# **BIOSECURITY SA** PIRSA

# **Compendium of branched broomrape** research

# Section 2. Host testing and host trials

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

**DECEMBER 2013** 







**Primary Industries** and Regions SA

# Compendium of branched broomrape research

Information current as of 3 December 2013

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See also the following publications:

Virtue J.G., DeDear C., Potter M.J., Rieger M. (2006) Potential use of isothiocyanates in branched broomrape eradication. 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. 629-632.

Virtue J., Prider J., Williams A. (2013) Host range of branched broomrape (*Orobanche ramosa* subsp. *mutelii*) in South Australia. Plant Protection Quarterly (in press).

# 1. Branched broomrape host list

#### October 2009

## Categorisation of host risk for the SA strain of branched broomrape, based on research and field observations.

Plant	Species	Family	Host Risk
			D = definite host
			L = likely host
			P = possible host
			N = likely non-host
Broad acre crops			
safflower	Carthamus tinctorius	Asteraceae	D
sunflower	Helianthus annuus	Asteraceae	D
canola	Brassica napus	Brassicaceae	D
white mustard	Sinapis alba	Brassicaceae	D
chick pea	Cicer arietinum	Fabaceae	D
faba bean	Vicia faba	Fabaceae	D
vetch	Vicia sativa	Fabaceae	D
coriander	Coriandrum sativum	Apiaceae	D
indian mustard	Brassica juncea	Brassicaceae	D
lentil	Lens culinaris	Fabaceae	Р
linola	Linum usitatissimum	Linaceace	Р
lupin	Lupinus angustifolius	Fabaceae	Test further
field pea	Pisum sativum	Fabaceae	N
lathyrus	Lathyrus cicera	Fabaceae	Ν
purple vetch	Vicia benghalensis cv. 'Popany'	Fabaceae	N
Vegetables	1		
carrot	Daucus carota	Apiaceae	D
broccoli	Brassica oleracea var. italica	Brassicaceae	D
cabbage	Brassica oleracea var. capitata	Brassicaceae	D
cauliflower	Brassica oleracea var. botrytis	Brassicaceae	D
tomato	Lycopersicon esculentum	Solanaceae	D
lettuce	Lactuca sativa	Asteraceae	D
eggplant	Solanum melongena	Solanaceae	D
potato	Solanum tuberosum	Solanaceae	N (excluding 'Shine' L)
cucumber	Cucumis sativus	Cucurbitaceae	Ν
pumpkin	Cucurbita maxima	Cucurbitaceae	Ν
rockmelon	Cucumis melo ssp melo	Cucurbitaceae	Ν
squash	Cucurbita pepo	Cucurbitaceae	Ν
watermelon	Citrullus lanatus	Cucurbitaceae	Ν
zucchini	Cucurbita pepo	Cucurbitaceae	Ν
onion	Allium cepa	Liliaceae	N
Pastures			
clover - white	Trifolium repens	Fabaceae	D
medic - disc	Medicago tornata	Fabaceae	D
medic - small burr	Medicago minima	Fabaceae	D
Balansa clover	Trifolium michelanium	Fabaceae	D
lucerne	Medicago sativa	Fabaceae	D (but not observed in field)
Persian clover	Trifolium resupinatum	Fabaceae	L
medic - annual burr	Medicago polymorpha	Fabaceae	L

Plant	Species	Family	Host Risk
ΓΙάΠ	opecies	ганшу	D = definite host
			L = likely host
			P = possible host
			N = likely non-host
medic - strand	Medicago littoralis	Fabaceae	
clover - sub	Trifolium subterraneum	Fabaceae	P
Native plants		1 4546646	· ·
common everlasting	Chrysocephalum apiculatum	Asteraceae	D
golden everlasting	Xerochrysum bracteatum	Asteraceae	D
poached egg daisy	Polycalymma stuartii	Asteraceae	 D
variable daisy	Brachycome ciliaris	Asteraceae	 D
variable groundsel	Senecio pinnatifolius	Asteraceae	 D
Sturt's desert pea	Swainsona formosa	Fabaceae	 D
cut-leaf daisy	Brachyscome multifida	Asteraceae	
sweet pea	Lathyrus odoratus	Fabaceae	
sticky goodenia	Goodenia varia	Goodeniaceae	L
scarlet mintbush	Prostanthera aspalathoides	Lamiaceae	
creeping boobialla	Myoporum parvifolium	Myoporaceae	
sweet apple berry	Billardiera cymosa	Pittosporaceae	
showy daisy bush	Olearia pimeleoides	Asteraceae	 N
creeping saltbush	Atriplex semibaccata	Chenopodiaceae	N
ruby saltbush	Enchylaena tomentosa	Chenopodiaceae	N
spiny saltbush	Rhagodia spinescens	Chenopodiaceae	N
desert cassia	Senna artemisioides	Fabaceae	N
golden wattle	Acacia pycnantha	Fabaceae	N
native lilac	Hardenbergia violacea	Fabaceae	N
running postman	Kennedia prostrata	Fabaceae	N
wild rosemary	Dampiera rosmarinifolia	Goodeniaceae	N
austral bugle	Ajuga australis	Lamiaceae	N
black anther flax lily	Dianella revoluta	Liliaceae	N
spreading emu bush	Eremophila divaricata	Myoporaceae	N
dryland tea tree	Melaleuca lanceolata	Myrtaceae	N
muntries	Kunzea pomifera	Myrtaceae	N
scarlet bottlebrush	Callistemon rugulosus	Myrtaceae	N
summer red mallee	Eucalyptus socialis	Myrtaceae	N
vorrell	Eucalyptus gracilis	Myrtaceae	N
lavender grevillea	Grevillea lavandulacea	Proteaceae	N
rock correa	Correa glabra	Rutaceae	N
Ornamentals			
nasturtium	Tropaeolum majus	Trapaeolaceae	D
sweet pea	Lathyrus odoratus	Fabaceae	L
gazania	Gazania sp.	Asteraceae	N
alyssum	Lobularia maritima	Brassicaceae	N
sweet william	Dianthus barbatus	Carophyllaceae	N
garden geranium	Pelargonium x domesticum	Geraniaceae	N
Italian lavender	Lavandula stoechas	Lamiaceae	N
petunia	Petunia x hybrida	Solanaceae	N
pansy	Viola arvensis	Violaceae	N
Weeds		11010000	
bathurst burr	Xanthium spinosum	Asteraceae	D
		, 1010100000	

Plant	Species	Family	Host Risk D = definite host L = likely host P = possible host
			N = likely non-host
capeweed	Arctotheca calendula	Asteraceae	D
cretan weed	Hedypnois rhagadioloides	Asteraceae	D
false sowthistle	Reichardia tingitara	Asteraceae	D
flatweed	Hypochoeris glabra	Asteraceae	D
skeleton weed	Chondrilla juncea	Asteraceae	D
smooth catsear	Hypochoeris glabra	Asteraceae	D
sowthistle	Sonchus oleraceus	Asteraceae	D
stemless thistle	Onopordum acaulon	Asteraceae	D
tolpis	Tolpis barbata	Asteraceae	D
common heliotrope	Heliotropium europaeum	Boraginaceae	D
salvation Jane	Echium plantagineum	Boraginaceae	D
sheepweed	Buglossoides arvensis	Boraginaceae	D
indian mustard	Brassica juncea	Brassicaceae	D
white mustard	Sinapis alba	Brassicaceae	D
wild turnip	Brassica tournefortii	Brassicaceae	D
rough poppy	Papaver hybridum	Papaveraceae	L

#### Also based on

- 1. Parasitic Weeds of the World, Parker & Riches CAB International, 1993.
- 2. Qaesem, J.R. and C.L. Foy, (2007), Screening studies on the host range of branched broomrape (*Orobanche ramosa*), Journal of Horticultural Science and Biotechnology, vol 82 (6), p885-89.

# 2. Summary of canola / branched broomrape research

Jane Prider Biosecurity SA January 2013

## Summary

Canola is a host crop of branched broomrape that is currently grown in the area infested by the weed in South Australia. Research throughout the eradication program has focussed on various aspects of this system to assess the potential impacts of branched broomrape on production and ways the system could be adapted to control branched broomrape. All canola cultivars tested were found to host branched broomrape. Canola releases the secondary metabolite 2-phenylethyl isothiocyanate. This compound stimulates branched broomrape germination and in high concentrations inhibits germination. Broomrape seed banks are likely to show some decline under canola crops as germination is promoted in much higher proportion to the successful attachment and further growth to maturity of attached broomrape plants, provided that further seed release can be prevented. In comparison to other hosts, such as vetch and cretan weed, canola varieties appear to be less susceptible to broomrape infection. It is perhaps due to this poor infection that no impacts of broomrape have been recorded on canola plants. The Clearfield varieties, that have tolerance to the Group B imizadolinone herbicides, have very low levels of broomrape infection. Trials have shown that prevention of broomrape emergence in Clearfield canola crops is achievable using low rates of the herbicides On Duty and ClearSol.

Although impacts of branched broomrape on canola production in the quarantine area have not occurred there is still some need for caution. Canola production occupies a very small proportion of the quarantine area. If the area of production was to increase and the Clearfield canola system not used there is the potential for branched broomrape abundance to increase. Relaxation of quarantine with the end of eradication will only exacerbate this problem. This has been the situation in France where the branched broomrape problem became critical with the rapid expansion of fields planted with canola.

## Background

#### **Review of problem in Europe**

Branched broomrape (*Orobanche ramosa*) is now recognised as one of the major pests of canola (oilseed rape) crops in France. Oilseed rape is a relatively recent crop in Europe and infection by broomrape has only occurred over the past twenty years (Brault et al. 2007), accelerating with the increase in oilseed rape production since 2005 (Veronesi et al. 2009). *Orobanche ramosa* was also a pest in oilseed rape crops in Spain in 1980 – 1981 (Sobrina Vesperinas 1982) but very little oilseed rape is grown there now. *Orobanche mutelii* was also reported from oilseed rape areas in Spain but the broomrape species (*ramosa* and/or *mutelii*) infecting oilseed rape was not specified. *Orobanche ramosa* is also an increasing problem in oilseed rape crops in Bulgaria (Shindrova and Kostov 2009), Greece (Economou et al. 2007, Tsialtas and Eleftherohorinos 2011) and Germany (Kohlschmid et al. 2011).

In France, there are several *O. ramosa* pathovars which have differing impacts on oilseed rape yield (Benharrat et al. 2005, Brault et al. 2007). In addition, cultivars of oilseed rape have varying susceptibility to broomrape infection (Buschmann et al. 2005). Infection by *O. ramosa* can result in decreases in

biomass of potted plants (Buschmann et al 2005), and dwarfism, chlorosis and early pod abortion of field grown plants (Gibot-Leclerc et al. 2012). A severe broomrape infection can result in up to 80-90% reduction in seed yield (Veronesi et al. 2009, Gibot-Leclerc et al. 2012). It is estimated that about 10% of the oilseed rape area in France is infested by *O. ramosa* (Veronesi et al. 2009).

#### Australia

Canola (*Brassica napus* ssp. *oleifera*) is a minor crop in the area affected by branched broomrape (*Orobanche ramosa* subsp. (aff. *mutelii*)) in South Australia. In 2010 and 2011, less than 1% of paddocks within the Quarantine Area (QA) were planted to canola (Survey X data base). In a cereal production system, canola provides a useful disease and weed break crop. This crop has the potential to increase in production in the area affected by broomrape and the surrounding region. This has implications for the persistence of broomrape in the area, the spread of broomrape and for the potential for yield losses as a result of broomrape infection.

#### **Research questions**

As one of the few crops within the QA that is a host of branched broomrape, there have been a number of research projects that have focussed on the canola/branched broomrape system. Initial host testing work was expanded to evaluate how this crop could be used to deplete branched broomrape seed banks. We have also used experiments to investigate the impacts of branched broomrape infection on canola production. GRDC-funded research investigated how branched broomrape could be controlled in canola crops. The following research questions have been addressed:

- 1. Are all canola cultivars hosts for branched broomrape or equally susceptible to infection?
- 2. Can the system be exploited as a catch crop or trap crop for branched broomrape control?
- 3. What is the impact of branched broomrape on canola?
- 4. How can branched broomrape be controlled in canola crops?

## Host testing

Canola was early recognised as a host of broomrape from observations of in-crop infestations. Most host testing has been done in conjunction with other research projects.

For hosts testing, three cultivation methods are used to confirm three stages of broomrape development; germination, host attachment and reproduction. As only the last stage can be observed in potted or field-grown plants a hydroponic cultivation method is used for observation of other developmental stages. Most testing of canola occurred from 2002-2003 as part of a broader Brassica project (Virtue et al. 2006), with supplementary testing and observations to 2010.

The hydroponic growth system comprised a filter paper enclosed in a polythene bag. Broomrape and host seed is sown on the filter paper and the host's shoots grow through the opened top of the bag. Observations can be made of broomrape germination and tubercle development on the host's visible root system.

Of the 15 lines of canola tested using this method, all promoted the germination of branched broomrape. Although there were some inconsistencies between tests most canola varieties stimulated from 20 -30% of broomrape seeds with a range of 15 - 65% (

#### Table 1).

Some observations of emerged broomrape from canola hosts have been made in either pot or field experiments. Varieties that have had emerged broomrape are Clearfield (2 lines), Mystic, Boomer TT and Tanami.

Several overseas studies have found that all tested oilseed rape lines are susceptible to *O. ramosa* infection (Sobrino Vesperinas 1985, Zehhar et al. 2003, Gauthier et al. 2012). This is in contrast to some other hosts *e.g.* sunflower, where at least some lines appear to be resistant to infection. For overseas infestations, the search for resistant varieties is important as a control method.

	Test						
Variety	1	2	3	4	5	6	7
Boomer TT							47.3 ± 10.2
Clearfield#		24.5 ± 8.4	23	25.8 ± 4.3	31.5 ± 3		
Clearfield 43C80#						49.3 ± 5.8	51.7 ± 7.9
Dunk-H		65.1 ± 8.3	24	35.8 ± 2.8			
Dunk-L		21.7 ± 2.8	23	34.3 ± 5.6			
GT61*						65.2 ± 4.1	
46Y20*						65.2 ± 4.4	
Hyola 601RR*						52 ± 6	
Karoo		26.9 ± 5.7	33	30 ± 3.8			
Kirkgard			30	28.1 ± 5			
Monty		15.6 ± 6.7	25	39.4 ± 4.6			
Nemcon		28.9 ± 3.5	22	31.1 ± 3.7			
New Kirk				20.3± 2.29			
Rainbow	22.3 ± 6.4						

Table 1. Percent germination of canola varieties using the hydrobag cultivation method in several tests conducted over ten years. Means  $\pm$  1 SE. Values marked in bold text also had broomrape tubercles.

Test

1. Host testing 1999/2000

2. Brassica experiment 1 2001

3. Brassica experiment 2 2002 (only means available)

4. Brassica experiment 3 2003

5. Host testing 2001

6. GM canola trial 2010

7. Trap crop experiment 2010

# Pioneer Seeds

\*Genetically Modified Canola

## Germination stimulation

*Brassica napus* does not produce strigolactones, the most common broomrape germination stimulant in other plants. The main germination stimulant of *Brassica napus* is possibly 2-phenylethyl isothiocyanate (2-PEITC) (Auger et al. 2012). This secondary metabolite is derived from the glucosinolate pathway. It is released from plant roots in response to tissue injury and has biocidal properties. 2-PEITC is also found in the rhizosphere of undamaged roots. It is suggested that *B. napus* roots release glucosinolate into the soil and it is converted by soil microbes into 2-PEITC. Therefore soil microflora could be involved in the infection of *B. napus* by *O. ramosa*. This has been confirmed with experiments in sterile soil where a reduction of *I. napus* by *O. ramosa* has been observed (Gauthier et al. 2012).

In a project to determine whether canola and other Brassica crops could be a tool for reducing the branched broomrape seed bank by inducing germination, a number of brassica lines were tested for their germination stimulatory activity (Virtue et al. 2006) (Brassica experiment 1, 2, 3 in Table 1). There was a significant positive relationship between the concentration of 2-PEITC in root tissue and germination stimulatory affect on broomrape germination but this effect was reduced with increasing 2-PEITC concentration, with concentrations of 100 ppm or more being inhibitory (Figure 2).

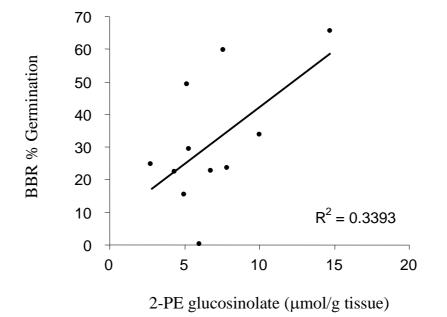


Figure 1. Branched broomrape seed germination and root concentration of 2- phenylethyl isothiocyanate (2-PEITC) for 11 Brassica lines (includes species in addition to B. napus). Root exudates were collected in a separate experiment and germination tested using the polybag system.

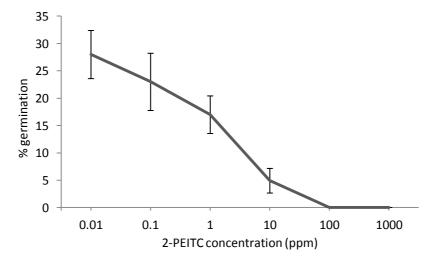


Figure 2. Branched broomrape germination response to concentrations of synthetic 2-PEITC from in vitro trials on filter paper in petri dishes. (n = 6) Means  $\pm 1$  SE

Due to the lack of consistency between trial results, the use of *Brassica* species (including canola) to reduce the branched broomrape seed bank is an unreliable method for control and was not taken up by the eradication program. However the demonstration of the effectiveness of isothiocyanates in either stimulation of broomrape germination at low concentrations or inhibition (or potential viability loss) at high

concentrations demonstrated the efficacy of using applications of ITCs for seed bank control. Formulations of the methyl-ITC releasing product, dazomet, were used in preference to 2-PEITC due their commercial availability and increased biological activity (necessary for conversion of dazomet to methyl ITC) in soils.

#### Trap crops



## Figure 3. Canola in a field trial to measure decline in branched broomrape seed bank under different crops in 2009.

In continuation of the work on germination stimulants, several projects over a number of years investigated the potential use of crops for reducing the broomrape seed bank. Canola was one of the crops trialled. The germination stimulant projects demonstrated that canola had high germination stimulatory activity compared with other broad acre crops cultivated in the guarantine area. Field trials (Figure 3) and pot trials examining broomrape infection from soils that had previously grown target crops failed to detect any decline in the broomrape seed bank for different crops. In 2011 a pot trial was conducted where a known number of broomrape seeds were mixed in the soil, the crops grown and the number of broomrape seeds remaining in the soil counted after the end of the growing season. This trial demonstrated a reduction in the broomrape seed bank after canola had been grown. There was a greater proportional reduction where there was a lower broomrape seed density (Figure 4). The lowest seed density represents the average seed bank density measured in infested fields. This project demonstrates that within a host crop there is some capacity for the broomrape seed bank to be reduced. For canola to be a true 'trap crop', the crop must stimulate germination but broomrape must not be able to develop further. As canola is a broomrape host it is more accurately a 'catch crop'. There is still a requirement for some other form of broomrape control to occur to prevent the production of further seed via either destroying the crop before broomrape emerges or controlling broomrape plants whilst attached to the host.

## Infection by broomrape in canola crops

Annual survey data shows that within Level 4 paddocks (paddocks where broomrape has previously been found) there has been an increase in the number of L4 paddocks planted with canola over time but there has been no increase in the number of paddocks reinfested by broomrape (Figure 5). Four of these L4 paddocks had large broomrape infestations so it can be assumed that these paddocks have a substantial broomrape seed bank. With the exception of one paddock, where the crop in the year of broomrape

discovery was canola, none of these paddocks have had an in-crop broomrape infestation in the years planted with canola. These findings imply that canola is not highly susceptible to broomrape infection (or that broomrape occurs at undetectable levels in these crops) and/or control of broomrape is very effective in canola crops.

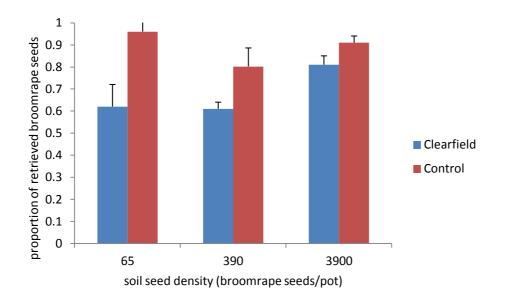


Figure 4. Proportion of broomrape seeds retrieved from pots sown with broomrape seeds of increasing density. Control pots were not planted and canola pots had one canola plant per pot. Seeds were counted in a 200 g subsample of soil from each pot (n = 5). Means + 1 SE. Each pot held approximately 1300 g of soil.

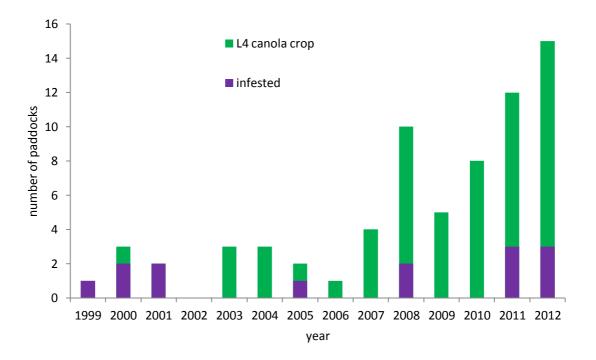


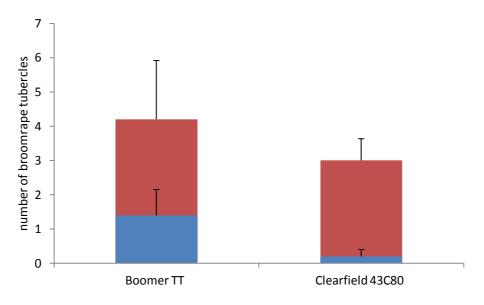
Figure 5. Level 4 paddocks that have been planted with canola for each year of the eradication program and the number of those paddocks that have been found reinfested by broomrape during annual surveys.

#### Canola susceptibility to infection

There is limited data on broomrape infection of canola in fields. Counts of emerged broomrape plants on five sampled host plants in three plots for a herbicide trial in 2007 found that canola was a less frequent host than cretan weed (canola  $1.4 \pm 0.9$ , cretan weed  $4.2 \pm 1.5$  emerged broomrape per 5 host plants, means  $\pm 1$  SE). Other observations from paddocks may be misleading as broomrape could have been hosting on weed hosts within these fields.

We have observed differences in the susceptibility of canola varieties to broomrape infection. These differences could occur at various stages in the infection process. Gauthier et al (2012) identified three stages in the infection process when this could occur: germination as a result of timing and quantity of stimulants released, at root attachment stage preventing the formation of haustoria, and after attachment resulting in delayed development and emergence.

A glasshouse pot experiment in 2009 found differences in broomrape emergence between two broomrape varieties (Figure 6). These two varieties had similar germination stimulatory activity (Table 1) and there was no significant difference in the number of broomrape plants attached. This suggests that there is delayed development of broomrape on the Clearfield variety such that fewer broomrape plants develop to the reproductive stage.



## Figure 6. Broomrape plants on two pot-grown canola varieties. The number of emerged plants is shown by the blue bars (n = 10). Means + 1 SE.

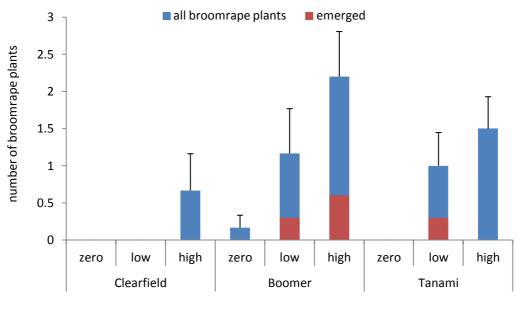
A field pot experiment in 2012 was conducted to confirm the above finding and to determine whether broomrape had any impacts on their canola hosts. Specifically we looked at differences in timing of canola reproductive development, leaf chlorosis, plant size and yield. Four canola varieties were grown in pots sown with either zero, a low density (9,000 seeds pot<sup>-1</sup>) or a high density (45,000 seeds pot<sup>-1</sup>) of broomrape seeds.

With the exception of Clearfield cv. Surpass (where only two plants grew successfully) all canola varieties hosted broomrape. No broomrape plants emerged on Clearfield hosts although there were broomrape plants found on the host roots at harvest in the high density treatment (Figure 7). Tanami and Boomer TT

varieties had emerged broomrape but not all plants were infected. There was no significant difference in infection with the density of broomrape seeds in the pot. Vetch and cretan weed hosts grown using the same method at the same time were all infected by broomrape and had emerged plants.

There were no differences in growth or reproduction detected between infested and uninfested canola plants but given the low levels of broomrape infection this was as expected.

This study and a number of observations related to the experience overseas suggest that the pathovars of branched broomrape present in Australia do not have a significant impact on the canola cultivars currently grown. Overseas findings do indicate that we need to be vigilant about the occurrence of broomrape in canola crops to respond quickly to any increases in broomrape abundance in these crops.



Canola variety

Figure 7. Occurrence of broomrape plants on canola hosts grown in pots with varying density of broomrape seed in the pot soil (n = 6). Means + 1 SE. Field soil was used in pots hence the infection of one of the "zero" Boomer treatments.

#### Yield losses

A 2009 glasshouse study of two cultivars of herbicide-resistant canola grown in small pots found that infected plants of the cultivar Boomer TT (tetrazine tolerant) had lower root and shoot biomass than uninfected plants (Figure 8). The biomass of the cultivar Clearfield 43C80 (imazamox tolerant) did not differ between infected and uninfected plants. Fewer Clearfield 43C80 plants were infected by broomrape than Boomer TT plants. This indicates that different varieties of canola may have different susceptibility to broomrape infection and it may be possible to select cultivars that are not likely to have a reduced yield if infected by broomrape. As described above, field experiments that were designed to test for yield differences between broomrape-infested canola varieties failed to achieve significant broomrape infection for comparison.

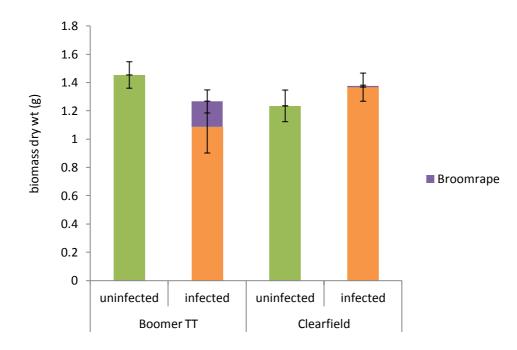


Figure 8. Total biomass (root and shoot dry weight) between two canola varieties infected or not infected by branched broomrape (n = 10). Mean  $\pm 1$  SE. Broomrape biomass is added to bars for infected plants.

In 1999, seed pods were collected from a canola crop in the QA that was infected by broomrape. Comparisons were made between canola plants that had emerged broomrape with plants that had no emerged broomrape. No difference was found in the number of canola pods, seeds or total seed mass between uninfected or broomrape-infected canola plants (Figure 9). In interpreting the results it should be noted that canola plants designated as uninfected may have had attached unemerged broomrape that could have impacted canola seed production but results of our hosts susceptibility trials imply that infection of canola by broomrape is poor.

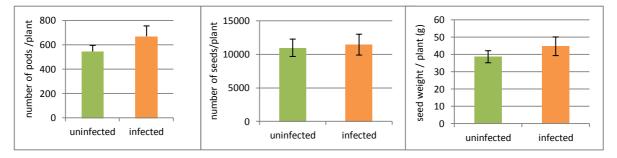


Figure 9. Comparison of reproductive yield of field crop canola plants infected or not infected by broomrape. (n = 33) Mean + 1SE.

#### Broomrape control in canola

Canola crops have been included in several GRDC-funded herbicide trials conducted by John Matthews. In 2001, glyphosate and On Duty™ (active ingredients imazapic and imazapyr) were trialled on a Clearfield and a non-Clearfield variety. Clearfield varieties have been developed for tolerance to imidazolinone compounds. On Duty applied at the rate of 20 g ha<sup>-1</sup> prevented emergence in one Clearfield trial. The same rate applied to Clearfield and Oscar canola decreased emergence to 0.07 broomrape ha<sup>-1</sup>. It is not known whether the broomrape was hosting on the canola or weeds in this crop. Glyphosate applied at the rate of 300 ml ha<sup>-1</sup> to Oscar canola reduced emergence to 0.19 plants ha<sup>-1</sup> in comparison with unsprayed plots that had 1.68 plants ha<sup>-1</sup> (Matthews 2002). Trials of Clearfield canola between 2002 and 2004 found that On Duty (rates of 30 and 40 g ha<sup>-1</sup>) and ClearSol (active ingredient imazapyr), at rates of 42, 56 and 84 ml ha<sup>-1</sup>, prevented broomrape emergence. Further trials with these two herbicides on Clearfield canola in 2006 also prevented emergence. This was a better growing season than 2004 and demonstrated the efficacy of these herbicides across a broad range of conditions. Trials in 2005 suggest that On Duty and ClearSol gave protection from broomrape recruitment for about 9 weeks but the method used to evaluate this is not described.

Although herbicide trials with non-Clearfield varieties have been limited, repeated trials have demonstrated the effectiveness of the Clearfield system for broomrape control. Although these varieties may in some situations incur a yield penalty, in most SARDI trials this has been found to be less than 10 % of the average canola yield across all varieties (SARDI 2012). The risk of an unknown effect of a potential broomrape infestation must be weighed up against the known losses incurred in a Clearfield system where broomrape can be controlled.

The GM canola system could also provide another alternative to the Clearfield system. GM Canola is glyphosate-tolerant and glyphosate is known to be effective for the control of broomrape without affecting the host when applied at low rates with correct timing. The three GM canola varieties trialled promoted broomrape germination and attachments formed on canola roots in polybags (see Table 1) but broomrape failed to infect potted plants. The effectiveness of this system therefore remains untested.

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# 3. Effect of branched broomrape on vetch

Jane Prider and Andrew Craig Biosecurity SA January 2013

## Summary

*Vicia sativa* is a minor crop in the area infested by branched broomrape in South Australia. In this field pot experiment we found that the vetch varieties Morava and Blanchefleur were readily infected by branched broomrape and the degree of infection was related to the density of broomrape seed in the soil. The *Vicia benghalensis* cultivar Popany was a poor host, with only one small dead broomrape plant found on one host plant. Branched broomrape had no effect on the biomass or pod yield of pot-grown Morava or Blanchefleur cultivars of vetch. Popany produced lower yields that the *V. sativa* varieties in this pot trial.

#### Introduction

Common vetch (*Vicia sativa*) is a host of *Orobanche ramosa* subsp. aff. *mutelii* in South Australia. Vetch is a minor crop in the area affected by branched broomrape. Over the past ten years, an average of 0.5% or 8 paddocks within the Quarantine Area (QA) were planted with vetch each year (Survey X data base). The recent development of disease-resistant vetch varieties provides additional crop choices for grazing and hay production in the drier rainfall regions of the state. This crop has the potential to increase in production in the area affected by broomrape and the surrounding region. This has implications for the spread of broomrape and for the potential for yield losses as a result of broomrape infection.

Vetch has long been recognised as a host for many broomrape species in the Mediterranean region (Parker and Riches 1993). Cultivars of *V. sativa* differ in their susceptibility to broomrape infection such that broomrape control in vetch production can be reasonably addressed by appropriate cultivar selection (Gil 1999). Our host testing has found that that all *V. sativa* varieties tested are hosts of branched broomrape but *V. benghalensis* cv. Popany is not.

Although vetch is grown as a broadacre host crops in the QA, it is difficult to estimate potential yield losses given the patchy nature of the broomrape seed bank. However pot experiments may give some idea about the relative yield losses or susceptibility to infection of different cultivars of vetch.

The objectives of this study were to:

- determine the susceptibility to broomrape infection of cultivars of vetch; and
- compare yield of vetch varieties, infected or not infected by broomrape.

The outcomes from this project will feed into risk assessments for commodities grown in areas infected by broomrape. This project will also inform primary producers/agronomists about vetch cultivars that can be used to reduce risk of broomrape infection or yield losses and to make informed decisions about relative yield losses attributable to broomrape.

## Methods

A field pot experiment was set up at the trial site at Mannum. This cultivation method has been used previously and we have generally achieved better plant growth than under glasshouse conditions. Plants are also subjected to the seasonal and light conditions they would experience in the field.

We used three densities of broomrape seed in pots in order to create a range of broomrape infection densities on host plants that reflect the range of *in situ* seed bank densities:

- 1. Zero
- 2. Low (0.1 ml per pot) 9,000 seeds pot<sup>-1</sup>
- 3. High (0.5 ml per pot) 45,000 seeds pot<sup>-1</sup>

We trialled four varieties of vetch:

- *V. sativa* varieties Blanchefleur, Morava, Languedoc
- V. benghalensis variety Popany

Six replicate pots for each broomrape seed density and each cultivar were prepared by half filling pots with 2 L field soil on May 22nd-25th 2012. Boomrape seed and 15 ml Nutricote fertiliser at half recommended rate was mixed by hand into 2 L of field soil and added to the half-filled pots. Pots held 5.6 kg of soil. Pots were buried in the ground to control temperature and reduce moisture loss. Vetch seeds were sown into pots on June 12<sup>th</sup>. Plants emerged by July 4<sup>th</sup>, except the variety Languedoc, and were thinned to one host plant per pot on July 13<sup>th</sup>. Pots were watered after seed sowing and when necessary during crop development.

When plants had commenced flowering they were checked weekly and reproductive stage and broomrape emergence was recorded for each pot.

Broomrape had emerged in pots by October 18<sup>th</sup> or at 1700 GDD. Vetch plants were harvested after pod formation on October 31<sup>st</sup> or 2000 GDD. Plants were cut at ground level and the soil washed from roots. Broomrape plants were removed from roots under a microscope and sorted into development stages with counts made of live and dead broomrape plants. Pods were removed from plants and counted, dried then weighed. Broomrape biomass and vetch root and shoot biomass were dried and then weighed.

#### Results

The Languedoc variety did not germinate. Blanchefleur plants commenced flowering on September 25<sup>th</sup>. Morava and Popany flowering commenced by 4<sup>th</sup> October. Both infected and uninfected plants were flowering. Pods were present on infected and uninfected Morava and Blanchefleur plants by October 12<sup>th</sup>. All plants, with the exception of two Popany plants, had pods at harvest on 31<sup>st</sup> October. There were no visible differences between infected and uninfected treatments.

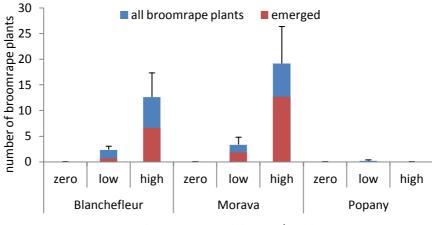
Only two Blanchefleur plants and one Morava plant had no infection with all other plants supporting several broomrape plants. The number of broomrape plants depended on broomrape seed density with more infections found where broomrape seed densities were high (Fig. 1). There was no difference between the number of broomrape plants on Morava and Blanchefleur. Infection of Popany was very low with one small dead broomrape found.

There was no significant difference between the root, shoot, or pod biomass of infected and uninfected vetch varieties (Fig. 2). There was no relationship between broomrape infection and measures of host biomass although there was a weak trend of reduced Morava biomass, particularly shoot biomass, with increasing broomrape density (Fig. 3). Popany vetch had less biomass than Blanchefleur and Morava (Fig. 2).

#### Discussion

Under the growth conditions in pots we did not detect any effects of *O. ramosa* subsp. *mutelii* on *Vicia sativa* cultivars Blanchefleur or Morava. The broomrape seed densities used in the experiment were chosen to represent typical densities that may be found in a paddock with low or high broomrape densities. Although these seed densities resulted in high infection rates of Morava and Blanchefleur there was no

reduction in vetch biomass or pod yield. The lack of any yield reduction in our study may be the result of the growing conditions in pots which would differ from a broadacre situation. However similar studies with other *Orobanche*-host systems using the same methods, measured yield reductions. In an experiment with *O. crenata* on faba bean hosts, reductions in pod yield occurred at 1,562 seeds kg<sup>-1</sup> soil (Linke et al 1991), fewer than our low rate which was equivalent to 1,600 seed kg<sup>-1</sup> soil or our high rate of 8,000 seed kg<sup>-1</sup> soil. Together with no reports of visible effects of broomrape in vetch fields in the QA there is no evidence that infection will result in production losses in vetch crops. This is consistent with overseas experience. For example, in the Mediterranean, *Orobanche* is not considered as a major risk to vetch crops although it is a host for several *Orobanche* species (Parker and Riches 1993). There is anecdotal evidence of crop damage with one report of "severe crop damage" by *Orobanche mutelii* on vetch in Israel (Jacobsohn et al 1991). *Orobanche crenata* infection resulted in a 60% reduction in pod number on a susceptible vetch variety in Spain (Perez-de-Luque et al 2004).



broomrape seed density / vetch variety

Figure 1. Broomrape infection of vetch varieties grown in pots with broomrape seed at three densities. Emerged includes broomrape plants that had developed underground stems that could have emerged post-harvest in addition to plants emerged and flowering at harvest. Each bar is the mean + 1 SE, n = 6.

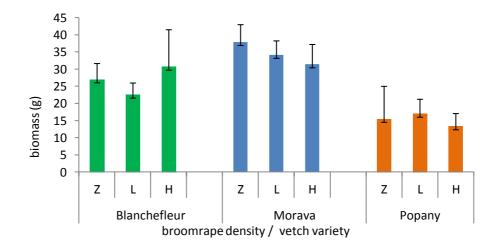


Figure 2. Total biomass of vetch varieties grown in pots with varying densities of broomrape seed. Bars are means + 1 SE, n=6.

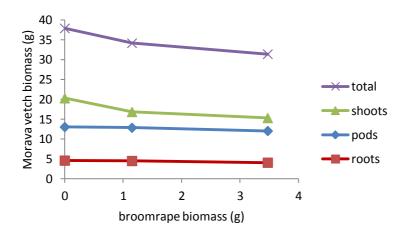


Figure 3. Components of Morava biomass plotted against broomrape biomass. Points are means, n = 6.

Orobanche ramosa subsp. mutelii failed to emerge in pots with Popany hosts. Popany is resistant to infection by other species of Orobanche as necrosis develops at an early stage when the parasite attaches to the host root (Goldwasser et 2000, Goldwasser et al. 1997). The single plant that we recorded on Popany was dead and had not developed a stem, suggesting a similar mechanism may occur in the interaction between *O. ramosa* subsp. *mutelii* and Popany. Our host testing of this variety detected low proportions of germination stimulation but no further testing in pots was done. As we did not detect emergence in this experiment there is no change in the status of Popany as a non-host.

The choice of less susceptible strains of vetch such as Popany could result in a yield penalty as this cultivar does not produce as much biomass as the *V. sativa* cultivars. However, as *V. sativa* cultivars are susceptible to broomrape infection, these crops could contribute to the persistence and/or spread of broomrape.

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# 4. Influence of soil type and sowing date on broomrape infection of carrot

Jane Prider and Andrew Craig Biosecurity SA August 2013

#### Summary

*Orobanche ramosa* subsp. *mutelii* occurs mainly on sandy soils within the area infested by the weed in South Australia. The ability of the plant to grow in other soil types remains untested. Vegetable crops are likely to be most at risk from broomrape infection and the main vegetable growing areas comprise soils that have higher clay content than soil where broomrape has been found growing. In this experiment we grew two varieties of carrot in the presence or absence of broomrape on sandy soils from Bowhill and Murray Bridge, within the current distribution of *O. ramosa* subsp. *mutelii*, and on more clay-rich soils from Virginia and Waikerie, outside this distribution. All soils were sourced from plots where carrots are cultivated. Broomrape was able to grow on both carrot varieties and in soil from all sources. Soils from Virginia grew the largest carrots and had the highest broomrape infection but we could detect no reduction in carrot biomass in broomrape-infested plants. Infection was higher following a spring sowing than a summer sowing and we consider that higher soil temperatures in summer reduce broomrape germination. Broomrape infection, may be poor under pot cultivation, similar results have also been reported from a field trail in 2006. The results from this study indicate that broomrape could grow in soils used for vegetable production in South Australia.

#### Introduction

Overseas experience has shown that intensively managed crops such as vegetables are particularly susceptible to broomrape impacts (Parker 2009). To date, branched broomrape has not resulted in production losses in vegetable crops in South Australia. Vegetable production is only a minor land use in the Quarantine Area (QA) but with a relaxation in containment and control measures, future spread of broomrape could present a risk to the State's premium vegetable production areas. Our host testing has found that a broad range of vegetables are broomrape hosts but the impact of the parasite on the yield of these hosts is not known. In addition, the ability for broomrape to establish in soil types outside the QA has not been tested.

Carrots are one of a number of vegetable crops that can be severely impacted by broomrape with crop losses of up to 60% (Jacobsohn and Kelman 1980, Bernhard et al. 1998, Eizenberg et al. 2001). The broomrape species that cause the most damage are *O. aegyptiaca* and *O. crenata* (Bernhard et al. 1998, Eizenberg et al. 2001) although *O. ramosa* (Zehhar et al. 2003) and *O. mutelii* (Jacobsohn et al. 1991) have also been reported from carrot crops in Israel. Overseas research has found that broomrape infection of carrot is strongly temperature-dependent (Jacobsohn and Kelman 1980, Jacobsohn et al. 1991, Eizenberg et al. 2001). In crops grown in Israel, autumn-sown crops are more severely affected than spring-sown crops. This has been attributed to a reduction in the susceptibility to infection in carrots grown in warmer soils.

In South Australia, carrots are grown on sandy loams and silts in the Riverland, the Adelaide Plains and the Mallee, and volcanic soils in the south-east (Coles and Wicks 2003). Our host testing has found that several varieties are hosts for the broomrape strain in the QA. In this study we will use carrots as a test vegetable type to assess whether broomrape can establish in high-risk commodities outside the current area of infestation. The aims of this study were to:

- Determine the susceptibility to broomrape infection of carrot in a range of soil types representative of the major vegetable growing areas of South Australia
- Evaluate whether carrot susceptibility to broomrape infection differs in relation to sowing date
- Measure impacts of broomrape infection on carrot hosts

This project will enable us to assess the potential for broomrape to establish in vegetable production systems outside the QA. This has implications for risk assessment and farm biosecurity planning in vegetable production areas.

## Methods

Carrots and broomrape were grown in pots of soil in a glasshouse. There were four factors in a full factorial experimental design; soil type, sowing date, carrot variety and broomrape infection. Soil was sourced from carrot growing areas in the QA at Murray Bridge and Bowhill, from the Riverland at Waikerie and from the Adelaide Plains at Virginia. Soils were collected from the surface of plots where carrots had been cultivated. The soils from the QA were sandy loams. The Waikerie soil was a silty clay loam and the Virginia soil was a clay loam. Soils were sieved before use to remove stones and break up clods. Carrots were sown in spring and summer. For the spring sowing, seeds sown on September 19<sup>th</sup> germinated poorly so were resown on October 8<sup>th</sup>. The summer sowing occurred on December 21<sup>st</sup>. We sowed the carrot varieties Nantes and All Seasons at both sowing dates.

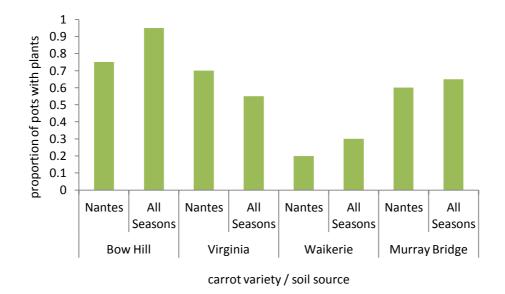
Pots of soil for broomrape treatments were prepared by thoroughly mixing approximately 6,000 broomrape seeds and 5 ml of Osmocote<sup>™</sup> fertilizer into 0.8 L of the collected field soil and filling 0.8 L deep, square pots. The same mixing method was used for the control pots but no broomrape seed was added. Carrot seed was sprinkled on the soil surface and covered with 5-10 mm of seed raising mix. 10 replicate pots were prepared for each treatment combination. Pots were kept well watered and soil temperature was monitored with T-Tech temperature loggers. Carrot plants were thinned to one plant per pot after the second set of true leaves appeared.

The spring sown carrots were harvested on January 14<sup>th</sup> and 15<sup>th</sup> at 1780 GDD. The summer sown carrots were harvested on March 27<sup>th</sup> and 28<sup>th</sup> at 2370 GDD. The carrot stems were removed and the soil washed from the root systems. Broomrape plants were separated from carrot roots. The number of broomrape plants at different stages of development was determined by examination under a microscope. Carrots were weighed fresh and after oven drying at 75 °C. Broomrape and carrot fine root dry weight was measured for the summer harvest.

## Results

#### **Spring sowing**

Both carrot varieties established poorly in some soils for the spring sowing. Overall only 59% of replicate pots had carrots due to repeated failures in germination or survival of young seedlings. Carrots established best in the Bowhill soils and established very poorly in the Waikerie soils (Fig. 1).



## Figure 1. Proportion of pots with carrot plants of the two varieties in the soils from different sources.

The infection of carrot roots by broomrape differed between the four soil types. The highest number of broomrape plants occurred in soil from Virginia, with the variety All Seasons having more broomrape plants than the Nantes variety (Table 1). Both varieties of carrots grown in other soil types had very low numbers of broomrape plants. All broomrape plants observed were in the underground stage of development and no emerged plants were found by harvest time at 1780 GDD.

		Broomrape plants		
Spring		Nantes	All Seasons	
Soil source	Bowhill	$0.43 \pm 0.43$	0	
	Murray Bridge	1.5 ± 0.76	0.56 ± 0.38	
	Virginia	50.9 ± 17.35	156.8 ± 54.9	
	Waikerie	0	2.8 ± 2	
Summer				
Soil source	Bowhill	0.1 ± 0.32	0	
	Murray Bridge	0	2.1 ± 1.6	
	Virginia	23.9 ± 13.2	34 ± 11.7	
	Waikerie	0.22 ± 0.22	0.22 ± 0.22	

Table 1. Number of broomrape plants (mean  $\pm$  SE) on carrot hosts of two varieties grown in soils from different sources at two sowing dates.

Both carrot varieties grew poorly in all soil types. The largest carrots grew in the Murray Bridge soil and the smallest in the Waikerie soil. The carrots grown in the Virginia soils were the only plants with sufficient numbers of broomrape plants to look at effects of broomrape on carrot. For this soil type, there was no difference in carrot fresh or dry weight between broomrape present or absent treatments (Fig. 2A).

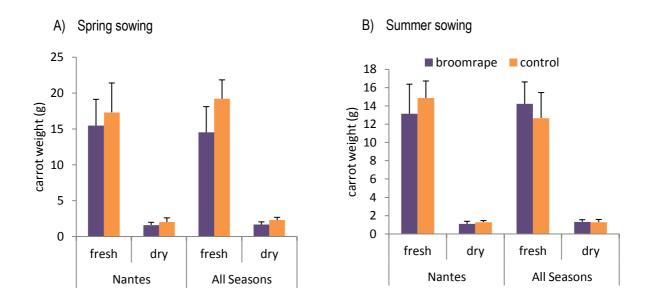


Figure 2. Fresh and dry weight of two varieties of carrot grown in the presence or absence of branched broomrape in soil from Virginia. Each bar is the mean  $\pm$  1SE.

#### Summer sowing

Carrots grew more successfully in the summer sowing. Nantes failed to establish in some pots of Murray Bridge and Virginia soils but All Seasons established in most pots.

Carrots were harvested at a later date for the summer sowing (2370 GDD) but there were no emerged broomrape plants at this time. Broomrape infection levels were low in soil from all sources except Virginia. For this soil type, there were fewer broomrape plants in the summer sowing than the spring sowing (Table1). Broomrape plants had reached similar stages of development on the two carrot variety hosts (Fig. 3). The majority of attachments were at an early stage of development where they were yet to develop stem buds or roots (Stage 3). Stage 5 attachments had stems that may have emerged given more time although harvest was delayed for the summer sowing to give plants the opportunity to mature.

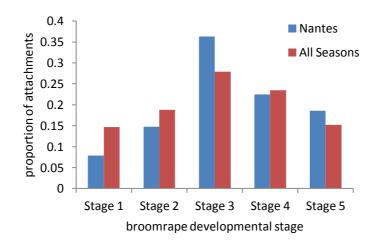


Figure 3. Proportion of broomrape plants at various stages of development on two carrot varieties in the Virginia soils. Each bar is the mean. Stage 1 – new attachments, Stage 2 – new tubercles, Stage 3 – tubercles without roots, Stage 4 – tubercles with roots and stem buds, Stage 5 – tubercles with stems, Stage 6 – emerged stems (not observed).

There was a significant difference in the fresh weight of carrots grown in soils from different sources (Table 2). Carrot fresh weight was smallest in the Bowhill soils and highest in the Waikerie and Virginia soils (Fig. 4). There was no difference in the fresh weight of the two carrot varieties or with broomrape infection (Table 2). The control/broomrape treatments for the Virginia soil were tested in isolation as there were low broomrape numbers in other soil types. There was no significant difference in carrot fresh or dry weight between broomrape infected and control treatments or between carrot varieties (Fig. 2B). Carrot fine root production did not differ between soil types (data not shown).

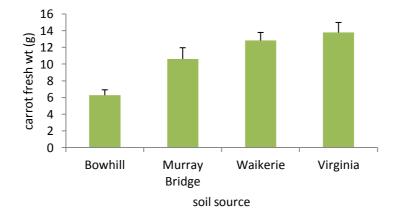


Figure 4. Fresh weight of carrots grown in soil from four sources. Data has been combined for two carrot varieties and broomrape infested and control treatments. Each bar is mean + 1 SE.

Table 2. Results of chi-square tests comparing the fits of Generalised Linear Models (Gamma distribution, reciprocal errors) for each of the experimental factors. The chi-square tests for differences in the residual deviance of each model fit. Models 2-4 are compared with Model 1.

	Factor	Residual df	Residual deviance	$\Delta  \mathrm{df}$	$\Delta$ deviance	Chi-square p-value
Model 1		142	83.81			
Model 2	Soil source	139	70.72	3	13.09	0.004*
Model 3	Broomrape infection	141	82.73	1	1.08	0.298
Model 4	Carrot variety	141	82.48	1	1.33	0.248

significant fit at α < 0.05</li>

#### Discussion

Carrot cultivation was not ideal in potted plants. The soils were prone to water logging and this was considered to be the cause of the poor establishment of carrots in the spring sowing. Pots were watered less frequently for the summer sowing and this may have improved carrot establishment and seedling survival of this second sowing.

Broomrape infection was poor in all soils with the exception of the soils from Virginia. These soils also produced superior carrots as although they were of similar fresh weight to carrots from Waikerie soils, they were of a longer shape. The Waikerie carrots were short, thick and stunted. Broomrape plants did not develop to emerge above the soil surface even though the summer harvest was delayed until after 2000 GDD. Emerged carrots have been observed in other pot experiments from 1500 GDD or 100 days after

sowing. Although our harvest dates were less than 100 days after sowing, broomrape stems were not sufficiently advanced to be approaching emergence.

We have shown that broomrape can grow in a variety of soil types, including heavier clay-rich soils. This was unexpected as in the QA broomrape is predominantly found on sandy soils. The low number of broomrape plants found in the other soil types was surprising given that two of the soil types were sourced from the current area of broomrape infestation in the QA.

Both carrot varieties were susceptible to infection by broomrape with higher infection of All Seasons in the Virginia soil in both the spring and summer sowings. The fewer attachments in the summer sowing may be the result of the higher soil temperatures at this time. Laboratory experiments have found that very few broomrape seeds germinate when the temperature exceeds 25 °C. The average soil temperature measured following the summer sowing was 25.4 °C. For the spring sowing it was 18.2 °C, falling within the optimal temperature for broomrape germination of 18- 22 °C. We measured similar soil temperatures in the different soil types therefore this cannot explain the higher infection rates in the Virginia soil. Could there be more efficient movement or persistence of germination stimulants in this soil type? Carrots grown in soils from Virginia did not produce more roots so this does not explain the higher infection rate. Broomrape is intolerant of water logging, which could explain the poor infection in the spring sowings given that soil temperatures were optimal for germination.

Carrot does not appear to be very susceptible to the broomrape strain present in South Australia. Although several carrot varieties are hosts, the number of broomrape plants that grow to emergence is very low. Trials in plots established at the Mannum Trial Site in 2006 found that only 0.05% of carrot plants had emerged broomrape, compared with 12% of cabbage plants and 3% of broccoli. Previous glasshouse experiments have also recorded low emergence on carrot hosts. In this experiment, our observations of high numbers of immature broomrape attachments on carrot roots suggests that host resistance occurs after the haustorium has formed, such that the development of broomrape becomes retarded. Work in Israel has found that *Orobanche crenata* and *O. aegyptiaca* development of carrot is arrested at higher temperatures and although broomrapes attach to carrot roots they fail to develop further (Eizenberg et al. 2001). Changes to host cells at the haustorial interface and necrosis of broomrape at early developmental stages have been observed in some carrot varieties grown at higher temperatures (Eizenberg *et al.* 2001). Our data supports this but low infection during spring when soil temperatures were low is still unexpected. Certainly the number of seeds present in pots should have been sufficient to result in infection. Bernhard et al (1998) found carrot production was affected with as few as 200 *O. crenata* or *O. aegyptiaca* seeds kg<sup>-1</sup>.

#### Conclusions

- We demonstrated that broomrape will grow in pots with carrot hosts in a variety of soil types, including soils with a high clay content.
- Carrot is not highly susceptible to broomrape infection and broomrape development to maturity is quite poor on carrot hosts, as demonstrated in pot trials and one field trial.
- We did not measure any yield losses in carrot as a result of broomrape infection in potted plants.

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