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Compendium of branched broomrape research

Section 7. Decontamination

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

DECEMBER 2013

PREMIUM
FOOD AND WINE FROM OUR
CLEAN
ENVIRONMENT



Compendium of branched broomrape research

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Table of Contents

1. Effect of Niproquat on branched broomrape (<i>Orobanche ramosa</i>) seed	4
2. Niproquat Corrosion Investigation	10

See also the following publications:

Craig A., Virtue J., Prider J., DeDear, C. (2014) Effect of Niproquat® (Didecyl dimethyl ammonium chloride) a quaternary ammonium based compound, on the germination and viability of branched broomrape (*Orobanche ramosa*) seed. (in preparation)

1. Effect of Niproquat on branched broomrape (*Orobanche ramosa*) seed

J. Virtue and C. DeDear

Department of Water Land & Biodiversity Conservation

Animal & Plant Control Commission

With amendments and additions by Jane Prider

Summary

The present study shows the effect of fifteen concentrations (0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1%) of Niproquat (Didecyl dimethyl ammonium chloride), three temperatures (15, 21 and 30°C) and three exposure times (12, 60 and 300 min) on the germination and viability of branched broomrape (*Orobanche ramosa*) seed. An initial experiment to test Formaldehyde 2%, with an exposure time of 20 min at room temperature proved to be less successful than comparative rates of Niproquat and was therefore discontinued in further trials. Discovering an effective wash-down agent that gives the user the confidence of total seed kill was a necessary tool for the property owners, departmental staff and anyone else working within the state imposed quarantine area in the western Murray-Mallee region of South Australia. Prior to a testing regime being completed, decontamination was performed with a 1% water solution of Niproquat, a protocol obtained from preliminary results. These results showed this dilution rate to be 100% effective. The final results have now shown that the effective rate of Niproquat can be confidently halved from 1% to 0.5%, for use between 15°C and 30°C. This temperature range is indicative of relevant environmental conditions likely to be encountered whilst using Niproquat as a decontaminant. This will also mean a significant economic benefit to those employing the product.

Introduction

Branched broomrape (*Orobanche ramosa*) is an underground parasite of broadleaf plants, attaching to host roots in order to obtain nutrients and water. Broomrape seed responds to chemical stimulants from host roots to both germinate and then attach to these roots up to 15 cm underground. It accumulates reserves in a tubercle (a swollen, storage organ) and develops a flowering bud during the winter season. After a period of time this bud goes into a phase of rapid growth, and a non-photosynthetic flowering stem emerges from the soil during early spring. Flowering and seed set takes place within 3-4 weeks after emergence. An individual broomrape plant can produce up to 20,000 dust like seeds, which are ovoid in shape and measure approximately 290 µm in length. They are extremely resilient and can remain viable for up to 13 years in storage but this figure is thought to be less under varying field conditions.

In 1999 a suitable chemical was required to help effectively control the spread of *Orobanche ramosa*, which is currently only found growing in a specific area in the western Murray-Mallee region of South Australia. This area is under quarantine and is subject to a 'Code of Practice', which places conditions on the movement of produce and machinery within and out of the quarantine area. Seed is commonly spread through various vectors including, but not exclusive to, produce, soil, machinery, livestock and wind dispersal.

Communication with the nursery industry identified several compounds that are used for sanitation. We also sourced a compound that is used in the United States for washdown procedures for the Witchweed Eradication Program. These quaternary compounds are typically active against bacterial and fungal organisms and act through disrupting the cell membrane.

The aims of this study were to

- identify a product that provided the best broomrape seed kill
- determine the exposure times and concentration that achieved the best broomrape seed kill
- determine whether the product would be active under conditions that are likely to be encountered under regular practice.

After sourcing various chemicals and undertaking preliminary laboratory research, it was determined that Niproquat, a quaternary ammonium based product was potentially the most effective.

Materials and methods

Plant materials

Broomrape plants were collected in the spring of 1999 from within the quarantine area in South Australia. These plants were dried at 40 °C in a drying oven for one week. They were then left at room temperature for some time in order for the seeds to mature, after which they were crushed and sieved to extract the seed. The seed was surfaced sterilised before use by immersing it in a 5% sodium hypochlorite solution for 5min. After this it was thoroughly rinsed with distilled water, then dried and stored at room temperature.

Conditioning

Before the viability of treated or untreated broomrape seeds could be tested using either one of the two methods as set out below, i.e. hydroponic bag test or GR24 germination stimulant, they needed to be conditioned. This was done by placing the seeds onto small GF/A glass fibre discs, moistened with sterile water and sealed in petri dishes at 21 °C for 10-12 days. After the conditioning period, the seeds were either, transferred into a suspension of sterile water and pipetted onto the roots of a host plant, or in the case of the GR24 stimulant method, the seed was allowed to dry down at room temperature and the GR24 chemical was added.

Germination stimulant GR24

A strigol analogue chemical stimulant, known as GR24, has been developed in the Netherlands with the ability to artificially stimulate parasitic weed seed. In particular it has the ability to stimulate very high percentages of *Orobanche ramosa* seed. As this chemical became available it was the preferred tool for testing broomrape seed viability as opposed to the hydroponic bag host test (see below), as this method proved to be tedious and time consuming. GR24, made up to a concentration of 1ppm, was applied to the pre-conditioned seed on the GF/A glass fibre discs and was kept at 24 °C for 7 days. The seeds were then assessed for germination under a microscope. A seed was considered to have germinated once a radicle had protruded through the seed coat.

Hydroponic polyethylene bag host test

The hydroponic host test method was used prior to the availability of a synthetic germination stimulant. It is a method that allows visual assessment of the treated seeds when exposed to the root system of any particular plant.

Initially host seeds were planted and allowed to germinate in sterile punnets of vermiculite. The seedlings were then transplanted into the polyethylene bags (35 x 20 cm) when they had grown to a suitable size to

ensure survival. The hydro bag was set up such that it contained an 11.5 x 28.5 cm rectangular insert of Whatman GF/A glass fibre paper. The glass fibre paper enabled the host plant to draw required quantities of nutrient solution that had been added into the bottom of the bag and also allowed for broomrape seed to be placed in close proximity to the plant's root system. A window flap was cut into the front of the bag to allow access to the root system but was closed with resealable tape to prevent it drying out. The top of each bag was folded over a rod and stapled. This then enabled it to be suspended vertically in a darkened tub and placed in a glasshouse or under artificial lighting. Observation of germination was done by carefully opening and folding back the taped window flap and viewed under a microscope. Cabbage or canola was used as a host plant.

Tetrazolium testing

To test whether ungerminated seed was still viable we used tetrazolium solution. Seeds were initially soaked in a 1 % solution of sodium hypochlorite and then rinsed. This sterilises the seeds and lightens the seed coat. When dry, the seeds were transferred to a 2 ml eppendorf tube with 1 % 2,3,5-triphenyltetrazolium chloride (tetrazolium) solution. Seeds were incubated in the dark for 14 days at 30 °C. Viability is scored by checking each seed under a microscope for pink to red staining which occurs in viable, respiring tissue. Unviable seeds remain unstained.

Trial 1. (1999) Quaternary compound screening

Six replicates of approximately 100 broomrape seeds were soaked for one hour in the following test solutions:

- water
- 1 % Quatrasan (a.i. c12-18 alkyl dimethyl ammonium chloride)
- 1% Kwiksax (a.i. c12-18 alkyl dimethyl ammonium chloride)
- 1% didecyl dimethyl ammonium chloride (e.g. Niproquat)
- 0.5% Quatrasan + 0.5% Kwiksax

Following soaking the seeds were rinsed twice with distilled water. Seed viability was assessed using tetrazolium solution.

Trial 2: (2000) Exposure time

Branched broomrape seeds were treated with four different concentrations of Niproquat; 0, 0.2, 1 and 5% [how does this compare with the 1% used above?], for three different exposure times; 12, 60 and 300 minutes. All treatments had three replicates. Seeds were immersed in the solutions in 25 ml flasks and then dried on filter paper. Germination and viability was assessed.

Trial 3: (2001) Niproquat concentration

Branched broomrape seeds were exposed for 12 minutes to eleven different concentrations of Niproquat; 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1%. Each treatment was replicated five times. Seeds were immersed in 50 ml tubes of the Niproquat solution. Seeds were placed on filter papers for 7 days to dry out. Seeds were rewetted and conditioned at 20 °C for 7-10 days before germination and viability testing.

Trial 4: (2003) temperature

Branched broomrape seeds were exposed to eight different concentrations of Niproquat; 0, 0.001, 0.005, 0.01, 0.05, 0.1, 0.5 and 1% by placing seeds on filter papers saturated with of Niproquat solution. Exposure time was until the filter paper dried out. Each treatment was replicated three times. Germination testing of branched broomrape seeds was done using GR24. Ungerminated seeds were then tested for viability using tetrazolium staining.

Results and discussion

Quaternary compound screening

Quaternary compounds with the active ingredient c12-18 alkyl dimethyl ammonium chloride (Quatrasan and Kwiksán) alone or in combination did not have any effect on the viability of broomrape seeds (Fig. 1). Didecyldimethyl ammonium chloride resulted in a significant decrease in seed viability in the treatment soaked for 15 minutes (ANOVA, $F = 6.4$, $p < 0.001$). This was not observed in the seeds soaked for 60 minutes.

The lack of a response in the seeds soaked for the longer period may have been the result of difficulties in determining whether seeds were stained. Many of the seeds treated with didecyldimethyl ammonium chloride stained poorly. It was decided to do further trials with this product but using a germination test in addition to a viability test to score seed deaths. Following these trials, Niproquat, a product containing didecyldimethyl ammonium chloride (120 g L^{-1}) was sourced.

Short exposure times of less than 12 minutes are effective for preventing germination and a high proportion of seeds are killed. However, unlike the previous trial, the product was not rinsed off the seeds after soaking hence exposure extended beyond the soaking time but under operational conditions this will be the situation.

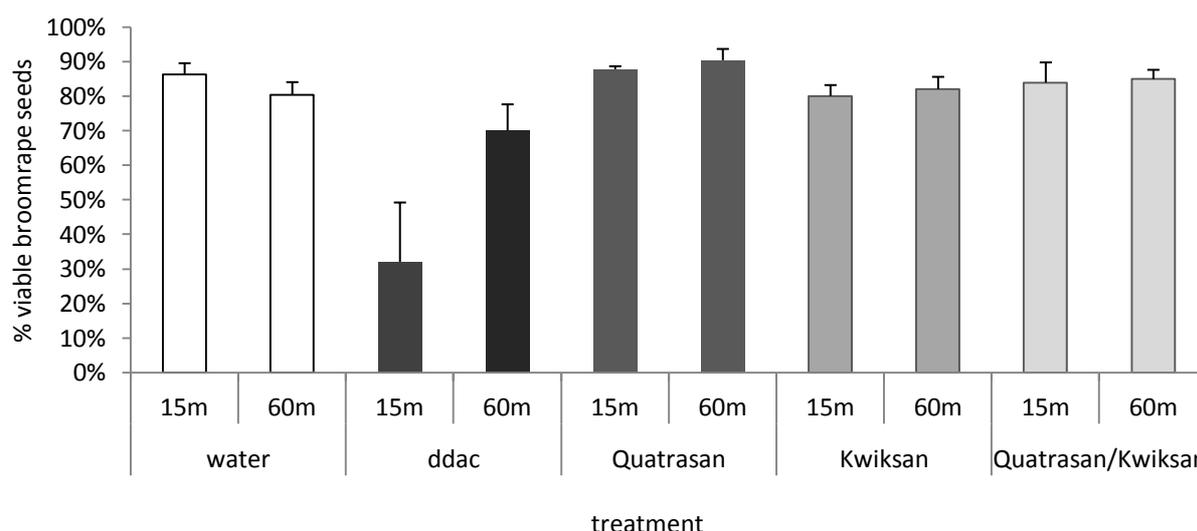


Figure 1. Viability of broomrape seeds after immersion in various quaternary compounds for 15 minutes or 60 minutes (mean + 1SE, $n = 3$). ddac = didecyldimethyl ammonium chloride.

Exposure time

No broomrape germination was observed following treatment with 1 or 5% Niproquat or 0.2 % Niproquat after 60 or 300 mins exposure. Application of Niproquat decreased broomrape seed viability compared with water alone (Fig. 1; ANOVA $F=23.87$, $p < 0.001$) but 100% seed kill was not achieved. All concentrations of Niproquat tested were equally effective and there was no increased seed death following longer exposure times to Niproquat (Fig. 2).

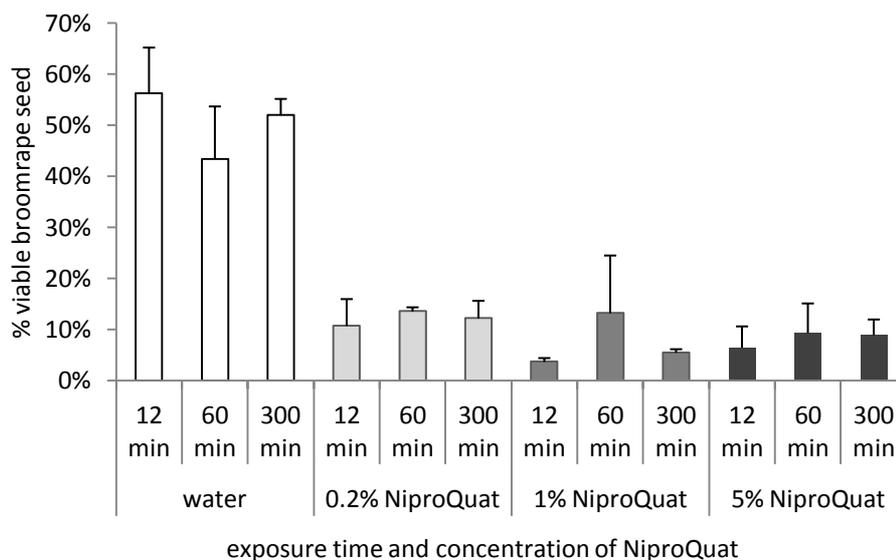


Figure 2. Viability of broomrape seed (germination plus viability testing) following exposure for increasing time periods to concentrations of Niproquat.

Niproquat concentration

Germination of branched broomrape was only observed in the control treatment, which contained no Niproquat. There was no difference in seed viability between seeds exposed to 0.1 to 1 % Niproquat concentrations (Fig. 3).

The registered rate of application for the product is a 1% solution. This trial demonstrated that under the experimental application conditions this was effective in killing all broomrape seeds. This result is not consistent with the previous trial but this may be the result of judgement in the degree of tetrazolium staining of a viable seed.

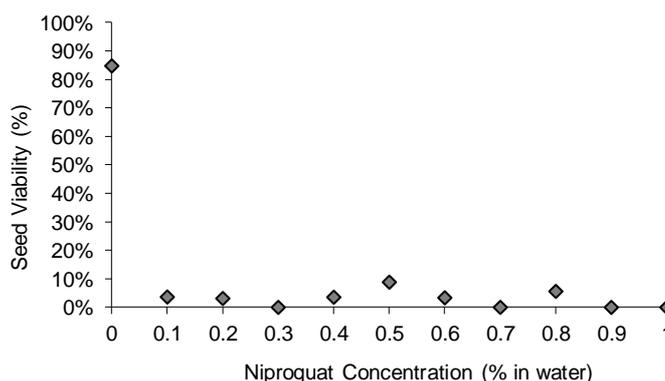


Figure 3. Viability of broomrape seed (germination plus viability testing) following exposure to low concentrations of Niproquat.

Temperature

Niproquat was equally effective at 15 and 30 °C (Fig. 4). No seeds survived treatments of 1 % Niproquat. Seeds on filter papers at 15 °C dried out after 3 – 4 hours whilst seeds at 30 °C dried out in one hour.

Temperature did not appear to affect the efficacy of Niproquat. The faster drying time of seeds at the higher temperature provided sufficient time for the Niproquat to penetrate the seed testa.

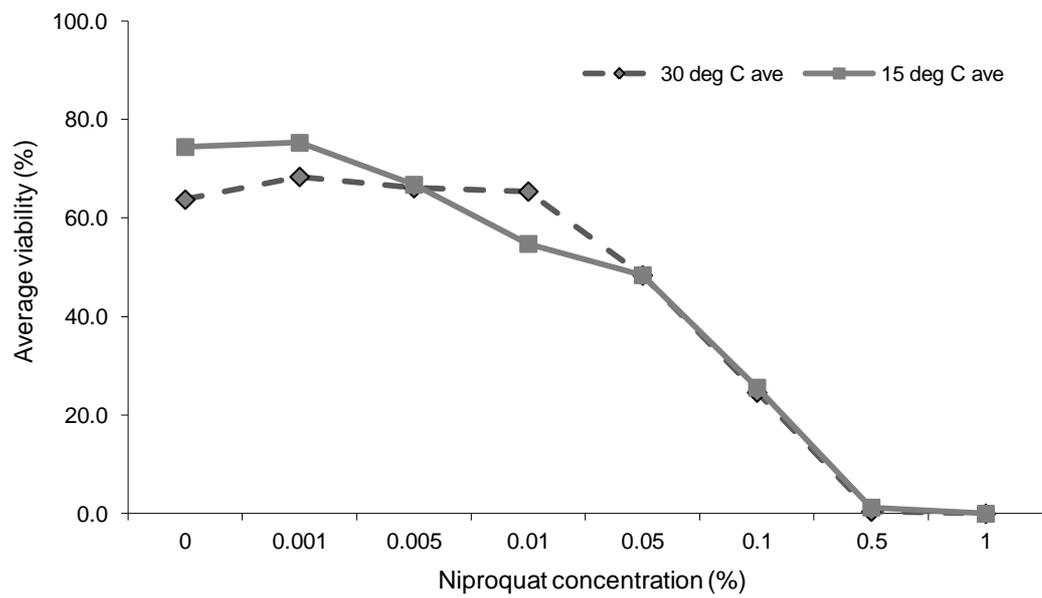


Figure 4. Viability of broomrape seeds placed on filter papers soaked with different concentrations of Niproquat solution at two temperatures.

2. Niproquat Corrosion Investigation

Monty Luke and Grant Doecke

Amdel Materials Services

November 2002

Summary

(by C. DeDear)

The question has been asked, "Is Niproquat corrosive to my equipment and machinery?" To answer this question Amdel Material Services was requested by the Broomrape program to carry out a corrosion test examining the effects of Niproquat on various materials. The testing was performed in accordance with ASTM G31 – "Standard Practice for Laboratory Immersion Corrosion Testing of Metals". The test used four solutions to run against the 'material specimens'. These were water (sourced locally), 1% Niproquat, 5% Niproquat and 100% Niproquat. The materials tested (coupons) were Cast Aluminium, Brass, Copper, Cast Iron, Steel and Polyethylene. The coupons were weighed to the nearest 0.0001 gram before they were immersed in the various solutions. They were then weighed after 30days in order to determine if any corrosion had occurred. It seemed that at the current rate of 1% Niproquat used for decontamination, four out of the six materials suffered less corrosion than the ones subjected to water only. The other two (cast aluminium and steel) suffered an insignificant amount of 0.05% more corrosion than the "water only" sample. The results were very similar again when using the 5% Niproquat. Although it is not necessary to ever use the Niproquat product at full strength, it was tested and was found to be up to four times corrosive as water. The conclusion we are able to draw from these tests is that the current use of Niproquat is not likely to be any more damaging than exposure to the weather.

Introduction

Amdel Material Services was requested by the Department of Primary Industries and Resources, Animal and Plant Control Commission to carry out a corrosion test examining the effects of Niproquat on various metal test coupons. Testing was carried out in accordance with ASTM G31 (Reapproved 1995) "Standard Practice for laboratory Immersion Corrosion Testing of Metals" using the four solution concentrations;

- Water only
- 1% Niproquat
- 5% Niproquat
- 100% Niproquat

Procedure

Test coupon materials and coupon arrangement were based on ASTM D1384. Material specimens were as follows:

- Cast aluminium
- Brass

- Copper
- Cast iron
- Steel
- Polyethylene

Test coupons were cleaned with P220 grit wet and dry paper by hand prior to being dried and weighed. The coupons were arranged in a bundle, each bundle consisting of one of each type of material. A bundle was fully immersed as well as half immersed in each solution for a test duration of 30 days (720 hours). A flea was used to continuously stir each test solution for the entire duration of the test. The test conditions were 20 ± 2 °C for the duration of the test. The solution was aerated no more than 5 min each day due to the frothing nature of the Nipro product.

The exposed samples were weighed to the nearest 0.0001 gram before and after testing. A cleaning control specimen was also used to eliminate any errors introduced by cleaning. The dimensions of the coupons were approximately 50 mm x 25 mm of various thicknesses.

Results

The details of the testing carried out are summarised in the following tables.

Client: The Department of Primary Industries and Resources **Test date:** 22-11-2002

Report No: 2AM1900
Client Ref: AP000523

Product code: NIPROQUAT
Test Specification: ASTM D1384-96

Figures in **BOLD** represent corrosion rates significantly worse than the control water

Figures in *ITALICS* represent corrosion rates significantly better than control water

Corrosion coupon type	Corrosion rate (mm per year)							
	water		1% Nipro		5% Nipro		100% Nipro	
	Half	Full	Half	Full	Half	Full	Half	Full
Cast aluminium	0.1261	0.2516	0.1384	0.1386	0.2887	0.1752	0.6377	0.1016
Brass	0.0136	0.0228	0.0079	0.0040	0.0122	0.0106	0.0432	0.0190
Copper	0.0145	0.0040	0.0049	0.0036	0.0085	0.0102	0.0372	0.0146
Cast iron	0.2763	0.4100	0.2351	0.3094	0.3642	0.3089	0.3274	0.1443
Steel	0.0723	0.2167	0.0821	0.0520	0.0704	0.0527	0.1679	0.0965
Polyethylene	-0.0017	0.0000	-0.0107	-0.0197	-0.0090	-0.0145	-0.0073	-0.0158

Corrosion coupon type	% weight loss							
	water		1% Nipro		5% Nipro		100% Nipro	
	Half	Full	Half	Full	Half	Full	Half	Full
Cast aluminium	0.75	1.67	0.83	0.81	1.60	0.97	4.15	0.61
Brass	0.15	0.25	0.09	0.04	0.14	0.11	0.48	0.22
Copper	0.15	0.04	0.05	0.04	0.09	0.11	0.39	0.15
Cast iron	1.41	2.14	1.19	1.60	1.89	1.46	1.67	0.72
Steel	0.78	2.17	0.83	0.52	0.79	0.54	1.67	1.10
Polyethylene	-0.01	0.00	-0.04	-0.07	-0.03	-0.05	-0.02	-0.05