BULLETIN 4747
ISSN 1833 7236

Strawberry root and crown rot disease survey

2005 and 2006 seasons

Government of Western Australia
Department of Agriculture and Food

Bulletin 4747
ISSN 1833-7236

Strawberry root and crown rot disease survey

2005 and 2006 seasons

Dennis Phillips, Development Officer,
and Hossein Golzar, Plant Pathologist

August 2008

Department of Agriculture and Food
Government of Western Australia

Summary

Reports of unusually high numbers of plant deaths in strawberry crops from growers in Western Australia prompted a survey to identify the cause. Plant and soil samples were taken from a range of growers and runner sources over a two-year period and tested for plant pathogens.

Fusarium oxysporum f. sp. *fragariae* was identified as the predominant pathogen, the first recording of this in Western Australia. Further work is planned to clarify the epidemiology of this pathogen and evaluate suitable methods of control.

Patches of dead and dying plants, typical symptoms in a strawberry plantation infested with fusarium crown and root rot

Introduction

The strawberry industry in Western Australia (WA) is small by world standards with an annual production of approximately 3,500 tonnes in 2004/05, and a wholesale value of A$17 million (ABS). Western Australia ranked third in the nation in 2004/05 for volume and value behind Queensland and Victoria, in an Australian industry producing around 24,000 tonnes annually, while 72% of Australia's exports were produced in WA.

Strawberry production is largely confined to two regions in WA, these being the acid sandy soils of the Swan Coastal Plain on the northern fringe of the capital city, Perth, and a range of soil types in the hinterland of Albany on the south coast.

Perth production districts include Wanneroo and Bullsbrook, which between them accounted for around 90% of total production in 2004/05. Typically in these districts, 'short day' strawberry cultivars are grown in an 'annual hill' production system for late winter and spring cropping on black plastic covered beds. Most strawberry farms are relatively small, and production is intensive, with the same land being continuously cropped to strawberries for 20 years or more in many cases. The crop is vegetatively propagated with certified runners bought in annually from specialist nurseries in eastern Australia. These runners are chilled and transported by road across the country in plastic-lined cardboard cartons holding approximately 500 runners each. The potential for soil-borne pathogens to establish in fruit production fields is high due to the lack of rotation; however soil fumigation is widely practised between crops in both runner nurseries and fruit production fields.

In the 2005 season, growers reported unusually high levels of plant death in crops in the Wanneroo and Bullsbrook districts. Samples from seven properties were sent to a pathology laboratory in Queensland in July and August 2005 and *Fusarium oxysporum* was isolated from all of the samples. A high prevalence of serious *Fusarium oxysporum* infection was found on plants of the cultivar Camarosa. Infections of *Fusarium* were also found on the cultivar Chandler but the symptoms were less advanced and the incidence of disease appeared to be far less than Camarosa at one site where both cultivars were grown.

Observations from local strawberry growers and allied industry people confirmed that there were problems with plant health, and on some properties these were significant. Whilst these symptoms had been noticed in previous years, in 2005 it seemed especially serious, and industry estimates of plant loss were of the order of one million plants out of a total of 10 million planted in these districts.

Root and crown disorders can be caused singly or by combinations of fungal pathogens including *Phytophthora*, *Fusarium, Colletotrichum*, *Verticillium, Rhizoctonia*, *Macrophomina*, and/or *Phoma*. These organisms have been isolated from strawberry plants in Western Australia in the past, as well as other strawberry growing regions in Australia.

Soil-borne pathogens of strawberries in Western Australia and elsewhere are commonly controlled by soil fumigation. The outbreaks near Perth in 2005 were observed in both fumigated and non-fumigated soil, though the incidence and severity varied greatly between properties where the soil was claimed to be fumigated.

These observations led the WA strawberry industry to fund a preliminary survey in conjunction with the Department of Agriculture and Food (DAFWA) to identify causes and severity of plant deaths observed, during the 2005 and 2006 seasons. The results of that study are reported here.

Materials and methods

Strawberry plants (root and crown) and soil from the root zone were randomly sampled from selected properties on three occasions over a two year period. Field symptoms were recorded and plants were taken back to the DAFWA plant pathology laboratory for testing. In early 2006, plants (runners) were sampled on arrival from eastern states nurseries, with the intention of identifying organisms present on plants from various runner producers, before they were planted in field soil. Crops planted with runners from these sources were revisited and tested later when fruiting.

15 September 2005 sampling

The first batch of samples was taken from five farms (Sites 1-5). At each site, four unhealthy and one healthy plant together with soil from the root zone, were collected for further tests. Roots were washed carefully under running tap water and the crown of each plant dissected lengthwise and disease symptoms were recorded.

The crown and roots of affected and symptomless plants (controls) were surface sterilised with sodium hypochlorite, rinsed in sterile water, then dried in a lamina flow cabinet. Pieces of root and crown were then separately plated on potato dextrose agar (PDA), water agar (WA) and selective media (PV10PH and P10VP) and incubated at 22 ± 3ºC. Emerged fungal colonies were subcultured onto carnation leaf agar, PDA and V-8 juice agar and incubated at 25ºC with a 12 hour light and dark cycle.

Soil samples were baited with *Eucalyptus seiberi* cotyledons for isolation of *Pythium* and *Phytophthora* species. Growth rate, colony morphology and morphological characteristics of the isolated fungi were determined.

A bulked soil sample from each site was also tested for plant parasitic nematodes.

Pathogenicity of the *Fusarium* isolates was tested on the strawberry cultivar Camarosa and also on *Lycopersicon lycopersicum* cv. Petula and *Cucumis sativus* (Lebanese cucumber) in a glasshouse experiment. Seedlings were inoculated by dipping the roots in a spore suspension at a concentration of 10^5/mL). Controls were dipped in sterilised water. Pathogenicity and morphological characteristics of the re-isolated fungus were confirmed by inoculation of healthy Camarosa plants and re-isolation from plants showing symptoms consistent with those observed in the field.

24 November 2005 sampling

The tests outlined above were repeated in November on two randomly selected samples (one with moderate, the other with severe symptoms) from each of the previous sites (except Site 3).

27 April/1 May 2006 sampling

Two runners were collected by DAFWA staff from growers at each of the sites detailed below (Table 1) from unopened cartons that had recently arrived from nurseries and were awaiting planting in the field. The intention had been to collect all samples prior to planting at this time, but while sampling, a crop was discovered that had been planted weeks earlier using ‘cool-stored (frigo)’ runners and plants were already showing symptoms of wilting (Table 2).

Sample analyses followed the same methodology as detailed for the first batch.

Table 1. Origin of runners and grower sites from which Camarosa runners were sampled

Grower site	Runner source
1	A
4	A
4	B
5	C
5	D
7	E

Table 2. Origin of runners and grower site from which wilting plants were collected

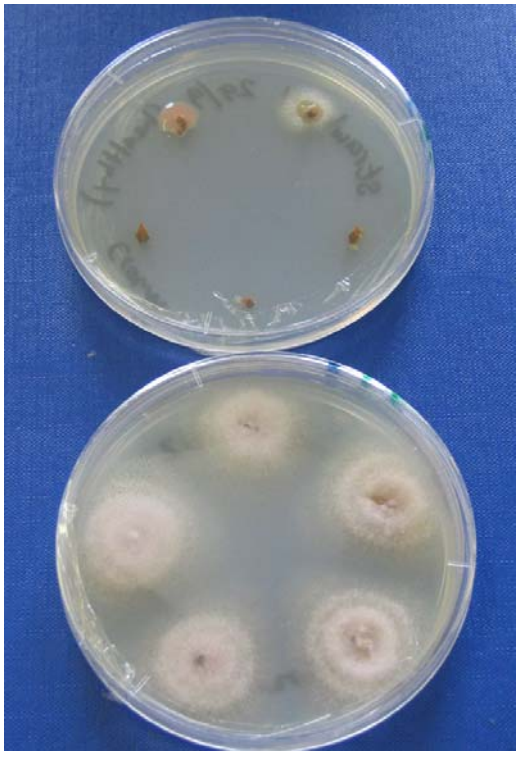
Grower site	Runner source	Cultivar	Plants sampled	Soil sampled
6*	B	Camarosa (frigo)	Mature plants dying in the field	Soil from roots of dying plants sampled for nematode test

* unfumigated soil

25 September 2006 sampling

Five plant samples (including one healthy) were collected from each site detailed in Table 3 during a return visit to the properties where unplanted runners had been sampled in April/May. At this sampling, one litre of soil from the root zone of each plant was also collected and tested in the laboratory. Sampling was extended to include three new sites in the Wanneroo area 3 (Sites 8, 9 and 10) where wilting symptoms were observed.

Sample analyses followed the same methodology as detailed for the September 2005 tests.



Examples of cultures grown from infected strawberry root and crown material (later identified as Fusarium oxysporum *f. sp.* fragariae)

Table 3. Origin of runners and grower sites from which strawberry samples were taken in September 2005

Grower site	Runner grower	Plant symptoms	Variety	Method of fumigation
Site 1	A	Wilting	Camarosa	Telone® C-35 strip
Site 4	A	Wilting	Camarosa	Telone® C-35
Site 4	A	Healthy	Camarosa	Telone® C-35
Site 5	Unknown	Wilting	Camarosa	Telone® C-35 full ground
Site 7	Unknown	Wilting	Ventana	Telone® C-35 strip
Site 8	Unknown	Wilting	Camarosa	Telone® C-35 strip
Site 8	Unknown	Healthy	Camarosa	Telone® C-35 strip
Site 9	Unknown	Wilting	Camarosa	Telone® C-35 full ground
Site 10	Unknown	Type 1 wilting	Camarosa (frigo)	Telone® C-35 strip
Site 10	Unknown	Type 2 wilting	Camarosa (frigo)	Telone® C-35 strip

Typical field symptoms of fusarium root and crown rot

Results

15 September 2005 sampling

A high level of *Fusarium* (80-90%) was isolated from the crowns of all unhealthy samples but only low levels (5-20%) were recovered from the crowns of apparently healthy plants (Table 4).

A moderate level of *Fusarium* (24-30%) was detected in approximately one third of the unhealthy root samples but only in 4-10% of root samples from apparently healthy plants. *Pythium* was also isolated from the roots of approximately one third of all soil samples, including those from around healthy plants.

Table 4. Incidence of *Fusarium* isolated from crown and roots (mean of five) of healthy and unhealthy strawberry plants from five sites

Site	*Fusarium* incidence (%)			
	Unhealthy plants		Healthy plants	
	Roots	Crown	Roots	Crown
1	28	89	10	20
2	24	75	5	10
3	38	90	5	15
4	24	85	4	5
5	30	81	10	5

Low levels (2-5%) of a range of other pathogens were also found on crown and root material as detailed in Table 5.

Table 5. Presence or absence of fungal pathogens other than *Fusarium*, isolated from crown and roots of healthy and unhealthy strawberry plants from five sites

Site	1	2	3	4	5
Macrophomina	✓	✓	✓	✓	✓
Rhizoctonia	✓		✓		
Phoma		✓	✓		✓
Colletotrichum	✓		✓		
Pythium			✓		

In addition to the fungal pathogens, plant parasitic nematodes were also isolated from some sites.

- Site 1 – *Aphelenchoides* sp.
- Site 2 – Various nematodes
- Site 3 – *Aphelenchoides* sp.
- Site 4 – Various nematodes
- Site 5 – *Pratylenchus* sp.

None of the nematodes was in high enough concentration to be considered a problem.

24 November 2005 sampling

The results were similar to those from the September sampling in that *Fusarium oxysporum* was the predominant fungal pathogen isolated from crown and root samples. Plant parasitic nematodes were found in samples from Sites 1, 2 and 4 but in very low levels and not considered to be an issue.

27 April and 1 May 2006 sampling

Table 6 details the results of this sampling. With the exception of Site 6 which was already planted in the field, all these samples of unplanted strawberry runners were negative for *Fusarium* sp. Most roots showed some sign of discolouration and stripping. *Pythium* and *Phytophthora* were also recovered in some instances. *Botrytis* was recovered from crowns of runners from two origins only.

Table 6. Results from 27 April and 1 May samplings of unplanted runners

Site	Symptoms	Pathogens detected
1A	Some discolouration and stripping of roots	No root pathogens, some *Botrytis* (crown)
4A	Slight discolouration of one crown, discolouration and stripping of roots on most plants	*Phytophthora* and *Pythium* (roots), *Botrytis* (crown), *Alternaria* (roots – probably secondary)
4B	Discolouration and stripping of roots on all plants	None
5C	Discolouration and stripping of the fine roots and dark lesions on medium size roots on all plants	None
5E	Discolouration and stripping of roots, dark lesions on roots close to the crown	*Pythium* (roots), *Alternaria* (roots – probably secondary)
6B (plant)	Stem and leaf lesions, crown discolouration and discolouration and stripping of roots	*Fusarium* (crown and roots), *Pythium* (roots), *Rhizoctonia* (crowns and roots), *Botrytis* (stems)
6B (soil)		Nematodes *(Helicotylenchus)* found at very low levels but not considered significant
7D	Discolouration and stripping of roots, lesions on roots	*Botrytis* and *Pythium* (roots)

25 September 2006 sampling

The final survey examined plants from some previously unsampled sites. A high incidence of *Fusarium* was isolated from the crowns and roots of most unhealthy samples. One sample returned only *Macrophomina* and another proved to be infected with *Phytophthora*. Two samples had infections of *Rhizoctonia* and *Phytophthora* respectively, in combination with *Fusarium*.

Pythium was isolated from all of the soil samples except one. *Phytophthora* was found in addition to *Pythium* in one sample. The pattern of infection was similar to that found in the 2005 sampling (see Table 7).

Table 7. Results from 25 September 2006 plant and soil sampling

Sample ID	Results from soil	Results from plant tissue		Comment
		Root	***Crown***	
1A	*Pythium* sp.	*Fusarium* sp.	*Fusarium* sp.	High level of *Fusarium* crown rot and moderate root rot
4A	*Pythium* sp.	*Fusarium* sp.	*Fusarium* sp.	Severe *Fusarium* crown rot and moderate root rot
4B	*Pythium* sp.	*Rhizoctonia* sp.	*Colletotrichum* sp.	Low level of crown and root rot
5	*Pythium* sp.	*Fusarium* sp. *Rhizoctonia* sp.	*Fusarium* sp.	Severe *Fusarium* crown rot
7	*Phytophthora* sp. *Pythium* sp.	*Phytophthora* sp	*Phytophthora* sp.	Severe *Phytophthora* crown and root rot
8 (healthy)	*Pythium* sp.	*Macrophomina* sp.	No pathogens detected	Low level of root rot
8 (unhealthy)	*Pythium* sp.	*Macrophomina* sp.	*Macrophomina* sp.	Moderate level of root and crown rot
9	*Phytophthora* sp. *Pythium* sp.	*Fusarium* sp.	*Fusarium* sp.	Severe *Fusarium* crown and root rot
10A	*Phytophthora* sp. *Pythium* sp.	*Fusarium* sp. *Phytophthora* sp. *Pythium* sp.	*Phytophthora* sp. *Fusarium* sp.	Severe *Phytophthora* and *Fusarium* crown and root rot
10B	No pathogens detected	*Fusarium* sp. *Rhizoctonia* sp.	*Fusarium* sp.	Severe *Fusarium* crown rot and *Rhizoctonia* root rot

Vascular discolouration is apparent on an unhealthy crown (right) compared with a healthy crown (left)

Discussion

This study found a high rate of crown rot with typical vascular discolouration in unhealthy plants collected from the field. *Fusarium oxysporum* was consistently isolated from 70% of samples tested and subsequent tests on those isolates confirmed them to be *Fusarium oxysporum* f. sp. *fragariae*. This fungus has been reported in the *Compendium of Strawberry Diseases, 2nd edition,* edited by JL Maas and published by the American Phytopathology Society, as an important pathogen of strawberry and found in Queensland and Japan.

Phytophthora cactorum was isolated from crown, root and soil samples. *Phytophthora cactorum* had not been reported as a crown rot on strawberries previously in Western Australia, however there are records of this fungus on strawberry fruit and other hosts.

These fungi were often isolated independently or in combination with *Pythium*, *Rhizoctonia*, *Colletotrichum* and *Macrophomina* spp.

There was a moderate incidence of *Pythium* and *Phytophthora* isolated from soil surrounding the roots of strawberry plants but without identification to species level it is not possible to determine their pathogenicity. However in most cases the incidence of these pathogens was identical in soil from the root zone of healthy and unhealthy plants and this would tend to suggest they are of low pathogenicity.

Limited testing of runners sampled before planting out in the field revealed a range of commonly occurring organisms that could be pathogenic or secondary but Fusarium was not among them.

Cultures of *Fusarium oxysporum* f. sp. *fragariae* and *Phytophthora cactorum* have been deposited in the WA culture collection.

Conclusions

The disease survey was conducted to provide baseline information to assess whether further investment in a much larger research project on this topic was justified. The finding that *Fusarium oxysporum* f. sp. *fragariae* is responsible for much of the plant death observed in strawberry crops is justification for a more detailed study being undertaken in the future.

This finding is a first record of this pathogen in strawberries in Western Australia and is a significant step forward in our understanding of the causes of strawberry plant death in the field.

The role of other potential pathogens in the symptoms observed is unclear and also requires further study.

Acknowledgements

Thanks to Michaela Antoine, Lachlan Chillman, Don Hutton, Sandy Mack, Aileen Reid and participating growers for helping with field and laboratory work, data analysis and editorial assistance.

Thanks also to Inge Tvede from The University of Western Australia for permission to use her photographs.