Scoping study to determine the soil borne diseases affecting Brassica crops

Trevor Wicks
SA Research &
Development Institute

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Level 1
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Telephone: (02) 8295 2300
Fax: (02) 8295 2399

E-Mail: horticulture@horticulture.com.au

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Scoping Study to Determine the Soilborne Diseases Affecting Brassica Crops

FINAL REPORT

HORTICULTURE AUSTRALIA LIMITED

VG05005

30th July 2006

By C.J. Hitch, B.H. Hall and T.J. Wicks
South Australian Research and Development Institute

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Final Report 31st July 2006

Project Title: Scoping study to determine the soilborne diseases affecting brassica crops.

HAL Project Number: VG05005

Research Organisation: South Australian Research and Development Institute
GPO Box 397, Adelaide, SA 5001

Project Leader: Dr Trevor Wicks
Phone: (08) 8303 9563
Fax: (08) 8303 9393
Email: wicks.trevor@saugov.sa.gov.au

Key Personnel: Catherine Hitch

Barbara Hall

Dominic Cavallaro

Dr Mark Sosnowski

Soilborne diseases of brassica crops occur every year with varying degrees of severity, and affect all stages of growth from seedlings to mature plants. In some years plant losses of 70-80% occur in cauliflower plantings in South Australia losses also occur in other brassica growing areas of Australia. Additional economic losses occur as a result of increased harvesting and sorting costs. Crop loss in recent years has been caused by the disease complex known as brassica stem canker. The aims of this project were to investigate the cause, extent and economic impact of this disease complex.

We acknowledge the Vegetable Industry and the Commonwealth Government for funding this project through Horticulture Australia Limited.

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MEDIA SUMMARY

Soilborne diseases of brassica crops occur every year with varying degrees of severity and can affect all stages of growth from seedlings to mature plants. In some years South Australian growers reported losses of 70-80% in cauliflower plantings. Losses are also occurring in other brassica growing areas of Australia. This crop loss is caused by a disease complex known as brassica stem canker first observed in South Australia in 2000.

The aims of this project were to determine the extent of the problem in Australia, to investigate whether the problem arises in the nursery or mainly in the field after planting, identify the soil fungi causing plant death in brassica crops and to determine the economic importance of brassica stem canker.

Brassica stem canker is a disease complex of several fungi causing symptoms that range from superficial scurfing, russetting and discrete lesions on stems to complete stem rot and plant collapse. Fungal pathogens involved in the disease complex included *Phoma* (Black leg), *Rhizoctonia*, *Fusarium* and *Pythium*.

Surveys conducted on 113 brassica crops throughout Australia showed stem canker occurred in all mainland states. While worst in cauliflower it was also detected on Brussels sprout, broccoli, green and red cabbage. In some plantings in South Australia, 100% of stems were affected and complete plant collapse occurred by harvest in the spring of 2005.

The survey of 38 winter cauliflower plantings in South Australia showed that an estimated $309,000 per week was lost due to unmarketable and collapsed plants.

This scoping study has provided an understanding on the causes of the canker, but further research is required to formulate management strategies that will be vital for the continued sustainability of the industry.

TECHNICAL SUMMARY

Brassica stem canker is a disease complex first observed in South Australia in 2000 where it causes crop loss of 70-80% in cauliflower crops. This 12 month study has investigated the extent of the problem throughout Australia, the major fungal pathogens involved and the cost to the industry.

1) Symptoms typical of brassica stem canker range from superficial scurfing and russetting on lower stems, discrete lesions covering 25% of the stems to complete stem rot and plant collapse. Both scurfing and discrete lesions often progress to complete stem rot.

2) Seventeen properties were surveyed in South Australia which included 52 cauliflower, 3 red cabbage, 3 broccoli and 6 Brussels sprout plantings. Four cauliflower plantings in Queensland, 12 in Victoria, 11 in Western Australia and 13 in New South Wales were surveyed, as well as 4 red cabbage, 2 green cabbage, 1 broccoli and 2 Brussels sprout plantings in NSW.

3) Brassica stem canker was found in all mainland states, it is more severe in cauliflower but also affects Brussels sprout, red and green cabbage and broccoli. No differences were observed between brassica cultivars for all crops assessed.

4) The incidence of brassica stem canker was highest in South Australia where up to 100% of some plantings were affected in spring 2005 with complete plant collapse. Incidence levels ranged from 0-100% in South Australia, 1-25% in Qld, 0-38% in Vic, 0-28% in WA and 0-74% in NSW.

5) Continuous surveys were carried out on 22 winter and 7 summer cauliflower plantings in South Australia. Surveys began 2 to 4 weeks after planting and plants were assessed every 2 weeks up to harvest. Symptoms typical of brassica stem canker were first observed 2-4 weeks after planting and increased in incidence and severity as plants mature.

6) No disease was observed in nursery speedlings. Treatments with fungicides such as Terrachlor® and Rovral® in nursery/pre-field for *Rhizoctonia* have minimal effect on disease levels.

7) Over 500 plants were collected tested for the presence of fungal pathogens with over 1,000 fungal isolates recovered from brassica plants, roots and soil.

8) The fungi *Rhizoctonia*, *Phoma*, *Fusarium* and *Pythium* were frequently isolated from diseased plants. *Sclerotinia*, also a known pathogen did not seem to play a role in the disease complex. Other fungi isolated from plants included *Phytophthora megasperma*, *Peronospora parasitica* (downy mildew), *Alternaria* sp., *Stemphylium* sp., *Cylindrocarpon* sp., *Ulocladium* sp., *Pleospora* sp., *Colletotrichum* sp., *Epicoccum* sp., *Cladosporium* sp. and numerous unidentified fungal species.

9) *Rhizoctonia* was recovered from the roots of weeds including fat hen, knotweed, thistles, rye grass, sour sob and paddy melon during the survey but no obvious symptoms typical of brassica stem canker were found. *Fusarium*, *Alternaria* and *Cladosporium* were recovered from volunteer potatoes.

10) Pathogenicity tests were carried out using cauliflower seedlings which were inoculated with 62% of the isolates collected. Symptoms typical of brassica stem canker developed on plants inoculated with either *Rhizoctonia*, *Phoma*, *Fusarium* and *Pythium*.

11) All *Rhizoctonia* isolates were pathogenic causing wirestem symptoms after 21 days. The Anastomosis Groups (AG) of 63 out of the 102 *Rhizoctonia* isolates were determined by AG PCR techniques. AG 2.1, 2.2 and 4 were detected with over 50% as AG 2.1.

12) Further research is necessary to develop management strategies to sustain the future of the industry. Investigations are now required to determine the relative importance of each pathogen in the complex, the environmental conditions conducive to disease development and effective management strategies such as chemical and bio-control, bio-fumigation, crop rotations and irrigation management.

INTRODUCTION

Soilborne diseases of brassica crops occur every year with varying degrees of severity and can affect all stages of growth from seedlings to mature plants. In some years losses are severe, with South Australia growers reporting losses of 70-80% in cauliflower plantings. In recent years much of this crop loss has been caused by a disease complex known as brassica stem canker, first observed in South Australia in 2000. Fungi such as *Rhizoctonia*, *Fusarium*, *Pythium*, *Phoma*, and *Sclerotinia* have been recovered from an increasing number of diseased plants submitted to the diagnostic service at the South Australian Research and Development Institute. These fungi are pathogens of crucifers, causing damping off, root rot and stem damage of both seedlings and mature plants. However it is not uncommon to recover several fungi from the same affected plant, and it is uncertain what role each plays in the disease complex.

The aim of this project was to determine the extent of the brassica stem canker in Australia, investigate whether the problem arises in the nursery or mainly in the field after planting, identify the soil fungi causing plant death and determine the economic importance of brassica stem canker.

DISEASE SURVEY

Introduction

Extensive surveys of brassica plantings throughout Australia were carried out in 2005/06. The survey was undertaken predominantly in cauliflowers as symptoms typical of brassica stem canker are more prevalent in this host.

Survey methods

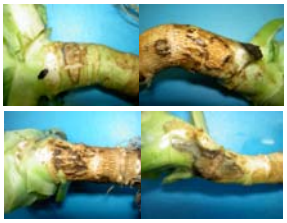
In July 2005, cauliflower crops on two properties on the Northern Adelaide Plains were checked for symptoms typical of brassica stem canker and samples collected for isolations. Growers were contacted and brassica crops were also assessed for the incidence of symptoms typical of brassica stem canker. Brassica crops in all mainland states were assessed from July 2005 to May 2006. Mature crops were examined 0 to 2 weeks before harvest by counting the number of affected stems out of 100 plants from 10 areas of 10 plants selected at random within each planting.

The stem symptoms were categorised as described in Figure 1, and samples collected and returned to the laboratory to isolate and identify possible causal organisms. Other affected brassicas plants which were not surveyed were also collected for processing.

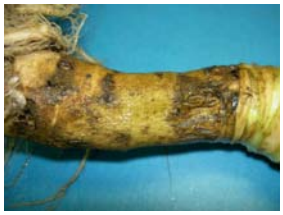
1: "Sclerotinia like" lesion high on stem / white mycelial growth.
2: Stem lesion including scurfing / russetting / watersoaked lesions.
3: Stem lesions which have pitted or cankered the stem.
4: Typical "Phoma like" lesions high on the stem, including leaf lesions.
5: Water mark lesions on stem.
6: Stems with more than one symptom were given this classification and the 2 or 3 symptoms noted.

Figure 1. Disease symptom ratings used for assessing winter brassica plantings 2005/06.

Symptom 1



Symptom 2



Symptom 3

Symptom 4

Symptom 5

Symptom 6

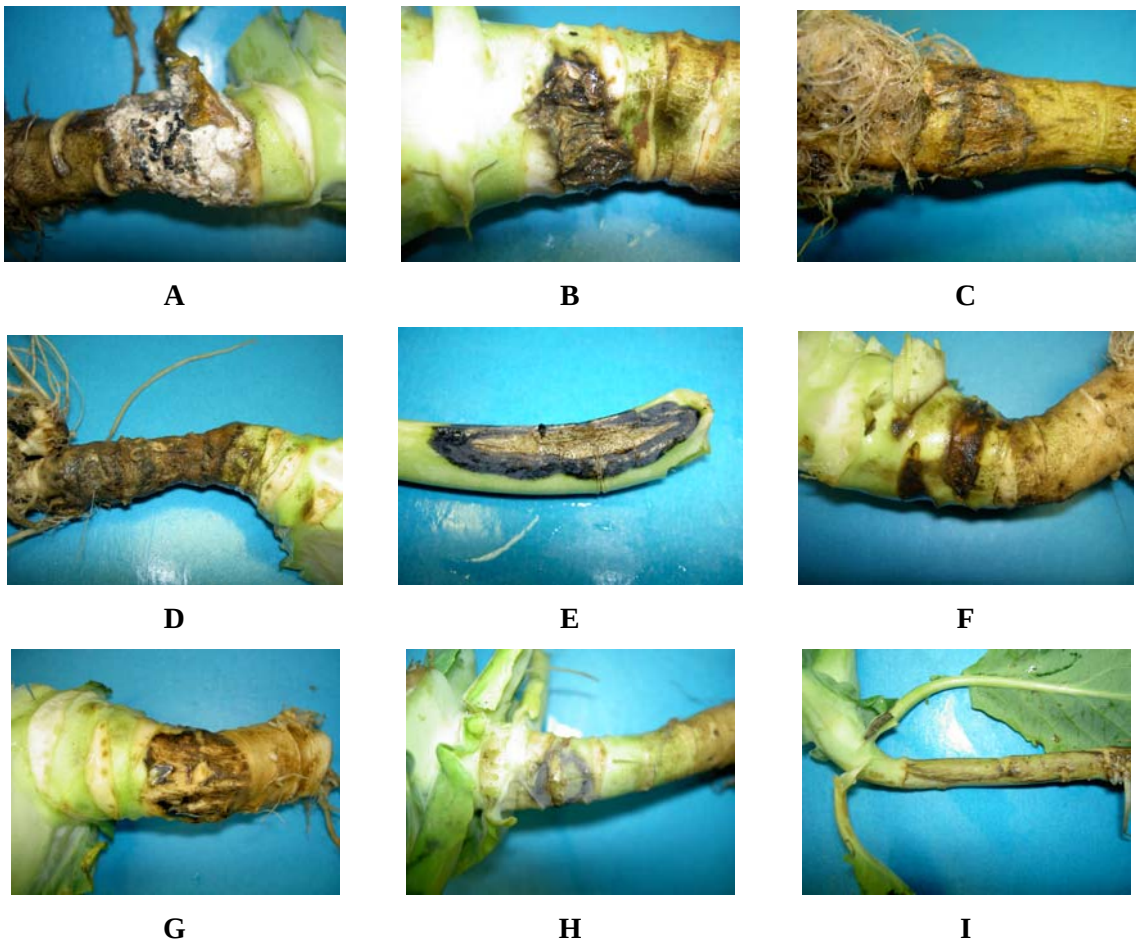
Initially all brassica stems with symptoms were collected and returned to the laboratory. Stems were washed, photographed, symptoms described and examined microscopically for evidence of fungi and bacteria before isolations were made onto various media.

As the survey continued the symptoms on brassica stems were categorised into more descriptive groups described below (Fig 2). These new assessment groups provided a more effective way to compare symptoms to the fungal organisms isolated from these symptoms. A representative plant sample showing the symptoms was collected and returned to the laboratory for processing as previously described.

The stem samples that were collected and processed using the original rating groups were re-categorised into the new symptom groups using the sample photographs taken. Symptoms and fungal organisms could then be analysed on all the data collected during the survey.

A: *Sclerotinia* lesions
B: "Phoma like" lesions high on stems.
C: Soft stem russetting.
D: Severe stem canker.
E: Leaf lesions.
F: Lesion where leaves removed.
G: Hard black cracked russetting.
H: Water soaked lesions high on stem.
I: Water mark lesion on stem (Symptom 5, figure 1).

Figure 2. Disease symptom ratings used for assessing summer brassica plantings, 2006.



Surveys interstate were undertaken at times when the symptoms typical of brassica stem canker were more prominent in the field. The symptom assessment categories used in South Australia were also used interstate. Surveys were carried out on cauliflower crops in WA, Qld, NSW and Vic.

Weeds and volunteer potatoes were collected from 3 sites during the survey and assessed for symptoms typical of brassica stem canker and fungal organisms.

Survey Results

A total of 113 brassica crops were surveyed for symptoms typical of brassica stem canker in South Australia, Victoria, New South Wales, Queensland and Western Australia. Crops included cauliflower, Brussels sprouts, red and green cabbage and broccoli. The incidence of symptoms typical of brassica stem canker ranged from 0-100% (Table 1).

Table 1. Summary of the incidence of symptoms typical of brassica stem canker seen on brassicas during the survey 2005/06 (Refer to Appendix 1 for complete survey data).

State	Crop	No. plantings assessed	Infection (%)
South Australia	Cauliflower	52	0-100
	Brussels sprout	6	64-100
	Red Cabbage	3	49-83
	Broccoli	3	0-7
New South Wales	Cauliflower	13	4-74
	Brussels sprout	2	2-6
	Red Cabbage	5	0-51
	Green Cabbage	1	15
	Broccoli	1	1
Victoria	Cauliflower	12	0-38
Western Australia	Cauliflower	11	0-28
Queensland	Cauliflower	4	1-25

Symptoms typical of brassica stem canker were found in all states surveyed with the incidence of disease varying between states. The worst affected crops were found in South Australia where up to 100% of cauliflower stems were affected in some plantings. Complete plant collapse occurred on the Northern Adelaide Plains in spring 2005. While growers interstate commented that the symptoms of brassica stem canker were more severe in late summer, collapsed plants were not seen during the survey in other states. All symptoms were found in plantings to varying degrees of severity, from plants affected with stem lesions but growing to their full production potential to plants with severe canker that collapsed before the end of the growing season.

All brassica crops surveyed were affected with varying degrees of severity by symptoms of brassica stem canker. Cauliflowers were the most susceptible brassica crop surveyed, along with Brussels sprouts and red cabbage. While the incidence of symptoms was low in broccoli and green cabbage.

No obvious differences were seen between crop cultivars. All cultivars of cauliflower examined were susceptible to brassica stem canker. Cultivars surveyed included Artic, Discovery, Skywalker, Nova, Nautilus, Chaser, Summer Love, Aviron, Forte, Aviso, Monarch, Ascale, Fremont and Freeman 7. Brussels sprout cultivars were assessed including Adagia, Mistral, Abacus, 1999 and Oliver. While the incidence of symptoms in the cultivar Oliver was much lower than Adagia, Mistral, Abacus and 1999 no direct comparison can be made as the Oliver sprouts were grown in NSW not South Australia.

No differences in disease levels were observed between different nursery supplier. Three hundred plants from two nurseries in South Australia were tested and no fungal pathogens were found. No difference in disease incidence was seen in the field with both nursery transplants affected by

brassica stem canker. Treatments with fungicides of Rovral® or Terrachlor® in nursery/pre field planting for *Rhizoctonia* had minimal effect on disease levels.

The incidence of stem canker was similar in crops planted into either virgin, rotation or continuous cropping ground.

Weeds sampled during the survey included volunteer potatoes, fat hen, rye grass, thistles, sour sob, paddy melon, stinging nettles, marshmallow, knotgrass and fennel. No stem lesions were seen on volunteer potatoes or on the weeds surveyed within the crops however fungal mycelia was recovered from roots.

Economic loss

The assessments carried out on the 38 cauliflower plantings in South Australia were used to estimate the economic loss caused by brassica stem canker (Table 2). The numbers of plants with severe stem canker (symptom D, figure 2) were used for these calculations.

The following assumptions were made to estimate the cost of brassica stem canker.

1) The plants assessed with severe canker would not be harvested due to plant collapse.
2) A planting contained 10,000 plants.
3) A planting was planted every week.
4) Assessing 100 plants per planting gave an adequate indication of planting infection.
5) Cauliflowers were sold for $2 each.

Table 2. Calculation of economic losses caused by brassica stem canker – Winter 2005.

Grower	Number of plants assessed	Number of plants with severe canker	% with severe canker	Number of plantings assessed	Total number cauliflowers not harvested	Estimated loss ($)
1	500	262	52.4	5	26,200	52,400
2	300	178	59.3	3	17,790	35,580
3	200	129	64.5	2	12,900	25,800
5	700	300	42.9	7	30,030	60,060
7	200	78	39	2	7,800	15,600
8	300	203	67.6	3	20,280	40,560
9	200	133	66.5	2	13,300	26,600
10	192	167	86.9	2	17,380	34,760
11	200	17	8.5	2	1,700	3,400
12	200	43	21.5	2	4,300	8,600
13	300	16	5.33	3	1,599	3,198
14	200	1	0.5	2	100	200
15	100	2	2	1	200	400
16	200	13	6.5	2	1,300	2,600
			Total	**38**	**154,879**	**$309,758**

The 38 plantings assessed over winter 2005 cost growers an estimated $309,758. This figure is an approximation however the total indicates that brassica stem canker is dramatically reducing the potential earnings of growers.

DISEASE PROGRESSION

Continuous survey

Continuous monitoring of cauliflower crops throughout the growing season on 28 plantings. Twenty one winter plantings on 4 properties and 7 summer plantings on 1 property were assessed every 2 weeks with assessments beginning 2 to 4 weeks after planting and continuing until harvest (approximately 14-18 weeks after planting). One hundred plants per planting were assessed for symptoms typical of brassica stem canker using both rating assessment methods described previously.

Results

Symptoms typical of brassica stem canker were seen on cauliflower plants at 2 weeks after planting however incidence levels were under 6% (Table 3). Ten to 12 weeks after planting over 50% of cauliflower stems had developed symptoms typical of brassica stem canker. This trend was seen in all winter plantings. All properties surveyed showed similar progression trends with up to 100% of stems affected by harvest and plant collapse evident in the plantings (Table 3 & 4).

The incidence of stem symptoms in the summer plantings was less than those in winter plantings with 50% of cauliflowers affected by harvest (Table 5). Crops planted in December were more affected than those planted in February and March with 80% and 50% of stems affected respectively.

Table 3. Incidence of symptoms typical of brassica stem canker in winter cauliflower plantings, Northern Adelaide Plains, 2005.

Planting	**Plot 1**	**Plot 2**	**Plot 3**	**Plot 4**	**Plot 5**	**Plot 6**	**Plot 7**
Planting date	11/5/05	25/5/05	8/6/05	25/6/05	13/7/05	27/7/05	10/8/05
Survey date	**% of plants infected / (Age of plants in weeks)**						
25/08/05	98 (15)	31 (13)	16 (11)	25 (9)	33 (6)	9 (4)	6 (2)
08/09/05	81 (17)	63 (15)	57 (13)	44 (11)	27 (8)	12 (6)	1 (4)
22/09/05	H	86 (17)	96 (15)	68 (13)	17 (10)	12 (8)	0 (6)
06/10/05		100 (19)	96 (17)	84 (15)	36 (12)	11 (10)	5 (8)
20/10/05		H	H	94 (17)	91 (14)	65 (12)	33 (10)
03/11/05				H	94 (16)	83 (14)	73 (12)
17/11/05					H	89 (16)	87 (14)
						H	H

H: Crop harvested.

Table 4. Incidence of symptoms typical of brassica stem canker in winter cauliflower plantings, Northern Adelaide Plains, 2005.

Planting	**Plot 2**	**Plot 3**	**Plot 4**	**Plot 5**	**Plot 6**	**Plot 7**	**Plot 8**	**Plot 9**	**Plot 10**	**Plot 11**	**Plot 12**	**Plot 13**	**Plot 14**	**Plot 15**
Planting date	5/4/05	19/4/05	3/5/05	17/5/05	31/5/05	28/6/05	5/7/05	19/7/05	28/7/05	12/8/05	25/8/05	6/9/05	13/9/05	22/9/05
	Property 1									Property 2			Property 3	
Survey date	**% of plants infected / (Age of plants in weeks)**													
10/08/05	98 (18)	88 (16)	87 (14)	69 (12)										
25/08/05	84 (20)	64 (18)	74 (16)	24 (14)	52 (13)	41 (8)	0 (7)	55 (5)	3 (4)					
08/09/05	62 (22)	62 (20)	69 (18)	21 (16)	65 (15)	72 (10)	30 (9)	63 (7)	7 (6)					
22/09/05	H	H	H	52 (18)	86 (17)	60 (12)	49 (11)	54 (9)	7 (8)	2 (6)	8 (4)	0 (2)		
07/10/05				H	82 (19)	98 (14)	95 (13)	59 (11)	26 (10)	6 (8)	8 (6)	6 (4)		
20/10/05					H	100 (16)	96 (15)	89 (13)	59 (12)	34 (10)	19 (8)	0 (6)		
03/11/05						99 (18)	100 (17)	75 (15)	94 (14)	77 (12)	39 (10)	35 (8)		
17/11/05						H	H	H	H	99 (13)	67 (12)	41 (10)		
01/12/05										H	81 (14)	81 (12)	66 (11)	95 (12)
15/12/05											H	H	77 (13)	98 (14)
													H	H

H: Crop harvested.

Table 5. Incidence of symptoms typical of brassica stem canker in summer cauliflower plantings, Northern Adelaide Plains, 2006.

Planting	**Plot 1**	**Plot 3**	**Plot 5**	**Plot 7**	**Plot 9**	**Plot 11**	**Plot 13**
Planting date	5/12/05	23/12/05	5/1/06	18/1/06	3/2/06	16/2/06	3/3/06
Survey date	**% of plants infected / (Age of plants in weeks)**						
17/01/06	7 (5)	2 (3)	0 (2)				
31/01/06	56 (7)	10 (5)	5 (4)	0 (2)			
14/02/06	88 (9)	75 (7)	20 (6)	1 (4)			
28/02/06	79 (11)	100 (9)	81 (8)	13 (6)	0 (4)	0 (2)	
14/03/06	61 (13)	85 (11)	89 (10)	78 (8)	9 (6)	0 (4)	
28/03/06	H	87 (13)	81 (12)	89 (10)	26 (8)	3 (6)	1 (4)
11/04/06		H	H	49 (12)	27 (10)	17 (8)	0 (6)
26/04/06				H	56 (12)	20 (10)	5 (8)
09/05/06					19 (14)	42 (12)	12 (10)
23/05/06					H	40 (14)	34 (12)
06/06/06						H	45 (14)
20/06/06							H

H: Crop harvested

Symptom Progression

Two summer cauliflower plantings were used to follow the progression of disease during the season. Plants were tagged when stem symptoms first appeared and rated according to symptom type and photographed. Cauliflowers which were planted 4 and 5 weeks prior to assessment were tagged on the 31st January 2006 and 14th February 2006 and assessed every 2 weeks until 28th March 2006 where cauliflowers were harvested. Plant stems were collected and returned to the laboratory for a final stem assessment. Soil was also collected from the base of each plant and tested for soil pathogens. No stem isolations were made from diseased stems.

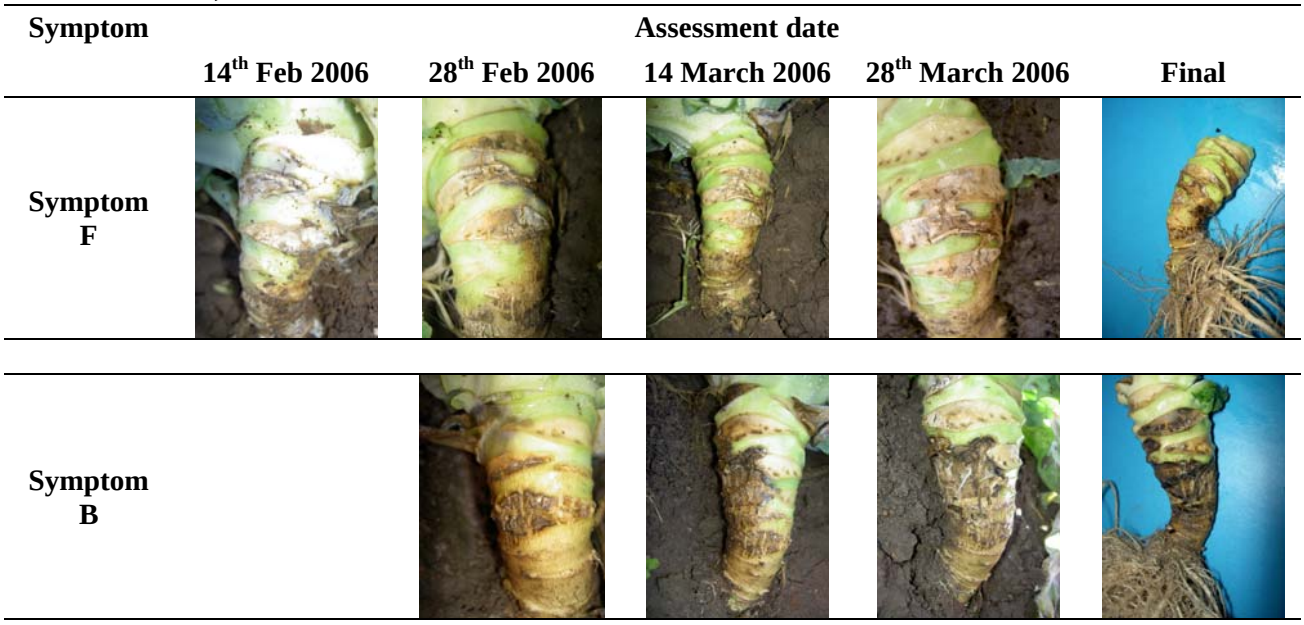
Results

The incidence of symptoms typical of brassica stem canker on cauliflowers increased dramatically between 31st Jan and 14th Feb. For example, only 25 cauliflower stems showed symptoms when plants were first tagged compared to 75 plants 2 weeks later.

While the severity of disease symptoms increased (Fig 3) they did not progress at the same rate in the winter plantings where severely cankered plants collapsed by harvest. Due to the high incidence of stem canker on the grower's property in previous plantings, a biological control agent was applied to these cauliflowers during the growing season. While the canker symptoms were still present in the plantings the severity of symptoms did not progress to that seen in other plantings surveyed. The symptoms dried and the severity did not increase. Many factors apart from the bio-control agent could have restricted the progression of symptoms in these summer plantings including high temperature, short growing period compared to winter crops, property location, cultivar susceptibility and soil type.

Pythium was detected in 22% of the soil samples collected from the affected plants.

Figure 3. Symptom progression on two cauliflower stems affected with symptom F and B, Northern Adelaide Plains, SA 2006.



PATHOGEN IDENTIFICATION

Materials and Methods

Isolation of organisms from stem canker symptoms

Diseased tissue from the stems and roots of affected plants were surface sterilised using 4% sodium hypochlorite solution, rinsed thoroughly, dried in a laminar flow cabinet and plated onto a range of different media including, Corn Meal Agar (CMA), Potato Dextrose Agar (PDA) at both full and ¼ strength, Tap Water Agar (TWA) and a *Phytophthora* specific agar (P10VP+) (1). Plates were incubated at 25^{o}C for 10-14 days with a 12 hour photoperiod and then examined for the presence of fungal pathogens.

Soil was baited with *Eucalyptus sieberi* cotyledons to detect *Pythium* and *Phytophthora*. Approximately 50g of soil collected from the root zone of brassica plants was placed in a small plastic tub with 200ml of demineralised water. The cotyledons were floated on the surface for 7 days, then removed, dried and placed onto CMA and P10VP+. After 7-10 days incubation the plates were examined for the presence of *Pythium* or *Phytophthora*.

All isolates were cultured and stored at 4^{o}C until required for pathogenicity testing. Isolates that were commonly recovered from affected stems were sent to mycologist M. Priest at the Department of Agriculture, Orange, NSW for formal identification. Stems with bacteria were forwarded to Ms. D Noble, bacteriologist with the NSW DPI for identification.

Weed samples were washed and the roots examined microscopically for the presence of fungal mycelia. Any mycelia found was picked off with fine-point tweezers and plated onto TWA and incubated for 7 days for fungal identification.

Pathogenicity

Pathogenicity tests were carried out on a selection of the isolates recovered from affected brassica stems. Speedling trays were seeded at a commercial nursery with cauliflower cultivars of either Aviron, Skywalker or Chaser and collected before any fungicide treatments were applied. Cauliflowers were germinated and grown in a shade house for 4 to 11 weeks before being inoculated with fungal isolates. Five seedlings were inoculated with each isolate.

Two 8mm plugs of mycelia from 7-10 day old cultures grown on PDA or TWA were used to inoculate cauliflower seedlings. All isolates were tested by burying the mycelial plugs in the root zone of each plant. Seedling stems inoculated with “Phoma like” isolates were wounded by lightly scratching the surface before the mycelia plugs were added to the soil. Seedlings were maintained at either 15^{o}C (*Rhizoctonia*) or 25^{o}C (remaining isolates tested) and assessed every 2 weeks for stem canker and plant death.

Two pathogenic *Rhizoctonia* isolates were also used to inoculate older cauliflower plants 12 and 19 weeks of age using four 8mm plugs of mycelia as previously described. Plants were maintained in a growth room at 15^{o}C and assessed every 2 weeks for disease symptoms.

Rhizoctonia grouping

Rhizoctonia isolates were grouped using AG specific PCR techniques for the Anastomosis Groups 2.1, 2.2, 3, 4, and 8 (2).

Combination Testing of Isolates

Two *Rhizoctonia* isolates, 1 virulent and 1 non-virulent were tested in combination or alone with 3 isolates of *Phoma* which had different growth patterns on PDA. Inoculation techniques were the same as those used for individual isolate pathogenicity tests. Two trays of infected plants were set up and maintained in either 15 or 25^{o}C and assessed for symptoms typical of brassica stem canker.

Results

Brassica stem canker causal organisms

Over 500 brassica plants affected with symptoms typical of brassica stem canker and over 1,000 fungal isolates were recovered from the stems, roots and soil. These were grouped morphologically by cultural characteristics and five known pathogens of brassicas were identified including *Phoma* (Black leg), *Rhizoctonia*, *Sclerotinia*, *Fusarium* and *Peronospora parasitica* (downy mildew). Other fungi including *Phytophthora megasperma*, *Alternaria* sp., *Stemphylium* sp., *Cylindrocarpon* sp., *Ulocladium* sp., *Pleospora* sp., *Colletotrichum* sp., *Epicoccum* sp., *Cladosporium* sp. and numerous unidentified fungal species were recovered.

The main fungal groups affecting brassica crops in each state varied (Appendix 2). South Australian and Victorian crops were commonly affected with *Phoma*, *Rhizoctonia*, *Pythium* and *Fusarium*. *Rhizoctonia*, *Pythium* and *Fusarium* in Queensland crops, *Phoma* and *Fusarium* in New South Wales and Western Australian crops affected by *Fusarium*. Cauliflower stems in Western Australia may be affected by other fungi however samples received were rotten and the recovery of fungi was difficult due to bacteria.

Pythium was commonly recovered from the soil of infected plants.

Rhizoctonia was frequently recovered from fat hen roots and was also isolated from thistles, rye grass, sour sob and paddy melon. Fungi recovered from volunteer potatoes included *Alternaria*, *Fusarium* and *Cladosporium*.

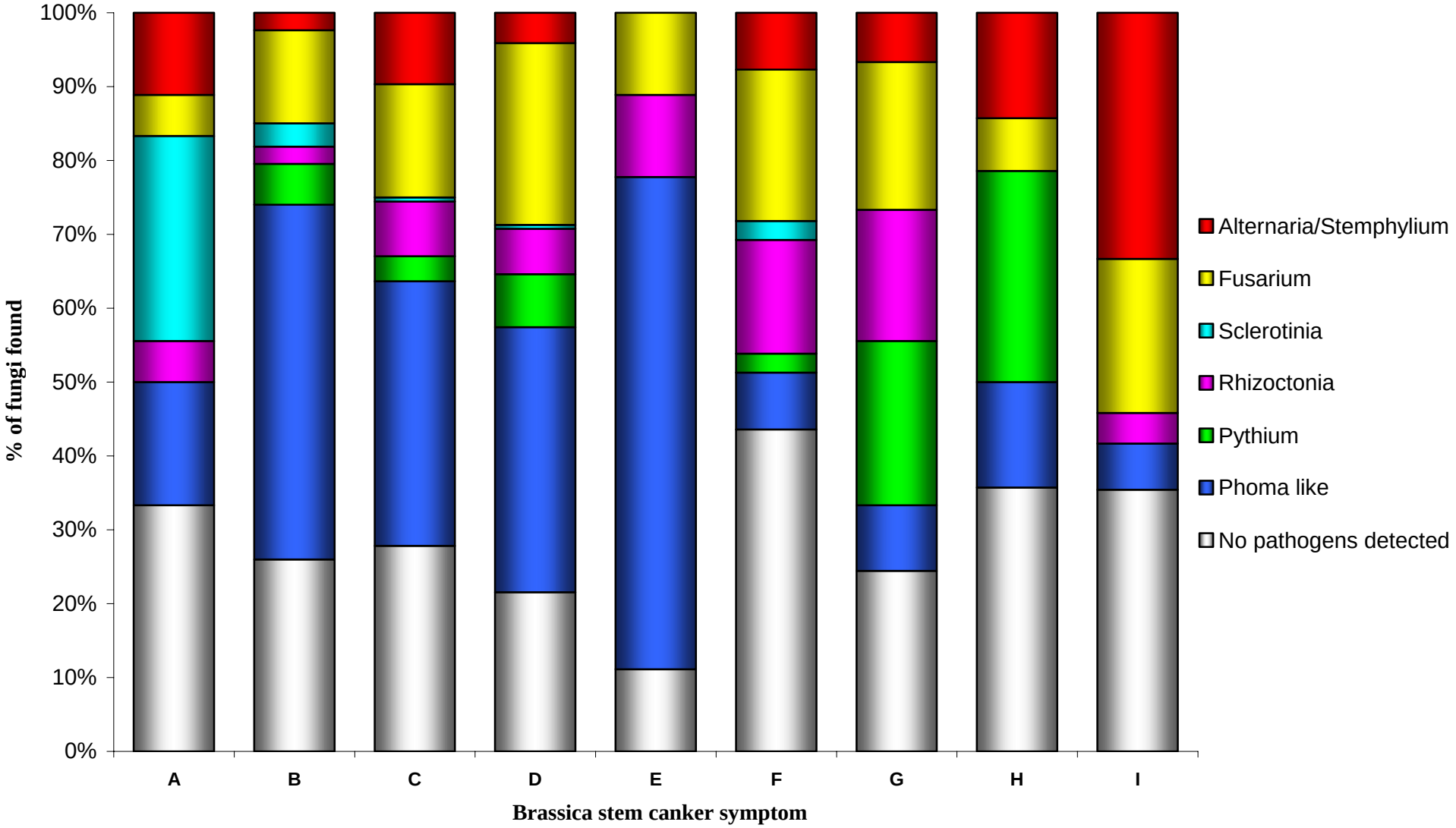
Fungal isolations and stem canker symptoms

While a number of different fungi were isolated from symptoms typical of brassica stem canker, trends were seen with some symptom categories. *Sclerotinia* was commonly recovered from symptom A, "Phoma like" isolates recovered from symptom E and *Pythium* recovered from symptom H. Fungi recovered from Symptom G were commonly *Fusarium*, *Rhizoctonia* or *Pythium* (Figure 4).

"Phoma like" isolates were the most frequently recovered fungi from all the symptom groups with 67, 49, 40 and 37% recovered from symptom E, B, C and D respectively.

The *Alternaria* and *Stemphylium* isolates recovered were categorised as secondary invaders (until further pathogenicity testing) and were grouped together.

Figure 4. Percent of plants in each symptom group with various fungi isolated.



Major fungal pathogens

Phoma

A total of 249 "Phoma like" isolates were recovered from brassica crops throughout Australia. Four have been identified as *Leptosphaeria maculans*, the cause of Black leg in brassica crops. The majority of the isolates have similar growth patterns on PDA and are also likely *Leptosphaeria maculans*. Two other isolates have been identified as *Phoma nebulosa* and the other a *Phoma* sp..

So far 208 *Phoma* isolates have been tested for pathogenicity and 97 of these caused stem cankers, produced pycnidia and stunted seedlings. Pathogenicity tests are continuing with further assessments required before the total number of pathogenic isolates recorded. *Phoma* was reisolated from infected seedlings confirming Koch's postulates.

Pathogenicity testing has been carried out on seedlings of various cultivars and seedling ages. No obvious differences were seen between cauliflower cultivar with symptoms typical of brassica stem canker seen on all cultivars tested. A minor difference was observed with seedling age at the time of infection with younger seedlings becoming symptomatic sooner than older seedlings. Seedlings of various ages all produced symptoms typical of brassica stem canker.

Rhizoctonia

A total of 105 *Rhizoctonia* isolates were recovered from stems and roots. The Anastomosis Groups of 63 of the *Rhizoctonia* isolates collected were identified as AG 2.1, 2.2 and 4. Over 50% of the isolates were classified as AG 2.1.

Pathogenicity tests have been carried out on all the *Rhizoctonia* isolates collected and all caused symptoms typical of brassica stem canker on cauliflower seedlings. The virulence varied between isolates, with some causing severe wirestem symptoms within 7 days whereas others produced localised lesions and stem discolouration after 21 days. *Rhizoctonia* was recovered from inoculated plants confirming Koch's postulates.

Inoculation of mature 12 and 19 weeks old plants did not cause symptoms typical of brassica stem canker after 8 weeks. In tests with cauliflower seedlings of various ages (4-11 weeks) and cultivars (Aviron, Skywalker and Chaser), no differences were detected between seedlings of different age and cultivar as all seedlings were infected. This indicated that *Rhizoctonia* is more of an issue early in the growing season.

Fusarium

A total of 119 *Fusarium* isolates were collected from brassica stems and roots. Three isolates were identified as *Fusarium equiseti*, F. *oxysporum* and F. *reticulatum*.

Pathogenicity tests 111 of these isolates are in progress. So far 19 of these isolates were pathogenic and caused stem lesions, canker and stunted plants. *Fusarium* was recovered from the inoculated plants confirming Koch's postulates.

No differences have been observed between cultivar and seedling age.

Pythium

A total of 65 *Pythium* isolates were recovered from stems, root and soil of brassica crops. Pathogenicity tests ha on 60 isolates showed that all were pathogenic causing slothing of roots and stems, stunting and stem lesions. *Pythium* was recovered from inoculated seedlings confirming Koch's postulates.

No differences have been observed between cultivar and seedling age.

Sclerotinia

Sclerotinia does not seem to be part of the brassica stem canker disease complex. *Sclerotinia* infected plants occurred at random throughout plantings. Plants infected with *Sclerotinia* were stunted and wilted with lesions covered with white mycelia and black sclerotes. Two species of *Sclerotinia* have been recovered *Sclerotinia sclerotiorum* and S. *minor*. Eleven isolates have been collected during the survey from affected cauliflower plants.

Combination isolate testing

The virulent isolates of *Rhizoctonia* tested alone and in combination with *Phoma* isolates were severely cankered within 11 days, initially showing typical wirestem symptoms of *Rhizoctonia* infection. Symptoms were more severe in plants which were maintained at 25^{o}C. The seedlings inoculated with *Phoma* alone were not affected with symptoms typical of brassica stem canker at 11 days, indicating the early infection came from the *Rhizoctonia*. However, after 4 weeks all seedlings inoculated with *Phoma* isolates either individually or in combination had symptoms typical of brassica stem canker. Symptoms on some plants inoculated with combination had progressed beyond the *Rhizoctonia* wirestem symptom to show severe cankers extending up the stem with *Phoma* pycnidia observed on some stems. Further combination testing including mixtures of *Fusarium*, *Pythium*, *Rhizoctonia* and *Phoma* are continuing.

TECHNOLOGY TRANSFER

Brassica Project Committee Meetings

- 30th June 2005
- 15th August 2005
- 8th February 2006

Survey Property Visits

- South Australia, July 2005 – April 2006.
- Bathurst NSW, April 2006.

Workshops

- Brassica Disease Workshop, Victorian DPI Extension Road Show, Virginia Horticulture Centre November 2005.

Articles

- Determining the soilborne diseases affecting brassica crops. WA Grower Bulletin/Magazine – Better Brassica. August 2005.
- Brassica diseases under the microscope. PIRSA Open Gate December 2005.
- Brassica stem canker. The Grower, November 2005.
- Stem canker of brassica crops. Good Fruit and Vegetable Magazine March 2006.
- Vegetables Australia July 2006
- Understanding stem canker disease. National Brassica IPM Newsletter. Issue 9, August 2006.

Conference Presentations

- Brassica Stem Canker (Poster) Australian Vegetable Industry Conference 2006 – A New Vision, Brisbane May 2006.
- Identifying the cause of brassica stem canker (Poster) 4th Australasian Soilborne Diseases Symposium (2006) September.

RECOMMENDATIONS

This project has provided a good understanding of the cause of the stem canker affecting brassica crops. Further research is necessary to provide control and management strategies to sustain the future of the industry. Further research needs to include:

Disease parameters (infection pathways and establishment)

Seed borne infection
Seed from the main brassica seed supplying companies need to be tested for pathogens and if present, hot water treatments and fungicide/chemical slurries need to be evaluated as control measures.

Soilborne infection
1) Rapid and sensitive tests need to be developed to determine *Rhizoctonia* and *Phoma* levels in the soil using molecular tests. Threshold levels of pathogens which cause disease need to be determined and how these levels act alone and in conjunction with other pathogens in the disease complex. Studies will also need to be undertaken to determine how long the fungi survive in the soil and on infected plant debris.
2) The effect of burial of diseased plant debris on the development of disease needs to be investigated. Investigating both the depth of burial, survival of pathogens on the debris at various depths and determining whether pathogens have established in the soil from the debris.
3) The influence of soil/plant nutrition, soil water levels, irrigation, temperature and other environmental conditions need to be investigated by measuring levels of nutrients in soil and plant material and correlating to the amount of disease observed. Environmental conditions need to be monitored and the interaction of these factors correlated with the plants susceptibility to disease.

Alternate hosts
Weeds collected in this study showed *Rhizoctonia* to be present on several weed species in diseased crops. The potential of common weeds in brassica crops acting as alternate hosts need to be investigated.

The susceptibility of rotation crops to pathogens found during the survey also needs to be investigated. including potatoes, cereals, pasture and other vegetables.

Alternate methods of contamination
The spread of disease through irrigation and run off water needs to be investigated to determine whether pathogens are present in water and the capability of diseased water samples infecting plants.

Management strategies

Chemical treatments, bio-control
The effect of fungicides applied as seed treatments, foliar sprays and soil drenches both before and after infection to control *Rhizoctonia* and *Pythium* needs to be investigated. Fungicide evaluations will need to incorporate individual fungi involved in the complex as well as a combination of the fungi. Bio-control agents available commercially such as various formulations of Trichoderma and others applied as foliar sprays and soil additives will be tested in similar fashion.

Resistant varieties
The susceptibility of cultivars needs to be more thoroughly investigated to determine whether resistant cultivars are grown.

Bio-fumigation, rotations
Rotation crops offer an alternative host for pathogen survival. Pathogen levels in the soil need to be tested before and after the rotational crop is used. Viable alternative rotation crops need to be tested and whether mustard brassicas will act as a bio-fumigant.

Nutrition and Watering
The nutritional status of infected plants will need to be investigated to determine if the addition or subtraction of different nutrients will affect the levels of disease. Similarly watering will be applied at different levels to determine the effect of disease and whether excessive soil moisture will contribute to disease establishment and progression.

ACKNOWLEDGEMENTS

We wish to thank the brassica growers of Australia for their co-operation in allowing surveys to be conducted on their properties especially Frank Musolino and George Pannucio. A special thank you to Frank Musolino and son Steven for allowing continual surveying of their properties and providing their valuable time to transfer information and answer all my questions. Gino from Gino's Nursery for supplying the seedlings for all the pathogenicity tests.

A big thank you goes to Maria Siguenza for all her help during the surveying of brassica crops and Judy Bellati for all her help in Bathurst and in the laboratory.

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All the technical staff interstate for carrying out surveys including Liz Mitchinton, John Duff, and Kristen Stirling. A big thank you to Stephen Wade for all his help during the Bathurst survey.

We wish to thank Michael Priest and Dorothy Noble for the New South Wales DPI for identifying fungi and bacteria isolates. Herdina and Andrew Taylor (Diagnostics, Crop Pathology SARDI) for their time identifying *Rhizoctonia* isolates.

DISCLAIMER

The results in this report are submitted on the basis that the tests were conducted by SARDI in accordance with the protocol requested by or agreed to by your company, and using test chemicals based on scientific information currently available to SARDI.

Neither SARDI nor its officers accept any liability resulting from this interpretation or use of the information contained herein. Use of information is at the risk of the user to the extent permissible by law.

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(3) Integrated pest management for Cole Crops and Lettuce, (1985). University of California Publication #3307.

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APPENDICES

Appendix 1. Complete data set from brassica stem canker survey in Australia 2005/06.

	Grower	Location	Date	Crop	Cultivar	% infection
SA	6	NAP	10/8/05	Cauliflower	Artic	19
	GP		26/8/05	Broccoli		7
	9	NAP	17/10/05	Cauliflower	?	98
	9		17/10/05	Cauliflower	?	100
	10	NAP	24/10/05	Cauliflower	Artic	96
	10		24/10/05	Cauliflower	Artic	100
	8	NAP	28/10/05	Cauliflower	?	76
	8		28/10/05	Cauliflower	?	100
	1	NAP	3/11/05	Cauliflower	Artic	100
	5	NAP	3/11/05	Cauliflower	Artic	94
	7	NAP	10/11/05	Cauliflower	?	87
	7	NAP	10/11/05	Cauliflower	?	87
	17	Adelaide Hills	10/11/05	Brussels Sprout	Adagia	85
	17		10/11/05	Brussels Sprout	Adagia	90
	17		10/11/05	Brussels Sprout	Adagia	64
	17		10/11/05	Brussels Sprout	Mistral	100
	17		10/11/05	Brussels Sprout	Abacus	72
	17		10/11/05	Brussels Sprout	1999	77
	2	NAP	1/12/05	Cauliflower	Artic	81
	FM	NAP	1/12/05	Red Cabbage	Rosa/Cairo	58
	FM		1/12/05	Red Cabbage	Rosa/Cairo	83
	FM		1/12/05	Red Cabbage	Rosa/Cairo	49
	11	Adelaide Hills	14/12/05	Cauliflower	Chaser	24
	11		14/12/05	Cauliflower	Chaser	37
	11		14/12/05	Broccoli	Mascot	0
	11		14/12/05	Broccoli	Mascot	1
	3	NAP	15/12/05	Cauliflower	Skywalker/Discovery	98
	12	NAP	17/1/06	Cauliflower	Nautilus	90
	12		17/1/06	Cauliflower	Nautilus	90
	13	NAP	17/1/06	Cauliflower	Discovery	63
	13		17/1/06	Cauliflower	Discovery	66
	14	Adelaide Hills	22/1/06	Cauliflower	Artic	9
	14		22/1/06	Cauliflower	Artic	14
	15	Adelaide Hills	22/1/06	Cauliflower	Nautilus	30
	16	Adelaide Hills	1/3/06	Cauliflower	Discovery	31
	16		1/3/06	Cauliflower	Discovery	44
	4	NAP	28/3/06	Cauliflower	Nova	87
Vic	1	Werribee South	27/12/05	Cauliflower	Discovery	16
	2	Werribee South	27/12/05	Cauliflower	?	1
	3	Werribee South	27/12/05	Cauliflower	Discovery	38
	4	Werribee South	6/3/06	Cauliflower	?	1
	4		6/3/06	Cauliflower	?	1
	5	Werribee South	6/3/06	Cauliflower	?	2
	6	Werribee South	6/3/06	Cauliflower	?	4
	7	Werribee South	6/3/06	Cauliflower	?	1
	8	Werribee South	6/3/06	Cauliflower	?	2
	9	Werribee South	6/3/06	Cauliflower	?	0
	10	Bairnsdale	7/3/06	Cauliflower	?	2
	11	Bairnsdale	7/3/06	Cauliflower	?	4
	9	South Manjimup	7/3/06	Cauliflower	Summer love	28

Appendix 1 Continued.

	Grower	Location	Date	Crop	Cultivar	% infection
WA	3	Palgarup	8/3/06	Cauliflower	Aviron	11
	10	Palgarup	8/3/06	Cauliflower	Aviron	24
	8	West Manjimup	9/3/06	Cauliflower	Aviron	7
	11	Palgarup	9/3/06	Cauliflower	Aviron/Summer love	3
	7	West Manjimup	10/3/06	Cauliflower	Summer love/Moby	23
	2	Albany	14/3/06	Cauliflower	Aviron	13
	4	Albany	14/3/06	Cauliflower	Aviron	10
	5	Albany	14/3/06	Cauliflower	Summer love	2
	6	Albany	14/3/06	Cauliflower	Aviso/Summer love	17
	1	Myalup	17/3/06	Cauliflower	Fremont	0
Qld	3	Granite Belt	31/3/05	Cauliflower	Nova	25
	4	Amiens area	31/3/05	Cauliflower	Freeman 7	1
	1	Lockyer Valley	12/10/05	Cauliflower	?	7
	2	Lockyer Valley	13/10/05	Cauliflower	?	7
NSW	1	Bathurst	4/4/06	Cauliflower	Aviso	23
	1		4/4/06	Cauliflower	Aviso	24
	2	Lagoon	4/4/06	Cauliflower	Forte	74
	2		4/4/06	Cauliflower	Artic	29
	2		4/4/06	Red Cabbage	Rosa	21
	5	Lagoon	4/4/06	Cauliflower	Miscellaneous	7
	5		4/4/06	Broccoli	Grevillia	1
	6	Bathurst	4/4/06	Cauliflower	Aviso	9
	6		4/4/06	Cauliflower	?	44
	6		4/4/06	Red Cabbage	Red Cardinal	0
	7	Bathurst	4/4/06	Cauliflower	Aviron	41
	7		4/4/06	Cauliflower	Ascale	65
	7		4/4/06	Red Cabbage	Cairo	51
	3	Bathurst	5/4/06	Cauliflower	Monarch	15
	4	Bathurst	5/4/06	Cauliflower	Forte	4
	4		5/4/06	Cauliflower	Forte	9
	8	Yetholme	5/4/06	Cauliflower	Skywalker	4
	8		5/4/06	Red Cabbage	Red Cardinal	0
	9	Yetholme	5/4/06	Brussels sprout	Oliver	2
	9		5/4/06	Brussels sprout	Oliver	6
	10	Bathurst	5/4/06	Green Cabbage	Green Coronet	15
	10		5/4/06	Red Cabbage	Red Cardinal	0

Where NAP is the Northern Adelaide Plains and ? unknown cultivar.

Appendix 2. Percent of fungal isolates recovered from Brassica stems in each state, 2005/06.

			Phoma	*Rhizoctonia*	*Pythium*	*Fusarium*	*Sclerotinia*	No ID	Total
SA	1	Cauliflower	39	9	3	17	6	37	127
	2	Cauliflower	47	13	16	13	0	26	38
	3	Cauliflower	17	0	67	0	0	17	6
	4	Cauliflower	0	59	24	24	0	6	17
	5	Cauliflower	34	15	16	18	3	46	74
	6	Cauliflower	20	10	10	20	0	40	10
	7	Cauliflower	52	24	5	0	0	38	21
	8	Cauliflower	69	6	25	13	0	6	16
	9	Cauliflower	77	31	8	0	0	15	13
	10	Cauliflower	100	14	0	0	0	0	7
	11	Cauliflower	75	0	0	25	0	0	4
	12	Cauliflower	0	0	25	0	0	75	4
	13	Cauliflower	0	0	100	33	0	0	3
	14	Cauliflower	0	25	50	0	0	50	4
	15	Cauliflower	0	14	100	43	0	0	7
	16	Cauliflower	0	0	50	67	17	0	6
		Brussels sprouts	50	0	17	50	0	17	6
	17	Brussels sprouts	92	23	7.7	31	0	0	13
		Brussels sprouts	83	0	17	17	0	0	6
	11	Broccoli	11	22	11	67	0	22	9
	1	Red cabbage	75	0	0	13	0	25	8
Qld	1	Cauliflower	0	0	14	0	0	71	7
	2	Cauliflower	14	14	43	71	0	14	7
	3	Cauliflower	0	50	0	33	0	33	6
	4	Cauliflower	0	100	0	0	0	0	1
Vic	1	Cauliflower	70	20	0	40	0	10	10
	2	Cauliflower	0	100	0	0	0	0	1
	3	Cauliflower	100	20	0	20	0	0	10
	4	Cauliflower	0	0	0	50	0	50	2
	5	Cauliflower	0	0	40	40	0	40	5
	6	Cauliflower	16.7	0	17	33	0	50	6
	7	Cauliflower	0	14	14	71	0	29	7
	8	Cauliflower	0	0	11	33	0	67	9
	9	Cauliflower	0	0	0	0	0	100	3
	10	Cauliflower	20	40	0	40	0	20	5
	11	Cauliflower	0	100	0	100	0	0	1
WA	1	Cauliflower							NA
	2	Cauliflower	0	0	0	33	0	67	3
	3	Cauliflower	0	0	0	100	0	0	3
	4	Cauliflower							NA
	5	Cauliflower							NA
	6	Cauliflower	0	0	0	100	0	0	1
	7	Cauliflower	0	0	0	0	0	100	3
	8	Cauliflower							NA
	9	Cauliflower	0	0	0	0	0	100	3
	10	Cauliflower	0	0	0	0	0	100	1
	11	Cauliflower	0	0	0	100	0	0	1
NSW	1	Cauliflower	67	0	0	33	0	33	3
	2	Cauliflower	40	0	0	20	0	40	5
	3	Cauliflower	0	33	0	67	0	33	3
	4	Cauliflower	67	0	0	67	0	0	3
	5	Broccoli						-	NA
	6	Cauliflower	0	0	0	100	0	0	3
	7	Cauliflower	20	0	20	60	0	20	5
	8	Cauliflower	0	0	0	0	0	100	2
	9	Brussels sprout	0	0	0	100	0	0	1
	7	Red cabbage	0	0	0	0	0	100	1
	2	Red cabbage	100	0	0	0	0	0	1
	10	Green cabbage	0	0	0	50	0	50	2