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# Compendium of branched broomrape research

## Section 10. Seed mortality – temperature

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

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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 17 January 2014



## Disclaimer

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# 1. Microwave destruction of seeds in soil from the quarantine area

*Australian Microwave Technologies*

*University of Wollongong*

*February 2002*

## Introduction

Following discussions between Mr John Mathews of Adelaide University and Mr. David McLean of AMT, it was agreed that, AMT should submit a proposal for the staged development of a microwave process that will allow for the continuous sterilisation of broomrape weed seeds with a target kill rate of 100%.

Seed sterilization using microwaves is technical feasible provided seed temperatures can be raised to lethal levels (Vela 1984, Davis et al 1973). The practical application of such technology has in the past been considered impractical due to the severe attenuation of microwaves in soil, ineffective selective heating mechanisms and the high cost of capital and energy (Nelson, 1996). However, the unique situation surrounding that of the branched broomrape seed that since the seed is located in dry sandy soil, a situation exists where microwave attenuation in the soil is significantly reduced and the high cost of chemical treatment methods makes this investigation justifiable.

It is the goal of this report to establish the preferred processing method from both an economic and practical point of view so that a suitable microwave applicator system can be developed that will allow a series of trials to be undertaken with branched broomrape seeds.

It is the conclusion of this study that the most economic and practical method is that of temporary soil removal using some form of feedback control system to ensure lethal temperatures. A series of trials have been undertaken to verify the seed lethal temperatures and power densities required for seed similar to broomrape i.e. ryegrass, mustard and *Orobanche australis* and *O. minor.*

## Methods

The test equipment used to conduct trials on the test samples provided by Adelaide University is shown below. The apparatus is a commercial microwave oven that has been modified to allow continuous variable microwave power up to 1.2kW and has open waveguide chokes both on the top and bottom cavity walls (Fig. 1). The purpose of these trials is to confirm both the seeds lethal microwave temperature and any associated physical conditions deemed important.

**Figure 1. The 2.45GHz Microwave Test Apparatus.**

The soil samples were received as cyclinders approximately 15mm diameter and 30mm height, held in place by a cloth boundary.Each sample was weighed and its temperature measured. The sample was then placed in the cavity.

The output power levels from the generator were approximately set as indicated below;

- High power = 1,000 watts
- Medium power = 850 watts
- Low power = 200 watts

These powers do not correspond to the net power absorbed by the soil, they represent the total power delivered to the oven. This delivered power is then distributed amongst the following list:

- Power absorbed by the soil causing heating and water vapourisation
- reflected power due to impedance mismatching
- power absorbed by the wave guide and applicator walls
- heating of the crucible and surrounding insulation
- conducted losses
- radiated emissions.

Once the sample was placed into the oven, the required power setting is applied and left for a period of time untill the desired temperature is achieved. The time required at each power level and temperature condition was determined by experimentation. Each parameter, such as time, temperature and power setting, was measured and then recorded.

The samples were delivered by John Mathews in December 2002 in small calico bags. There was no other product information provided.

The microwave tests were carried out at a frequency of 2450MHz at power levels ranging from as low as 200W up to 1kW on various batches. The sample was placed inside an insulating crucible to limit the thermal losses. The trials were performed in a multimode batch oven using a full variable microwave generator.

Samples were heated for periods from 30 sec to 180 sec at various power levels thus achieving a range of final temperatures at various heating rates. Each completed sample was collected and taken for viability analysis by John Mathews. For rye grass viability, seeds were retrieved and potted into potting mix in pots. The germination percentage reported is the percentage of emerged plants. There are no viability results for other seed types.

## Results

Table 1 is the test data from the ryegrass trials.

| sample | Sample moisture | Meter reading | Heating rate (s) | Freq (ghz) | Temp (°C) (bag, sand) | Seed imbibed? | Sample weight (g) | Germination (%) |
|---|---|---|---|---|---|---|---|---|
| 1 | dry | 1022 | 180 | 2.45 | 102, - | N | 174 | 0 |
| 2 | dry | 1006 | 160 | 2.45 | 89, - | N | 178 | 0 |
| 3 | dry | 1000 | 140 | 2.45 | 77, - | N | 181 | 0 |
| 4 | dry | 1025 | 120 | 2.45 | 73, - | N | 164 | 3 |
| 5 | dry | 1010 | 100 | 2.45 | 67.5, - | N | 177 | 4 |
| 6 | dry | 980 | 80 | 2.45 | 58.5, - | N | 156 | 35 |
| 7 | dry | 980 | 80 | 2.45 | 55, 45 | N | 150 | 5 |
| 8 | dry | 980 | 100 | 2.45 | 67.2, 59.2 | N | 157 | 7 |
| 9 | dry | 980 | 120 | 2.45 | 68, 61 | N | 150 | 4 |
| 10 | dry | 993 | 140 | 2.45 | 79, 69 | N | 190 | 0 |

| sample | Sample moisture | Meter reading | Heating rate (s) | Freq (ghz) | Temp (°C) (bag, sand) | Seed imbibed? | Sample weight (g) | Germination (%) |
|---|---|---|---|---|---|---|---|---|
| 11 | dry | 980 | 160 | 2.45 | 106, 73 | N | 201 | 0 |
| 12 | dry | 990 | 180 | 2.45 | 90, 72 | N | 163 | 0 |
| 13 | wet | 1020 | 70 | 2.45 | 95, 95 | Y | 211 | 0 |
| 14 | wet | 1020 | 70 | 2.45 | 94, 90 | Y | 213 | 0 |
| 15 | wet | 1000 | 55 | 2.45 | 97, 95 | Y | 187 | 0 |
| *16* | wet | 1000 | *55* | 2.45 | 98, 98 | Y | 230 | 0 |
| *17* | wet | 1020 | *45* | 2.45 | 97, 96 | Y | 203 | 0 |
| *18* | wet | 1020 | *35* | 2.45 | 96, 95 | Y | 149 | 0 |
| *19* | wet | 847 | *30* | 2.45 | 87, 87 | Y | 130 | 0 |
| *20* | wet | 213 | *30* | 2.45 | 43, 39 | Y | 181 | 4 |
| *21* | wet | 210 | *45* | 2.45 | 46, 45 | Y | 199 | 11 |
| *22* | wet | 210 | *60* | 2.45 | 48, 48 | Y | 203 | 4 |
| *23* | wet | 210 | *90* | 2.45 | 61, 61 | Y | 227 | 3 |
| *24* | wet | 210 | *120* | 2.45 | 68, 65 | Y | 228 | - |
| *25* | *control* | - | - | - | - | N | - | 64 |
| 26 | *control* | - | - | - | - | N | - | 56 |
| 27 | *control* | - | - | - | - | Y | - | 20 |

The power values cannot be extrapolated in order to determine the energy requirements for a full scale production system. This is because the experimental set-up design was not energy efficient or in any way similar to a final production system.

Heating rate does not appear to influence the germination, however this may be due to the very short heating times of between 30 s and 180 s.

The single most important parameter from the data above appears to be temperature. The wide variations in temperature distribution imply that the seed bag and sand have different dielectric properties and as such heat at different rates. The exception is for the wet samples where the water dominates everything ensuring that the sand and bag temperatures are similar.

The lowest lethal temperatures were achieved in samples #10 (dry) and #24 (wet). These were 79, 69 $^{o}$C and 68, 65 $^{o}$C respectively.

## Conclusions and recommendations

The technical feasibility of using microwaves to achieve lethal seed temperatures has been successfully demonstrated for ryegrass seeds. The lowest lethal temperatures were achieved in samples #10(dry) and #24(wet). These were 79, 69 $^{o}$C and 68, 65 $^{o}$C respectively. The dielectric properties of various sand combinations have indicated that past hurdles, such as high soil attenuation, are not a consideration in the sandy broomrape seed case.

Consideration has been given to two conceptual designs both involving a systematic soil treatment where by the applicator is driven over the affect area. By far the most economical method involves removing the topsoil layer for continuous processing and then returning the treated soil immediately. To process 1 hectare using the top layer removal method would take 21.6 hours at a running cost of $5.9K/hectare.

In view of this very encouraging initial study, it is recommended that a larger scale trial be undertaken in order to establish the technical feasibility of each conceptual design. The study would require a scaling up of power by up to 30 times and the development of a radiating applicator and detailed systematic evaluation of energy requirements and process reliability of both concepts.

## References

Davis FS, Wayland JR, Robinson CW, Merkle MG (1973).Phytotoxicity of UHF electromagnetic field. *Nature* 241: 291-292.

Nelson SO (1996). A review and assessment of microwave energy for soil treatment to control pests. *Transactions of the Asae* 39: 281-289.

Vela-Múzquiz (1984). Control of field weeds by microwave radiation. *Acta Horticulturae* (ISHS) 152: 201-208.

# 2. Are conditions in waste disposal treatments such as composting lethal to branched broomrape seeds?

*Anna Williams and Andrew Craig*

*Branched Broomrape Eradication Program*

*February 2009*

## Introduction

The protocols that describe movement of potatoes from within the Branched Broomrape Quarantine Area require that potatoes go through an approved washing process after being removed from an infested paddock. This procedure must be met in order for potatoes to be given Approval to move outside of the Branched Broomrape Quarantine Area. Potatoes that are washed in an approved manner harbour very little soil after washing. At the same time, the washing process is very successful in removing soil from the potato tuber.

All potatoes from the quarantine area apart from those retained for seed are delivered to a packer or processor. The waste can be controlled to reduce the risk of establishment through this waste stream. Proper treatment of this waste product is critical to the exporter in managing the risk of spread. For example, if it is composted, deep buried or treated following the potato cyst nematode protocol very few (if any) seeds would survive this process.

In this experiment we examined whether the temperature and humidity conditions that may be generated during waste disposal treatment, such as composting, are lethal to branched broomrape seeds.

## Methods

In this series of trials we tested the survival of branched broomrape seeds after exposure for increasing periods of time to three temperatures 45, 55, 65 and 75 °C at 90% humidity and 55, 65 and 75 °C at 100% humidity. Humidity was maintained at 90% by adding a desiccant solution of 75 g of lithium chloride in 1 L of water to the base of a sealed chamber (Fig. 1). Water was placed in the chamber for the 100% humidity treatments. The container was then placed in an incubator heated to the desired temperature. The containers were left in the incubator overnight to equilibrate before the addition of seed treatments.

### Trial 1

Three replicates of approximately 100 broomrape seeds were placed in uncapped HPLC vials and exposed to the experimental 90%humidity and 45, 55 and 65 °C temperature conditions for either 1, 4, 8, 16, or 24 hours..

### Trial 2

Broomrape seeds were exposed to 90% humidity and temperatures of 65 or 75 °C for either 0.5, 1, 1.5, 2, 3 or 4 hours.

### Trial 3

Broomrape seeds were placed between two wetted 21 mm filter papers and added to the humidity chamber maintained at 100% humidity at 55, 65 and 75 °C for 0.5, 1, 1.5, 2, 3 or 4 hours.


**Figure 1. Humidity chamber used for experiments.**

After treatment, the seeds from the 90% humidity chambers were placed on filter papers in petri dishes. The seeds from the 100% humidity treatment were removed, placed in petri dishes, left to air dry and the upper most filter paper discarded.

To assess viability, 200 µL of water was added to the dry filter papers with seeds and the dishes were sealed with parafilm and placed in an incubator at 20 °C for 14 days to condition. A 200 µL aliquot of GR24 was added, dishes resealed, and seeds incubated at 20 °C for a further two weeks and then germination assessed. Ungerminated seeds were tested for viability using tetrazolium solution.

Seed survival was calculated as the sum of the proportion of germinated plus stained seeds in the viability tests.

## Results

In all trials, treatments that had viable seeds included seeds that germinated as well as seeds that did not germinate bur were still respiring, i.e. stained in tetrazolium viability tests.

### Trial 1

Temperatures of 45 °C at 90% humidity are not lethal to branched broomrape seeds after exposure times up to 24 hours. At 55 °C and 90% humidity, seeds survived after one hour exposure but exposure times of more than 4 hours at these conditions were lethal to some seeds. There were some inconsistencies in the results that warranted retesting (e.g. some survival in the 16 hour treatments but none in the 8 hour treatments). At 65 °C and 90% humidity, some seeds did not survive one hour exposure, very few seeds survived 2 hours exposure and exposures of more than 4 hours were lethal (Fig. 2).

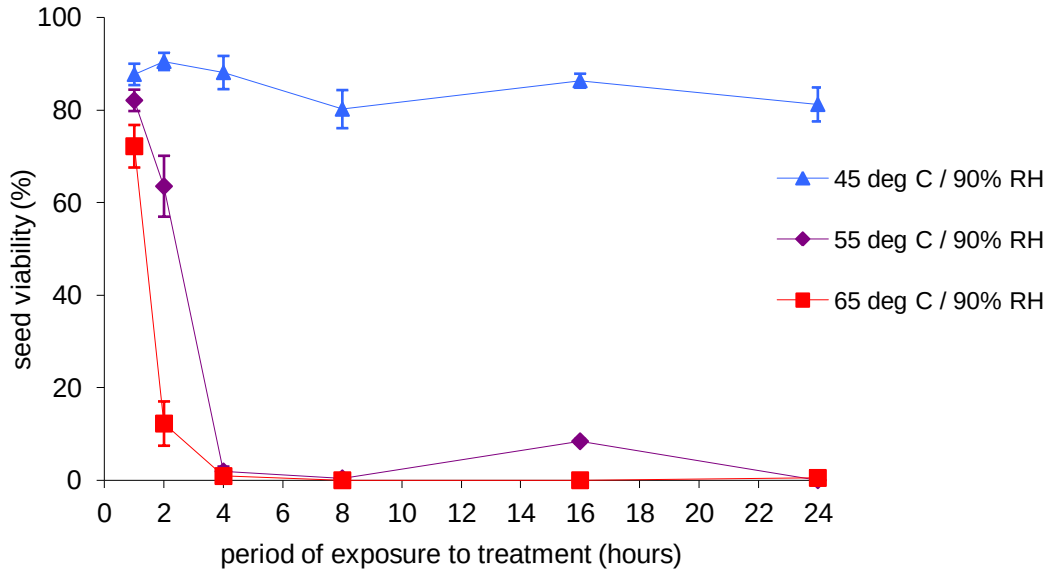


**Figure 2. Survival of branched broomrape seeds after increasing exposure to temperatures between 45 and 65 °C at 90% humidity. Points are mean ± 1 SE, *n* = 3.**

## Trial 2

Less than 1% of seeds survived 0.5 h exposure to temperatures of 75 °C at 90% humidity and no seeds survived longer exposure times (Fig. 3). At the lower temperature of 65 °C and 90% humidity less than 10% of seeds survived more than one hour exposure but 100% seed kill was not achieved after 4 hours.

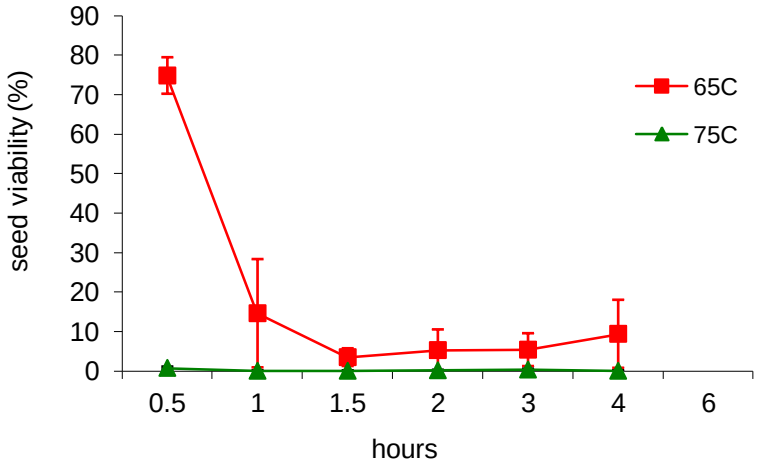


**Figure 3. Survival of branched broomrape seeds after increasing exposure to temperatures between 65 and 75 °C at 90% humidity. Points are mean ± 1 SE, *n* = 3.**

### Trial 3

At 100% humidity temperatures of 75 °C were lethal to broomrape seeds after exposure of 0.5 hours (Fig. 4). Less than 2% of seeds survived 0.5 hours of exposure at 65 °C and no seeds survived for 1 hour at this temperature. At 55 °C and 100% humidity a small proportion of seeds survived up to 6 hours exposure.

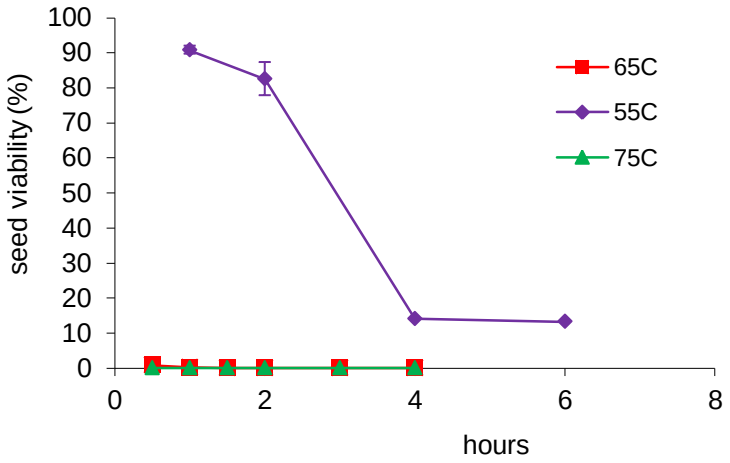


**Figure 4. Survival of branched broomrape seeds after increasing exposure to temperatures between 55 and 75°C at 100% humidity. Points are mean ± 1 SE, *n* = 3.**

## Summary

Provided that there is 100% humidity, unconditioned broomrape seeds are not likely to survive for more than 30 minutes at temperatures of 65 °C or higher. In this trial we did not determine the minimum exposure time.

At 90% humidity, not all seed will be killed after 4 hours exposure at 65 °C but at 75 °C these conditions are lethal for seeds after less than 30 minutes exposure.

Temperatures of 55 °C will not kill all broomrape seeds after 6 hours of exposure at 100% humidity and a higher proportion of seeds is expected to survive longer exposures at 90% humidity.

With moisture slightly less than saturation (90% humidity) broomrape seeds are not affected by exposures of up to 24 hours at temperatures of 45 °C.

# 3. Broomrape seed mortality with high temperature treatments

*Jane Prider*

*Branched Broomrape Eradication program*

*January 2011*

## Background

The processing of stock feed into pelletised form requires the application of very high temperatures in saturated conditions. The aim of this experiment was to determine whether broomrape seed would be killed after exposure to such conditions, i.e. at temperatures of 90 °C for periods of 1 minute or longer.

## Methods

Seed collected from the Mannum Trial Site in 2007 was used for the trial. Batches of approximately 200 seeds were placed in stainless steel baskets and surface sterilised. Five replicates were prepared for each treatment. After the seeds were dried, the seeds in their basket were immersed in a beaker of water held at 90 °C for 1, 2, 5 or 10 minutes. A control set of seed was not immersed in water and a second control was immersed in water at room temperature (23 °C) for two minutes.

Following treatment, the seeds were blotted on paper and then left to air dry. They were then placed in eppendorf tubes and approximately 500 µL of tetrazolium solution was added. The seeds were incubated for 14 days at 30 °C. Viability of seeds was assessed by checking for staining of actively respiring tissues.

## Results

No viable seeds remained in seeds that were immersed for 1 minute or more in water at 90 °C. Viability was low in control treatments, 22% for the unsoaked controls and 19% for the soaked controls.

The lethal exposure time for seeds at 90 °C in saturated conditions is less than one minute. Broomrape seeds are likely to be killed at the temperatures used during the processing of feed for stock pellets.