored in a container on ice for transporting to a freezer. The collection hole was re-filled with silicone.

Charcoal tubes were processed to determine MITC concentration as described in Section 8.5.

Experiment 1

Soil was collected from the Mannum Trial Site and dried in the oven. Containers were prepared in June 2008 as described for the pilot study. Basamid treatments received the equivalent of 120 kg ha^{-1} Basamid (0.853 g Basamid per container). Five replicate tubs were prepared as control treatments with no added Basamid. Water was added to the prepared containers to create the desired moisture levels; 8%, 10%, 12.5%, 15% and 20%. The control treatments had a soil moisture level of 15%. Five replicate containers were prepared for each soil moisture treatment.

Four sachets of broomrape seed constructed from filter paper were placed in each container during filling. These sachets had been conditioned for varying amounts of time by placing the moistened sachets in a 20 °C incubator for 1 day, 1 week, 2 weeks or 4 weeks.

Containers were regularly weighed and water added to maintain the target soil water levels. Soil temperature was recorded in one of the control containers.

Although soil air samples were collected they were not processed as the freezer in which they were stored was unintentionally switched off.

Seed sachets were retrieved after 4 weeks. The seeds were examined for germination and then processed as per usual protocol; 14 days conditioning, followed by germination assessment 14 days after GR24 addition and viability assessment 14 days after tetrazolium solution addition.

Experiment 2

The experiment was repeated in August so that MITC measurements could be made. Soil was collected from the field site and oven dried. For this experiment the water was thoroughly mixed into the soil using a cement mixer to create the soil moisture treatments.

The equivalent of 360 kg ha^{-1} of Basamid was added to containers as described previously. The soil moisture levels were 5, 7.5, 10, 12.5 and 15%. For the 10% level we included a control without Basamid and a 120 kg ha^{-1} Basamid treatment. The lower soil moisture levels were more closely matched to field conditions. Higher applications of Basamid were beginning to be introduced into operations at this time so they were used for this trial.

Soil air samples were collected as described for the pilot study from all containers immediately after Basamid application, and at 2-3 day intervals up to 30 days after application. Containers were maintained at the target soil moisture content by weighing boxes and spraying the surface with water if necessary. Soil temperature was recorded in one of the control containers using a T-Tec temperature logger (T-Tec Technology, Henley Beach, SA).

The charcoal tubes were processed as described in Section 8.5. However, each sample was spiked with 0.5 ml of 10 ug/ml MITC, to avoid readings that were below the detection limit of the GC.

Results

Pilot study

MITC was detected in soil air samples collected from containers (Fig. 1). MITC could be detected above the spiked concentration up to 7 days after Basamid addition. Samples for containers where no Basamid had been added were below the spiked concentration.

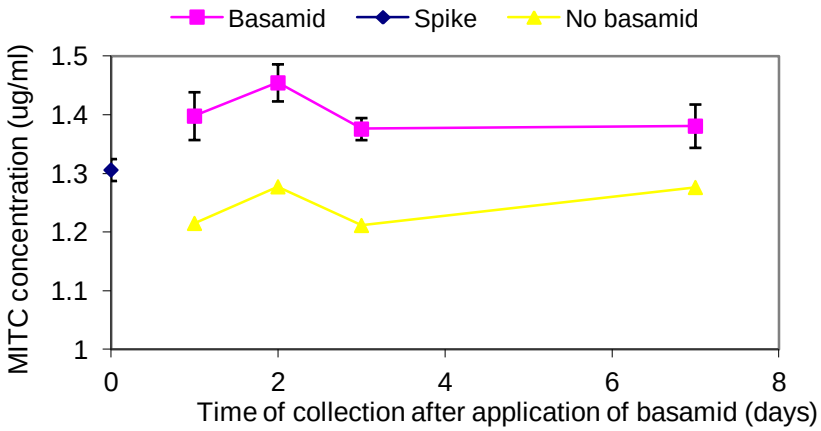


Figure 1. Concentration of MITC in soil air collected from containers in the pilot study. For Basamid treatments bars are mean + 1SE, n = 5.

Experiment 1

There was a decline in broomrape seed viability in Basamid treated soils as the soil water content increased (ANOVA, $p < 0.001$). Containers with a soil moisture level of 20% had very few viable seeds (Figure 2). Containers with soil moisture levels of 12.5% and 15% had lower viability than untreated seeds. There was low germination in all treatments, including controls. MITC in containers did not stimulate germination and germination was only observed after the addition of GR24 in the laboratory.

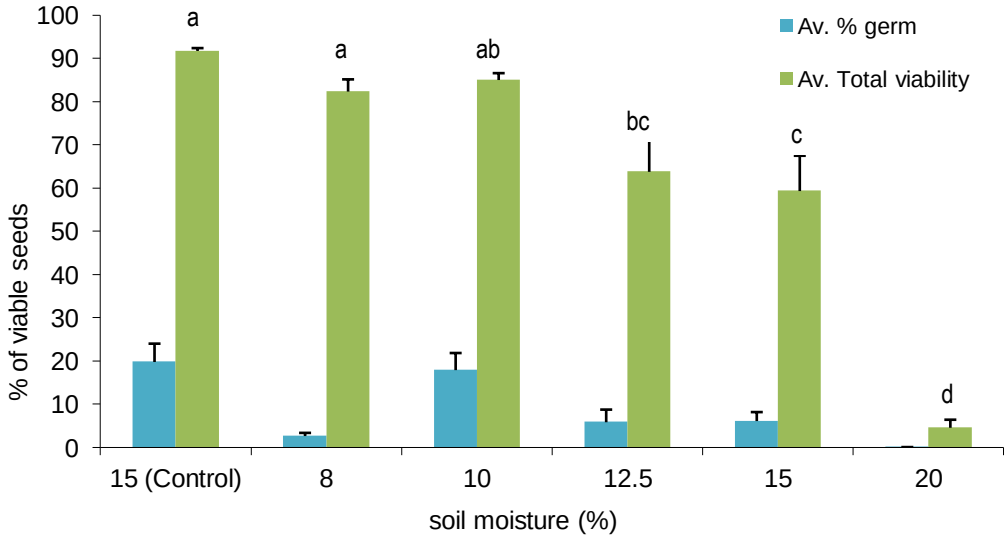


Figure 2. Viability (viability plus germination) and germination of broomrape seeds after exposure to the equivalent of 120 kg ha^{-1} Basamid in containers with different soil moisture content. Conditioning treatments have been combined, bars are means + 1 SE, n =20. Bars labeled with a different letter were significantly different at $\alpha < 0.05$, Tukey HSD tests.

There were not any discernible patterns in viability loss associated with pre-conditioning time before exposure to MITC in the containers (Fig. 3). Pre-conditioning period had no effect on the efficacy of MITC (ANOVA, $p = 0.589$).

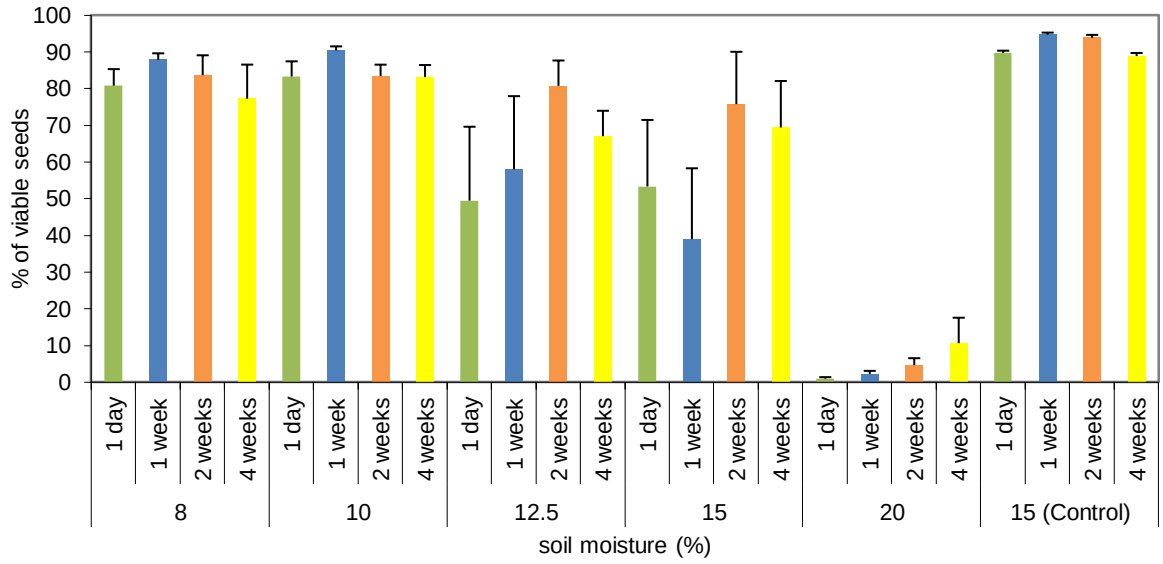


Figure 3. Viability (viability plus germination) of broomrape seeds conditioned for different lengths of time then after exposure to the equivalent of 120 kg ha^{-1} Basamid in containers with different soil moisture content. Bars are mean + 1SE, n = 5.

Experiment 2

The soil temperature in the containers averaged under 10 °C for the first 11 days of the experiment, frequently falling to a minimum of less than 5 °C during this period. Warmer temperatures occurred towards the end of the experiment (Fig. 4).

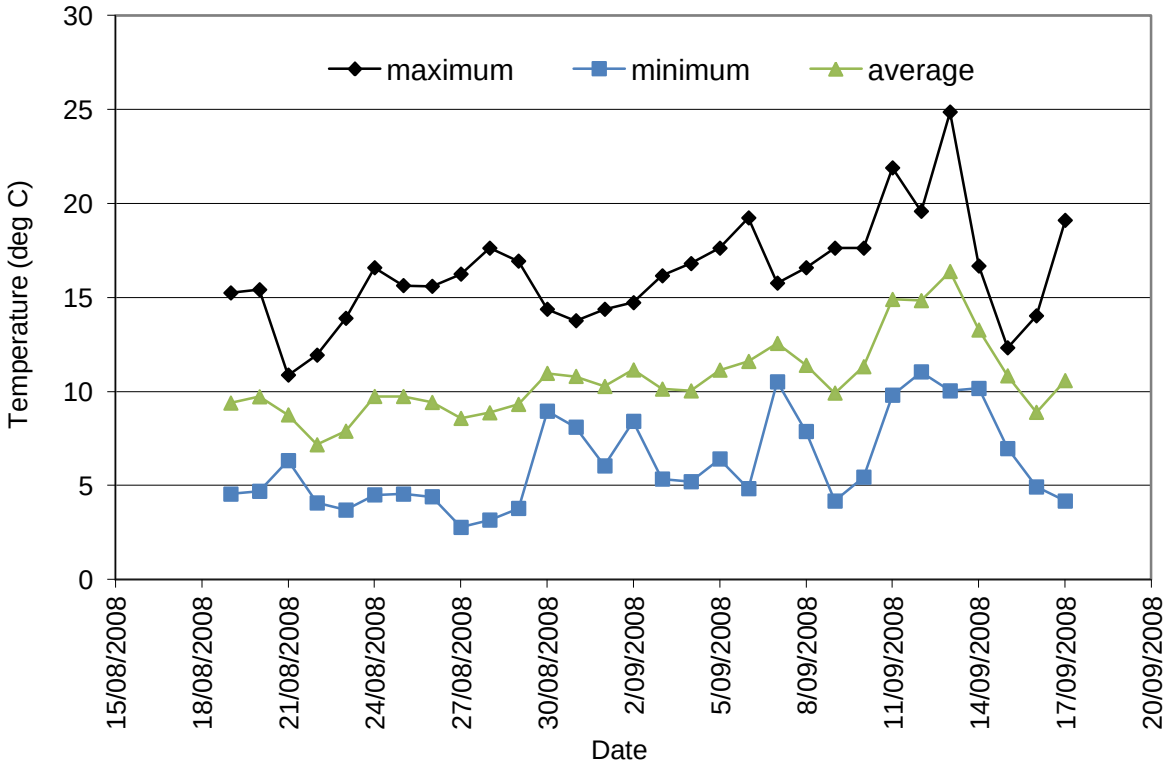


Figure 4. Daily soil temperatures measured in the untreated control container. The temperature was logged every 30 minutes.

The peak in MITC concentration occurred from 8-10 days after Basamid application. Higher concentrations were measured in containers with the highest soil water content and applications of the equivalent of 360 kg ha^{-1} Basamid (Fig. 5). MITC concentration in the driest soil treatment was similar to the concentration measured at the Basamid application rate of 120 kg ha^{-1} and the total concentration of MITC released (area under the curve) was similar. No MITC was measured in the 120 kg ha^{-1} treatment after 20 days but was still detectable in the 360 kg ha^{-1} treatments after 30 days, although the MITC concentration was very low in the wetter soil treatments.

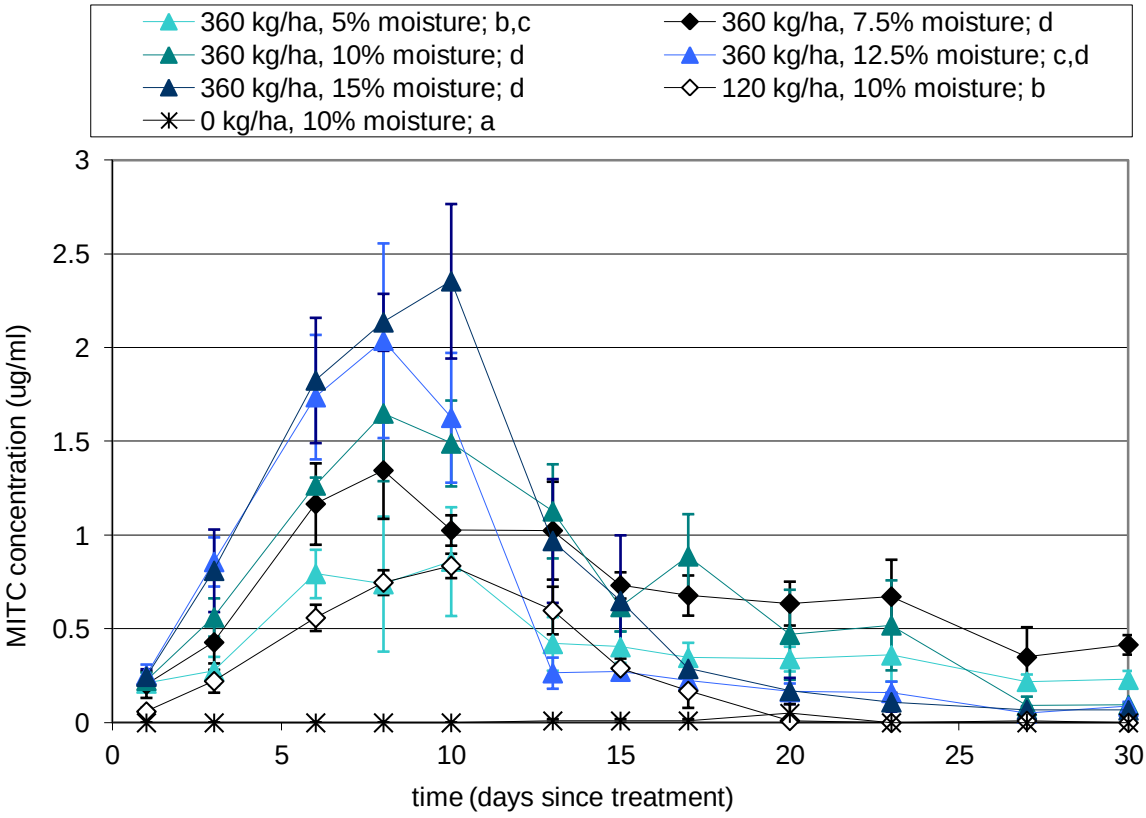


Figure 5. MITC concentration measured in soil air samples from containers of soil at different periods of time after the addition of Basamid. Points are mean ± 1SE, n =5. In the legend, different letters indicate significant differences between treatments for the calculated area under the curve at $\alpha < 0.05$ (Tukey HSD test).

Very few seeds remained viable in the Basamid treatments and there was no difference between soil moisture levels or Basamid rates although the results were more variable at the lower application rate (Fig. 6). There was no difference between conditioning periods prior to exposure to Basamid on seed survival ($p = 0.11$).

Discussion

The containers provided a satisfactory environment for the experiments. The pilot study demonstrated that soil air samples could be collected from the containers and MITC was detected in treated containers over the course of this study.

Seed viability results from the first experiment were disappointing, given that sachets remained in place for 30 days. However, this may have been the result of the method for adding water to the containers. Oven-dried soil could be water-repellent hence the water added to produce the soil moisture level treatments may not have effectively penetrated into the container to reach the layer of Basamid, which was at 8 cm

depth. Containers that received more water had some loss of viability, indicating that there was some MITC release but not enough to reach the concentrations required to be effective in killing a high proportion of broomrape seeds.

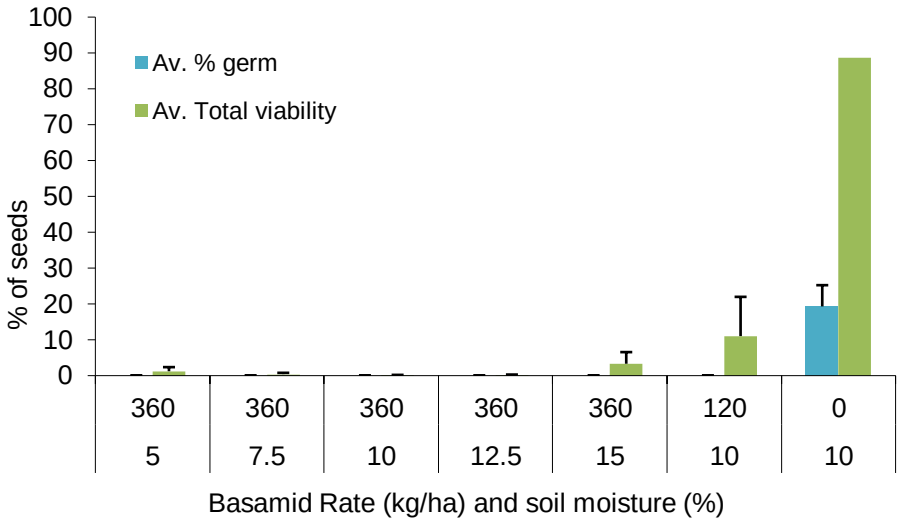


Figure 6. Viability (viability plus germination) and germination of broomrape seeds after exposure to the equivalent of 360 kg ha^{-1} or 120 kg ha^{-1} Basamid in containers with different soil moisture content. Conditioning treatments have been combined, bars are means + 1 SE, n = 20.

We found no evidence that MITC stimulated broomrape germination as has been found in laboratory experiments (Section 3.8). Low germination after the addition of GR24 in the laboratory was possibly a result of the onset of secondary dormancy in a high proportion of seeds. We found no difference in the response to MITC with pre-conditioning period in either experiment.

In the second experiment we demonstrated a relationship between MITC release and soil water content. In the treatments that received 360 kg ha^{-1} Basamid, MITC concentrations were higher in the wetter soils. However, the MITC concentration in all treatments was sufficient to destroy most broomrape seed. Applying a high concentration of Basamid to a dry soil was as effective as applying a lower concentration to a wetter soil.

It was expected that MITC release would occur immediately and then decline gradually over time. The delay in MITC release until 8-10 days after Basamid application was unexpected. However, the very cool temperatures during the early 11 days of the experiment could explain this delayed release. Temperatures below 10 °C slow the release of MITC (Swanson and May 2003). The optimum temperature range for Basamid application is 10 – 25 °C as MITC release is inhibited beyond this range. These cooler temperatures may also have influenced the residence time of MITC in the soil in containers, although the dissipation of soil from containers could occur at a slower rate than from the soil due to the restrictions of the container.

It may be possible to optimize a Basamid application rate to achieve maximum broomrape seed kill in dry soils whilst minimizing plant-back times. As the majority of seeds were killed in the driest soil moisture treatment (5%) could a rate lower than 360 kg ha^{-1} achieve the same results in dry soil? This was examined in another container experiment (see Section 8.10).

Reference

Swanson, B. and C. May. 2003. Manual for users of products containing metham sodium and dazomet. Department of Primary Industries, Melbourne.

10. Basamid container experiment: application rate in dry soil

Anna Williams, Andrew Craig, Darryl Miegel and Jane Prider

Branched Broomrape Eradication Program

2009

Aims

1. To calculate a concentration x time (ppm.hrs) measurement of MITC production when Basamid is applied at 120kg/ha, 180kg/ha, 240kg/ha, 300kg/ha and 360kg/ha to soil with low moisture content (5%).
2. To determine the lethal dose exposures for branched broomrape to MITC when Basamid is applied at 120kg/ha, 180kg/ha, 240kg/ha, 300kg/ha and 360kg/ha to soil with low moisture content (5%).

Background

The 2008 Basamid trial demonstrated that the reaction of basamid to MITC is dependent on soil moisture at depth (Section 8.6). Wetter soils generate a quicker reaction and dryer conditions extend the reaction time and subsequently extend the plant-back time for establishing a cover crop.

Application of Basamid at 360kg ha^{-1} was very effective at killing broomrape seeds (> 95% kill in all moisture treatments) even in low soil moisture conditions. However, MITC was still detectable in dryer soils (5% and 7.5% w/w soil moisture) after 30 days.

At 10% soil moisture MITC was not detectable after 20 days when basamid was applied at 120kg ha^{-1}, whereas at 360kg ha^{-1} MITC only became undetectable after 27 days.

Sustained soil moisture at depth during this reaction time is required to achieve these results. If soil moisture decreases during this period after application it would be expected that the reaction time would then increase, it would also be expected that the efficacy of the treatment would decrease.

Therefore, although >85% kill can be achieved when Basamid is applied at 120kg ha^{-1} to soil at 10% moisture this efficacy will drop if that moisture is not sustained.

Therefore, we need to determine the minimum rate of Basamid that can be applied in drier conditions and still achieve a high kill rate (> 95%) but minimise the reaction period (< 30 days).

Objective:

To determine the minimum rate of Basamid that can be applied in drier soil moisture conditions and still achieve a high kill rate (> 95%) of branched broomrape seeds but minimise the reaction period (< 30 days).

Methods

Field soil from the Mannum Trial Site was collected whilst it was dry and kept in plastic bags until required. Some field soil was oven dried at 75 °C for 48 h to create another treatment to test if the soil-drying treatment affected results. Containers were prepared as described for the previous Basamid container experiment (Section 8.6). Water was thoroughly mixed into the field soil to create a single soil water content of 0.05 g water g $soil^{-1}$.

Five replicates of the following Basamid treatments were applied:

- Control = 0 g Basamid
- 120kg ha^{-1} = 1.347 g of Basamid
- 180kg ha^{-1} = 2.020 g of Basamid
- 240kg ha^{-1} = 2.694 g of Basamid
- 300kg ha^{-1} = 3.367 g of Basamid
- 360kg ha^{-1} = 4.041 g of Basamid
- 120kg ha^{-1} = 1.347 g of Basamid to oven-dried soil

Soil air samples were collected onto activated charcoal at 2-3 days intervals up to 41 days after Basamid application using the method described in Section 8.6.

Soil temperature was logged using s sensor placed in a control treatment container. Soil water content was maintained by adding water to containers when their weight dropped below the starting container weight.

Soil air samples were processed by GCMS to determine MITC concentration by CSIRO.

Four sachets constructed from filter paper, containing broomrape seeds were placed in each container during filling, 5 cm below the surface. Seeds were conditioned by moistening the sachets and storing in a 20 °C incubator for 14 days before the experiment was set up.

Sachets were retrieved after 40 days and processed using the usual protocols; conditioned for 14 days at 20 °C, germination assessed 14 days following application of GR24, viability assessed 14 days following addition of tetrazolium solution. The data were pooled for the four sachets within a tub.

The experiment commenced on August 11th 2009.

Results

The soil temperature in containers over the course of the experiment fluctuated daily between 5 and 20 °C, with higher maximal temperatures at the end of the experiment (Fig. 1).

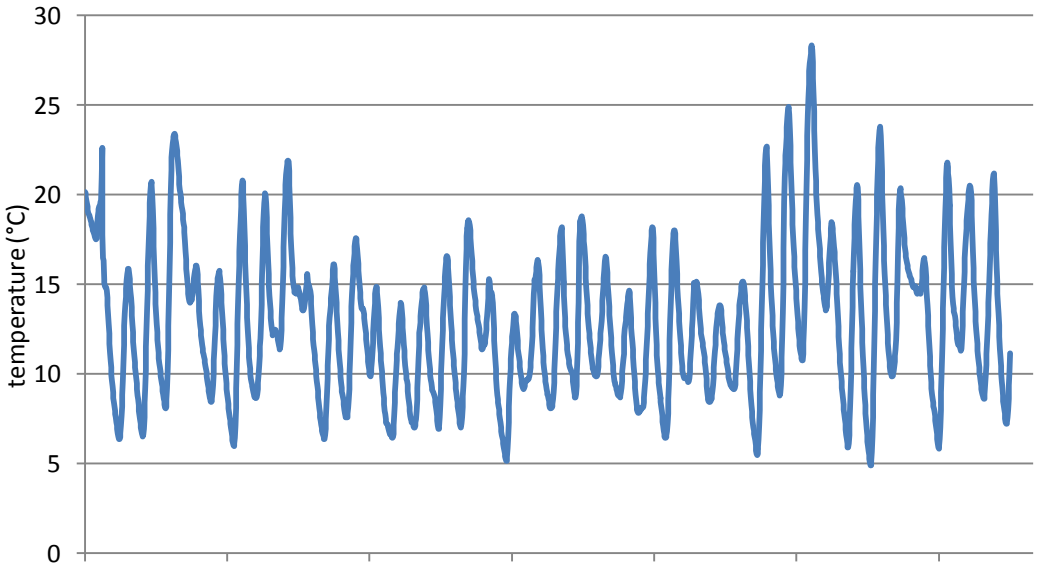


Figure 1. Daily fluctuation in soil temperature in containers over the course of the experiment. Temperature was logged at 30 minute intervals.

The oven-dried treatment had no effect on the results. MITC concentration and seed viability did not differ between oven-dried or air-dried treatments at the same Basamid application rate.

The highest concentrations of MITC were sampled in the day following Basamid application and the concentration declined gradually although not consistently over the following 41 days (Fig. 2). MITC was not detected at the lowest rate after 41 days but was detected in all other treatments at this sampling date (with the exception of untreated controls).

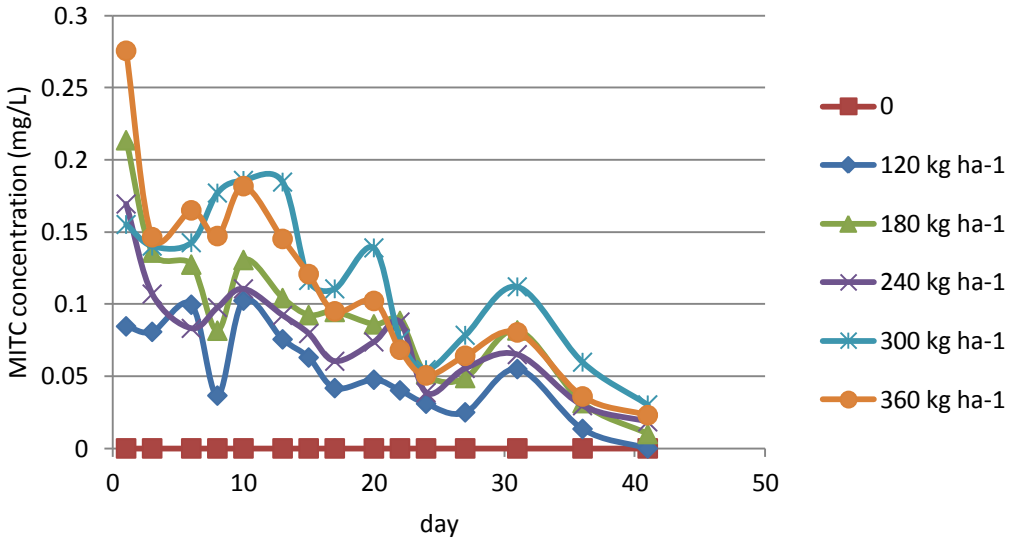


Figure 2. MITC concentration over time in containers of soil with different application rates of Basamid. Each point is mean, n = 5.

Less than 95% of broomrape seed remained viable in containers that received the equivalent of more than 120 kg ha^{-1} of Basamid. Survival of seeds at Basamid application rates of 120 kg ha^{-1} were higher ($p = 0.028$) but less than 10%.

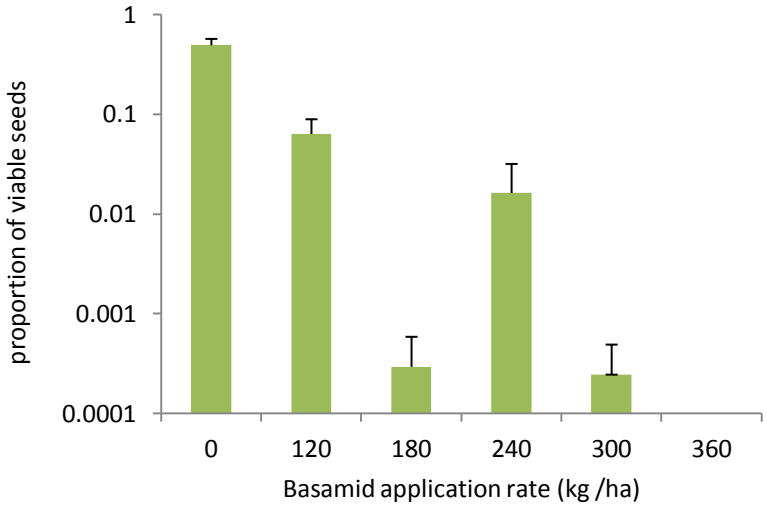


Figure 3. Proportion of viable broomrape seeds after exposure to Basamid treatments at different application rates. The y-axis is a logarithmic scale so that the very small values of the Basamid treatments can be seen. Bars are means + 1SE, n =5.

Discussion

The container experiment provides support that control of broomrape seeds is possible in relatively dry soils although MITC may persist at higher concentrations in a container so the efficacy may be over-estimated.

Lower concentrations of MITC were recorded in this experiment than the previous experiment (Section8.6) but were still sufficient to kill broomrape seeds. Small peaks in MITC production over the 41 day sampling period suggest that the release of MITC is not constant and given that soil moisture remained relatively constant this is probably related to changes in soil temperature.

MITC was present in containers at the higher Basamid application rates after 41 days. Plant back times in dry conditions are likely to be at least 40 days. However, MITC may persist for longer within the confines of the container than in a field situation.

This experiment demonstrated that in containers, rates of 180 kg ha^{-1} Basamid were as effective as a 360 kg ha^{-1} rate for broomrape seed destruction. Some survival of seed at the 240 kg ha^{-1} rate indicates that the even distribution of these small amounts of Basamid through the soil is difficult to achieve. Patchy application could allow seeds to survive in small pockets of soil. A higher application rate is therefore recommended to ensure that the Basamid is evenly distributed throughout the soil profile.

Although Basamid application should be applied when the soil profile is well-watered, this experiment has demonstrated that MITC release would occur as the soil profile dried out and even low concentrations are effective to kill seeds provided the exposure is over a long enough period.

11. Methyl iodide seed bank destruction field trial

Jane Prider, Andrew Craig and Anna Williams

Branched Broomrape Eradication Program

April 2010

Summary

Methyl bromide is currently used in the branched broomrape seed bank destruction program but its use is being phased out. In this field trial we evaluated methyl iodide + chloropicrin as a substitute fumigant for methyl bromide. We also compared it with the other seed bank destruction products currently in use; Basamid and pine oil. Methyl iodide + chloropicrin at both rates trialled, was comparable to methyl bromide and destroyed 97% or more of seeds in buried seed sachets. Basamid destroyed 95% of seeds in buried sachets whereas pine oil only reduced seed viability by 4% in comparison with controls. When methyl iodide is registered for use it will be a suitable substitute for methyl bromide for *O. ramosa* seed bank destruction. The application method is identical to methyl bromide. Of the other two seed bank destruction methods currently in use, Basamid achieves more consistent results than pine oil and should be used in preference to pine oil.

Introduction

The most common pre-emergence soil fumigants used for the control of broomrape are methyl bromide and formulations that release the active ingredient methyl isothiocyanate (metham sodium and dazomet) (Goldwasser *et al.* 1995; James 1976; Khalaf *et al.* 1994; Kleifeld *et al.* 1991). In general, these fumigants have been reported to be successful in preventing broomrape emergence, although often their efficacy in actually killing seeds under field conditions has not been tested. In addition, their efficacy is frequently inconsistent and has been reported to differ with physical properties such as soil type, soil moisture, temperature and with depth in the soil profile (Goldwasser *et al.* 1995; Kleifeld *et al.* 1991).

Destruction of the soil seed bank is an important component of the *O. ramosa* eradication program in South Australia, particularly for the prevention of the spread ofseed- infected soils. Several products for seed bank destruction have been the subject of field trials in South Australia (Matthews and Miegel 2004; Matthews *et al.* 2006; Williams *et al.* 2006). The eradication program routinely uses the fumigants, Basamid and methyl bromide, and a pine oil soil drench. The efficacy of these products is variable and this has been attributed to differences in soil type and soil moisture, and effectiveness of application. For all products, there is an 11-14% recurrence of broomrape in sites that have been treated (N. Secomb, unpublished data). As methyl bromide is likely to be phased out of production due to its ozone-depleting action, an alternative fumigant is required. Methyl iodide has been found to be as equally effective as methyl bromide for the control of plant fungal pathogens, nematodes and propagules of several weed species (Becker *et al.* 1998; Ohr *et al.* 1996; Zhang *et al.* 1998; Zhang *et al.* 1997). The aim of this study was to compare the effectiveness of methyl iodide in reducing the viability of *O. ramosa* seed with the products currently in use.

Materials and methods

The experiment was conducted at a single field site at Brinkley. The site is on the crest to southern aspect of a low east-west trending sand dune on a sandy loam with pH 6.9 and organic carbon content 0.78 mg kg^{-1}. The site has previously been used for cereal cropping. For the period of the experiment the mean

maximum soil temperature was 12.5 ºC and the mean minimum temperature was 7.7 ºC. 51 mm of rainfall occurred in the two months preceding the application of the first fumigants (2 April to 1 June 2009) and 31 mm rainfall occurred during the trial (2 June to 10 July 2009).

O. ramosa seed was collected from Mannum in 2006 and stored in the laboratory until use. To assess the effects of the products on the *O. ramosa* soil seed bank, we buried seed in sachets that could be later retrieved. Two different sachet types were trialled as the penetration of the product through the sachet materials was unknown. Sachets, 4 cm by 7.5 cm, were constructed from 100 µm nylon mesh (Tarpee PE) or glass fibre filter paper. A tag attached to the sachet with nylon string enabled relocation of buried sachets. Seed was surface sterilised in 1% NaOCl and rinsed in RO water. Approximately 300 *O. ramosa* seeds mixed with 5 ml of sieved, sterilised sand sourced from the region were added to each sachet. The inclusion of sand in the sachets facilitates contact between the seed and the treatment product held in the sand.

The experiment was set out as a randomised block design, with seed bank destruction treatments randomly assigned to plots within five blocks. Each plot was 25 m by 8 m with a 2 m gap between each plot and each block. The five treatments were replicated once in each block and included Basamid™ (active ingredient, methyl isothiocyanate), pine oil (Interceptor™), methyl bromide with chloropicrin (ratio 98:2), and methyl iodide with chloropicrin (ratio 50:50) at two rates.

Basamid was applied at a rate of 360 kg ha^{-1} with an air seeder with Morris tynes. Specialised seeding boots equipped with spreader plates dispersed the fumigant powder at the required depth. The plots were rolled with a ribbed roller after treatment to provide a surface seal. A 5% concentration of pine oil was sprayed onto the soil surface at the rate of 20,000 L ha^{-1}. Immediately after Basamid application, five nylon seed sachets and five filter paper seed sachets were buried at 5 - 10 cm depth at regularly spaced intervals within each treatment plot. Sets of sachets were also buried in control plots at the same time. Pine oil sachets were buried before pine oil was applied. Methyl bromide and methyl iodide treatments were applied a week later by a commercial applicator (R & R Fumigation) using a gas-pressurised tyne injection method. The plots were cultivated to a fine tilth and levelled beforehand. Methyl bromide was applied at a rate of 500 kg ha^{-1} and methyl iodide + chloropicrin at 500 kg ha^{-1} and 350 kg ha^{-1}. The plots were immediately covered in polyethylene sheeting. Small holes were cut in the polyethylene for the insertion and burial of the seed sachets and later resealed with cloth tape. A second set of sachets was buried in control plots at this time. Sachet tags were secured in place with wire after it was found that some sachets were being dug up by foxes.

Seed sachets were retrieved after one month of burial. Seeds were separated from the sand in the sachets by floating off in 40% w/w calcium chloride solution. Seeds were then briefly soaked in 1% NaOCl for 5 minutes and rinsed in RO water. This treatment surface sterilises seed and bleaches the seed coat to enable observation of the embryo within the seed. Seeds were placed on well-moistened glass fibre filter paper discs in petri dishes. The dishes were sealed and incubated in the dark at 20 ºC for two weeks to condition seeds. The synthetic germination stimulant GR 24 was then added to each disc, the petri dishes resealed, and the seeds incubated at 20 ºC for a further two weeks to promote germination. Seeds were counted as germinated if the radicle could be observed penetrating beyond the seed coat. To determine if seeds that had not germinated were still viable, they were placed in small eppendorf tubes containing 200 µl of 1 % 2,3,5-triphenyltetrazolium chloride solution (tetrazolium salt) and incubated in the dark for two weeks at 30 ºC. Seeds were scored viable if the embryo was stained either pink or red and unviable seeds remained unstained.

Analysis

We used the counts of germination and germination plus viability (viability) as a proportion of the total number of seeds retrieved as variables in the analysis. We fitted Generalised Linear Models with a binomial family and a logit link function to the data with the factors block, sachet type and treatment. We initially compared the two controls (that were buried at different times) and as they were significantly different we divided the analysis into two groups according to when treatments were applied. Hence

Basamid and pine oil treatments were assessed independently of methyl iodide and methyl bromide treatments. In most cases, we analysed fumigant or soil drench treatments in isolation from the controls, given the large difference between these treatments and controls. We used an iterative fitting procedure, including all factors and their interactions in the initial model and removing them sequentially. We used analysis of deviance with chi-square tests to compare the fits of each subsequent model.

Results

Viability

In all the analyses there were significant differences between blocks but these were not consistent between treatments or between sachet types. This was probably a result of inherent variation in either the application of the products or differences in soil characteristics across the site. The blocking factor is therefore a "nuisance" variable and ignored in the analysis results. The factors of importance are therefore treatment and sachet type. All statistical results are included in Appendix A.

There were significant differences in seed viability between the controls that were buried one week apart. Seed viability in the first controls (Control A) was higher than in the second controls (Fig. 1). Consequently, where applicable, further analyses have been split for comparison with the appropriate controls.

Basamid, methyl bromide, and methyl iodide + chloropicrin at both application rates reduced the proportion of viable seeds in comparison to controls (Fig. 1). Less than 5% of seeds remained viable in any of these treatments. The efficacy of Basamid was more variable but it provided similar levels of control as the methyl fumigants. Pine oil had a very small effect on seed viability (approx 4% reduction, Fig. 1).

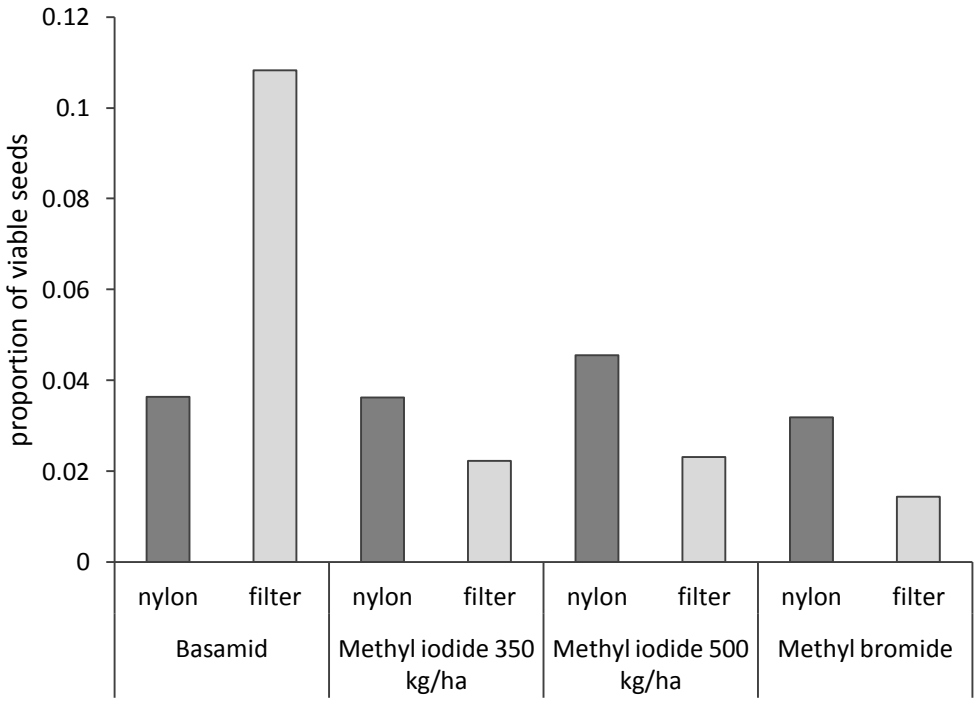


Figure 1. Proportion of *O. ramosa* seeds viable after treatment with soil fumigants and soil drench. MI high, methyl iodide + chloropicrin 500 kgha^{-1}, MI low, methyl iodide + chloropicrin 350 kg ha^{-1}, MB, methyl bromide. These treatments were compared with Control B. Pine oil and Basamid were compared with Control A.

Sachet type had significant effects on seed viability but there were differences in controls as well as fumigant or soil drench treatments. With the exception of Basamid treatments, seed viability was higher in seeds retrieved from nylon sachets than those retrieved from filter paper sachets. The difference between sachet materials was most marked for Basamid treatments (Fig. 2).

For the methyl bromide and methyl iodide + chloropicrin treatments there was an interaction between treatment and sachet type. Lowest viability occurred in seeds from filter paper sachets in methyl bromide plots. Viability was significantly lower in these sachets than in nylon sachets in the methyl iodide + chloropicrin 500 kg ha^{-1} plots (Fig. 2). As there was a loss of many of the filter paper sachets which could affect the analysis, the analysis was rerun on the nylon sachets alone. There was a statistically significant difference between treatments but the measured difference in seed viability was very minor and not likely to be significant in terms of effectiveness. Methyl bromide reduced overall seed viability to 2.4%, methyl iodide + chloropicrin 350 kg ha^{-1} to 2.8% and at 500 kg ha^{-1} to 3.1%.

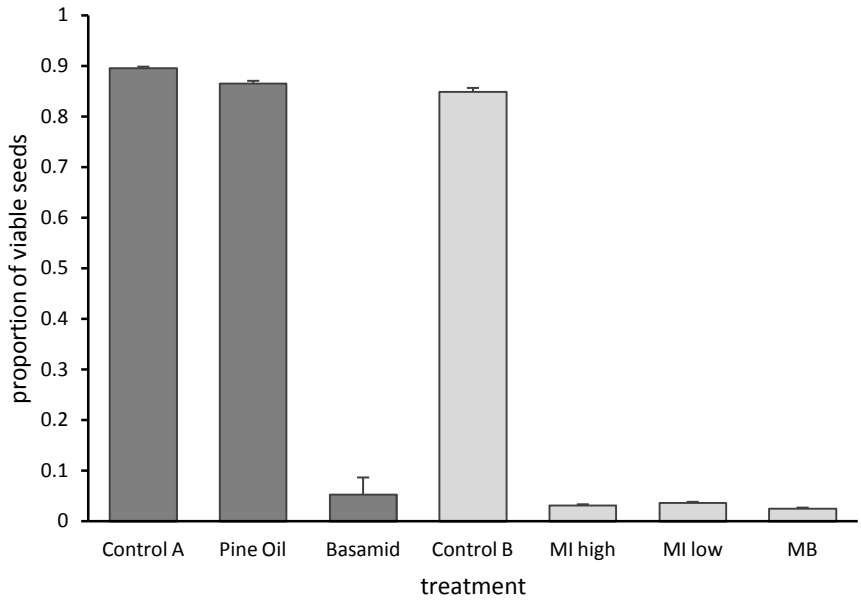


Figure 2. Difference in proportion of viable seeds retrieved from two sachet types after treatment with fumigants (Pine oil data not shown).

Germination

With the exception of pine oil and controls, only a very small percentage of seeds germinated. More seeds germinated from nylon sachets than filter paper sachets, apart from in Basamid treatments where germination was higher in filter paper sachets (Fig.3). Germination was reduced in pine oil treatments compared to controls but only in the filter paper sachets (Fig. 4).

Germination was significantly higher in methyl iodide + chloropicrin 350 kg ha-1 treatments than at the higher methyl iodide + chloropicrin rate or in the methyl bromide treatment. However, the germination proportions were extremely low at less than 0.05% (Fig. 3).

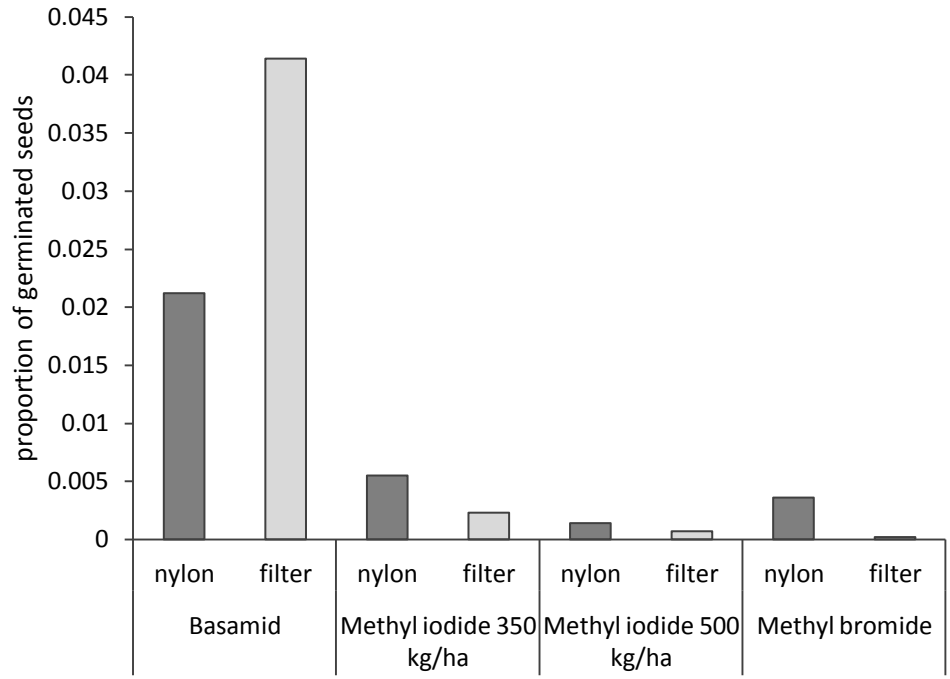


Figure 3. Germination proportion of seeds retrieved from nylon or filter paper sachets after treatment with fumigants.

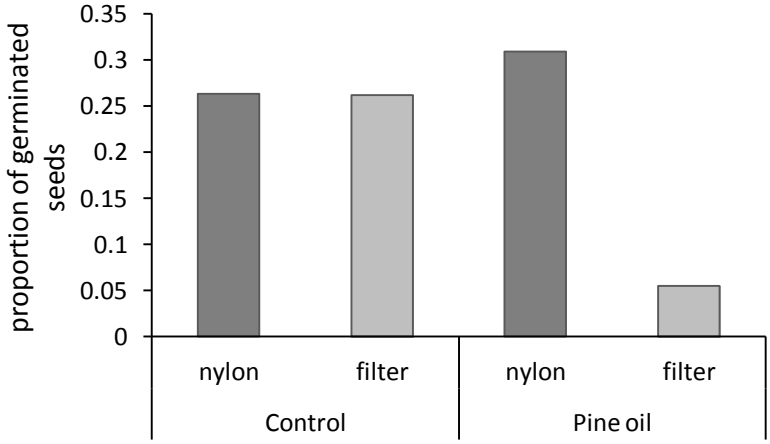


Figure 4. Germination proportion of seeds retrieved from nylon or filter paper sachets in controls or after treatment with pine oil.

Discussion

Methyl iodide + chloropicrin and methyl bromide provide effective control of *O. ramosa* seeds when applied under field trial conditions. There was a reduction in the viable seed bank up to approximately 97% of controls after the application of both fumigants. In this trial, these fumigants gave more consistent results than Basamid, which although almost equally effective with a seed viability reduction of 95%, had slightly more variation. The poor effectiveness of pine oil was surprising as the soil drench had demonstrated higher levels of seed destruction in other field trials (Matthews & Miegel 2004, Matthews 2009), although results have not been consistent. As pine oil is a lipophilic product, its movement down

the soil profile may have considerable effects on its activity on the seed bank. Pine oil is more effective at shallow depths and the addition of extra water post-application can improve effectiveness by distributing the pine oil deeper into the soil profile (Matthews 2006, 2009). Our seed sachets may have been buried at depths beyond the penetration depth of the pine oil.

Basamid efficacy in this trial was consistent with previous pot trials and more effective than other field trials (Williams *et al.* 2006). Early field trials used low rates of Basamid, where it may be difficult to achieve a uniform distribution of the product through the soil profile. Methods for quantifying seed viability have also improved over the course of these trials and this may partly explain some of the differences in the results. Broomrape seeds are difficult to work with quantitatively due to their small size and patchy distribution in the soil profile. Enclosing the seeds within sachets facilitates their retrieval and assessment of viability. We were concerned that the sachet material may interfere with the activity of the fumigants or soil drench and this was found to be the case. There were differences between sachets in all treatments and in one set of controls. However, even given these differences the fumigants were still effective in destroying seeds.

Of the seed destruction products currently in use, methyl bromide gave the best control of *O. ramosa* seeds. Methyl iodide + chloropicrin was equally effective and its efficacy did not differ between the two application rates trialled, 350 kg ha^{-1} and 500 kg ha^{-1}. The advantage of this fumigant mixture is that it is less detrimental from a human health and environmental perspective. It is therefore a viable alternative to methyl bromide in economically important control situations such as high-value crops or satellite infestations of the parasite. The commercial cost of application of methyl iodide is greater than methyl bromide; methyl iodide + chloropicrin at 350 kg ha^{-1} is 20% more than the cost of methyl bromide at 500 kg ha^{-1} (see Appendix B). There were no problems or issues encountered during application. The gas-pressurised tyne injection method is identical to methyl bromide application which has been routinely applied in the Quarantine Area. Unfortunately, methyl iodide is not currently registered for use in Australia under the APVMA Act.

Methyl iodide and chloropicrin mixtures are routinely used to increase the spectrum of pests that are controlled. Without further trials, it cannot be assumed that formulations with different proportions of chloropicrin will be equally effective at destroying *O. ramosa* seeds. Both methyl iodide and chloropicrin are believed to have the same mode of action but chloropicrin is less effective for the control of weeds (Hutchinson *et al.* 2004). However, this is probably species-specific with effective control depending on the hardness of the seed coat (Haar *et al.* 2003) or the moisture status of the propagule (Hutchinson *et al.* 2004). In addition, fumigant mixtures may have synergistic effects. Hutchinson et al (2004) found that methyl bromide was as effective as methyl iodide in controlling *Cyperus esculentus* but only with the addition of chloropicrin.

Recommendations

Methyl iodide + chloropicrin (50:50) is a suitable substitute for methyl bromide for the destruction of *O. ramosa* seed in the soil seed bank but is not yet registered for use. The low rate (350 kg ha^{-1}) is as effective as the higher rate (500 kg ha^{-1}).

Of the other two seed destruction treatments currently in use, Basamid is more effective than pine oil. Given the high cost of pine oil and the inconsistent results for seed bank destruction, Basamid is the preferred product.

There is still a requirement for an effective *O. ramosa* seedbank destruction product that is non-toxic and can safely be used in sensitive areas where there is a health risk to humans and livestock or a risk of environmental contamination.

Nylon sachets are more robust for field work but it should be recognised that the sachet material can either impede or facilitate the movement or absorbance of gases or liquids. Sachet material should be tested prior to any large field trial of a new seed destruction product.

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12. Ethanedinitrile *in vitro* trials

Che DeDear and John Virtue

Animal and Plant Control Commission

May 2002

Aim

To determine the toxicity of ethanednitrile (cyanogen) on broomrape seed

Method

This is what Che said he did

Three concentrations of ethanedinitrile were trialled: 50 mg, 100 mg and 150 mg L-1. These concentrations of gas were injected into bottles containing broomrape seeds or pots with soil. Seeds and media comprised both wet and dry treatments. The four treatments were:

- Dry seed in 10 ml bottle
- Dry seed in pot of 0.5 L dry soil
- Wet seeds in 150 ml bottle
- Wet seed in pot of 0.5 L wet soil

Each treatment was replicated. Seeds were exposed to the fumigant for 24 hours and then aerated for a further 24 hours. There was also a set of wet and dry controls.

Following treatment seeds were placed on filter paper discs and conditioned before 5 ppm GR24 was added. Seeds were scored for germination. Ethanedinitrile was applied by Dr Yonglin Ren and Dr M. Sarwar (CSIRO Entomology).

This is in Ren's report

Glass Petri dishes containing broomrape seeds (>1000) were placed at 3 levels of depth in a pot (2.5 L capacity) filled with dry or moist sandy soil. EDN was injected into the soil from bottom of pot at the rate of 25, 50 and 100mg/L. After 24 hours fumigation, the soil was aired and the broomrape seeds were collected for assessment of germination.

The broomrape seeds were 100% controlled at 25mg/L of C_2N_2 in moist soil. However, C_2N_2 could not kill the broomrape seeds in dry soil even at 100mg/L, indicating the benefit of moistened soil.

Results

These are the actual results that do not fit exactly with either design! Concentrations are Che's but seem to be all in pots as per Ren's report

Cannot find the raw data so do not know what the counts were – what is 100% germination? – we have never recorded this.

dose		pot/vial	depths		
			2cm	6cm	10cm
50mg/L	Wet	R1	0	0	0
		R2	0	0	0
100mg/L	Wet	R1	0	0	0
		R2	0	0	0
150mg/L	Wet	R1	0	0	0
		R2	0	0	0
Control	Wet	R1	100	100	100
		R2	100	100	100
50mg/L	Dry	R1	100	100	100
		R2	100	100	100
100mg/L	Dry	R1	100	100	100
		R2	100	100	100
150mg/L	Dry	R1	100	100	100
		R2	100	100	100
Control	Dry	R1	100	100	100
		R2	100	100	100
Figures above represent percentage of germination possible					
Moisture levels in soil		Before	After	moisture g	
Dry		341.27	340.7	0.57	
Dry		300.58	300.2	0.38	
Dry		501.4	500.79	0.61	
Wet		613.2	575.18	38.02	
Wet		431.38	409.25	22.13	
Wet		557.51	535.14	22.37	

13. Results from an application of ethanedinitrile (C2N2) for broomrape (*Orobanche* spp.) control

YongLin Ren and M Sarwar

CSIRO Entomology

June 2004

Aim

Update on progress of the second field trial of C_2N_2 in a commercial agricultural setting, with comments from above people.

Introduction

Broomrapes, *Orobanche* species, are parasitic weeds of broadleaf plants. Two species of broomrape are currently found in Australia: branched broomrape, *O. ramosa*, and clover broomrape, *O. minor*. Broomrape is a very serious weed pest in terms of both production losses and threats to export markets. The main industries at risk are pulses, oilseeds and vegetables and vetch, particular the seed industry for these crops. The indirect impact on the cereal seed industry could also be devastating if broomrape seeds were found in export produce due to the broomrape growing on broadleaf weeds within cereal crops. Broomrape seed is spread by livestock – through the gut or on their feet – farm machinery via soil, contaminated fodder and seed, contaminated soil and sand, flood water and wind dispersal especially in a sand dune blow-out.

Fumigation with methyl bromide has an efficacy of close to 100% in destroying branched broomrape at very high dose (500-1000 kg/ha and covered with plastic). However, there is only one season left to allow the use of methyl bromide for soil treatment until January 2005 (methyl bromide phase out).

Primary Industries Standing Committee held a meeting in Sydney regarding "Progress of the branched broomrape eradication program" (meeting No. 6, Item OOS 13), recently. The Committee has indicated that - Alternative for methyl bromide are being investigated. A replacement (ethanedinitrile, C_2N_2) for methyl bromide that is acceptable to both conventional and organic producers appears promising and will be escalated to field trial testing this year.

Trial design

A commercial scale field trial on application of C_2N_2 for broomrape control (sandy soil) was conducted in Mannum, SA on 15-16 July 2003 (see Section 8.2 for trial design). The trial was conducted in collaboration with the SA DPI (Dr John Virtue) and K & B Adam (fumigator). The trial was designed to determine:

- Control of broomrapes
- Control of weeds
- OH&S during application
- Any interaction between fumigant and wheat (phytotoxicity)
- The movement of C_2N_2 in soil

- The residues of C_2N_2 and HCN in soil

Results

1. During application the levels of C_2N_2 in the environment were 0.1-0.5ppm (near by plastic covered plots) and 0.5-2ppm (near by without plastic covered plots), much lower than the TLV of 10ppm, and have no HCN in the air.
2. C_2N_2 penetrated the soil very quickly (<5 min after application, C_2N_2 was evenly distributed through the soil from the surface to a depth of 25cm).
3. C_2N_2 and HCN residues in soil were 75-120ppm and 1-5ppm in plastic covered plots, and 45-85ppm and 0.5-3ppm in without plastic covered plots respectively, 20 hours post application. Two days post, both C_2N_2 and HCN residues had declined to indistinguishable levels.
4. Broomrape and weeds were well controlled by C_2N_2 at 25 g/m^2 in plastic covered plots, similar to results achieved C_2N_2 at 50 g/m^2 in without plastic covered plots and methyl bromide at 50 g/m^2 in plastic covered plots.
5. Telone-35 and sprayed herbicide show herbicide/fumigant residual phytotoxictity. After 4 weeks treatment, planted wheat almost killed and 2-3 months post treatment broomrapes and other weeds were grown when soil was wetted (Fig. 3).

The phytotoxicity studies were also conducted in green house scale trial. The results indicate plant-back could be as short as two days. It would be a significant strength if realised in commercial use (Fig. 2).

Figure 1. Dr M Sarwar (CSIRO Entomology); taking gas samples.

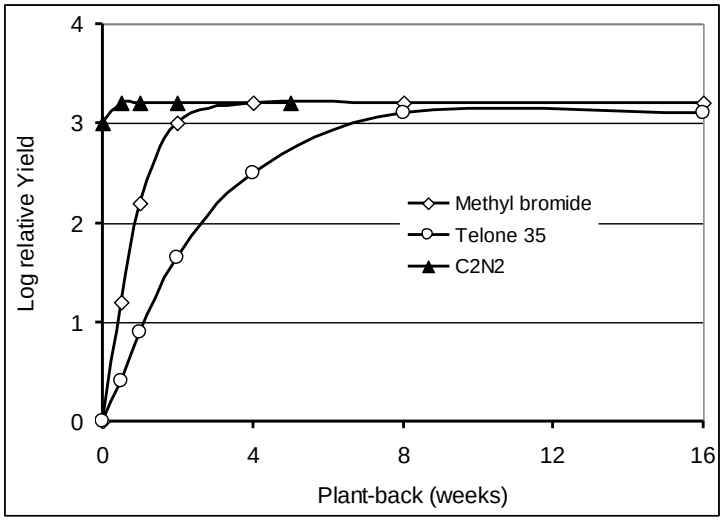

Figure 2. Relationship between plant-back and yield of strawberry fruit

Untreated plot, planted wheat

C_2N_2 25g/m^2 with plastic covered plots

C_2N_2 50g/m^2 without plastic covered plots

Methyl bromide 50g/m^2 with plastic covered plots

Telone-35 50g/m^2 with plastic covered plots

Sprayed herbicide

Figure 3. Wheat growth in treated plots, planted 4 weeks after fumigant or herbicide treatment

14. Ethylene as a germination stimulant

Andrew Craig and Jane Prider

Branched Broomrape Eradication Program

December 2011

Introduction

Ethylene has been shown to stimulate germination of *Striga hermonthica* seeds (Logan and Stewart 1991) . Injected into the ground, ethylene has stimulated *S.hermonthica* seed germination approximately 1 metre away from the injection site (Eplee 1975).

It is unclear if the same would be seen in *Orobanche ramosa* ssp. *mutelii* , with one laboratory based study showing ethylene had no effect on *O. ramosa* germination (Zehhar et al. 2002) while a reduction in *O. ramosa* emergence was observed in a tomato field after ethylene treatment (Chun et al. 1979). However, it has been shown that *O.crenata* (Kasasian 1973)and *O. aegyptiaca* (Edwards et al. 1976) germination cannot be induced by ethylene. Therefore, it would seem that the effect of ethylene on *Orobanche* germination differs amongst species.

Aims

This study examined the effect of differing concentrations of ethylene on the germination of *O.ramosa* ssp. *mutelii* seeds. Ethylene was supplied in the form of Ethephon, which decomposes to ethylene gas in the presence of water at a pH of more than 5. The study was done under optimal conditions in the laboratory for the germination of *O. ramosa.*

The outcomes will establish if ethylene can be used as an effective seed bank reduction agent through the suicidal germination of *O.ramosa* ssp. *mutelii* seeds.

Method

Experimental design

For broomrape seed to germinate, it needs to be conditioned for two weeks, during which the seed imbibes water, then a germination stimulant is added and germination occurs over a further two weeks. For any non germinated seed, viability is checked with the addition of Tetrazolium salts.

In this study we used two different seed lots, D 2006 and W 2008 seed, with each treatment consisting of 5 replicates of between 100 to 400 seeds. 6 different concentrations of ethephon were used; 0.01, 0.1, 1, 10, 100, 1000 mM. Control seeds were also set up with the negative control using RO water for the stimulant and the known *Orobanche* stimulant GR24 used for the positive control.

Seeds were placed on 22 mm filter paper discs in 50 mm x 9 mm Petri plates, 200 µl RO H_20 was added and the plates sealed with parafilm and seeds were conditioned for 14 days at 20ºC.

After the conditioning period, the seeds were air dried and the treatment was added, 200 µl of ethephon solution or control. The plates were resealed and incubated for a further two weeks at 20ºC.

Germination was recorded as the presence (germinated) or absence (non germinated) of a protruding radicle from the seed.

In order to check the viability of the treated seed, the non germinated seeds of all treatments were immersed in a 1% tetrazolium salt (2:3:5 triphenyltetrazolium chloride) solution for fourteen days at 30ºC.

Respiring tissue reduces the colourless tetrazolium salt solution to a red formazen compound, leaving viable seeds stained red.

The experiment was repeated using the W 2008 seed lot and a different seed lot, collected from Brinkley in 2010. In this experiment, the pH was adjusted to 7 with the addition of sodium hydroxide to the ethephon solution. This step had been omitted from the previous experiment.

Statistical analysis

The effect of seed lot and Ethephon concentration on seed viability was tested with a two-way Analysis of Variance (ANOVA). Where there were significant Ethephon concentration effects the difference between treatment levels were tested with Tukey HSD tests.

Results and discussion

Germination of D 2006 or W 2008 seed was not seen at any concentration of Ethephon during the study. In contrast, positive controls resulted in germination rates of 90%-92% for the two seed lots when stimulated with GR24.This shows *Orobanche ramosa* ssp. *mutelii* seed is not stimulated to germinate by ethylene released from Ethephon degradation. This is in contrast to the findings of Zehhar *et al.* (2002). They found that although exogenous ethylene failed to induce *O. ramosa* germination, ethephon concentrations of from 250 – 1000 mg L-1 stimulated germination in a concentration-dependent response.

Viability counts indicated that seeds had diminishing viability as the concentration of the ethephon solution increased (ANOVA, $F = 18.41$, $p < 0.001$). This occurred to the extent that there were no viable seeds in concentrations of 100 mM or higher (Figure 1). Ethephon concentration of 10 mM showed a significant decrease in viability when compared to those in the 0.001 mM to 1 mM range.

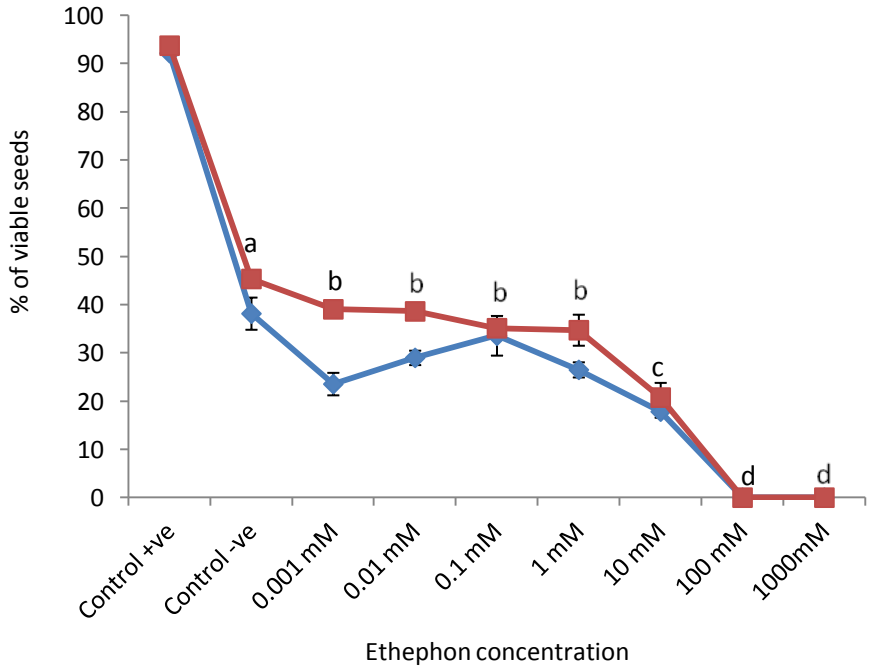

Figure 1. Viable seeds after treatment with differing concentrations of Ethephon. Each point represents the Mean ± 1SE, n = 5. Points labelled with different letters were significantly different, seed lots combined (Tukey HSD test). The different lines represent two seed lots (blue, D 2006; red, W 2008).

While ethephon failed to induce germination in either seed lot, the effect seen was the decline of seed viability with increasing ethephon concentration. This would suggest that Ethephon is a potential candidate to use for the reduction of the *Orobanche ramosa* ssp. *mutelii* seed bank. Further studies would confirm this potential. As the pH was not checked in the first experiment, and ethephon is very acidic, the pH may have resulted in seed death. Low pH can affect the germination of other *Orobanche* species (van Hezewijk et al. 1994) but the effects on viability are not known. The experiment was repeated with adjusted pH. In this experiment no germination was observed in any of the ethephon treatments. There was a loss of viability in the ethephon treatments of 10 ppm or higher but this was only in one seed lot (Brinkley) (Fig. 2). The W 2008 seed lot that was tested previously did not have a decrease in seed viability in the ethephon treatments.

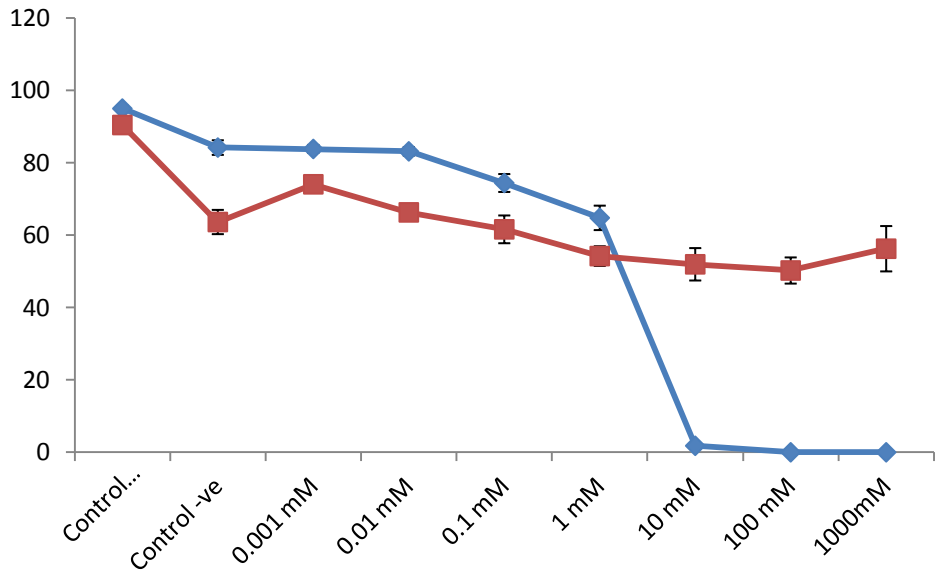

Figure 2. Viable seeds after treatment with differing concentrations of Ethephon. Each point represents the Mean ± 1SE, n = 5. The different lines represent two seed lots (blue, Brinkley 2006; red, W 2008).

Conclusions

Ethephon did not stimulate germination of *Orobanche ramosa* ssp. *mutelii* at concentrations in the range of 0.001 mM to 1 M. The effect of ethephon on seed viability differed between seed lots, reducing its applicability for the control of *O. ramosa* subsp. *mutelii* seed banks.

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