THE EFFECT OF HEAT ON SURVIVAL OF *PHYTOPHTHORA CINNAMOMI, P. CITRICOLA* AND *ARMILLARIA LUTEOBUBALINA* IN COLONISED ORGANIC MATERIAL

B.J. Smith and E.M. Davison

Department of Conservation and Land Management,
50 Hayman Road, Como WA 6152

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B.J. Smith and E.M. Davison
Department of Conservation and Land Management
50 Hayman Road, Como WA 6152

Summary

The survival of *Phytophthora cinnamomi, P. citricola* and *Armillaria luteobubalina* was determined at a range of temperatures. Millet seeds colonised by at least four isolates of each fungal species were incubated in either water or dry soil at temperatures between 30°C and 60°C for up to 120 hours. Different isolates of the same species behaved in a similar manner. In water *P. cinnamomi*, *P. citricola* and *A. luteobubalina* were killed after 1 hr at 42.5°C, 52°C and 47.5°C and after 24 hr at 40°C, 45°C and 45°C respectively. Limited experiments using colonised pine plugs gave similar results. The fungi survived at higher temperatures in dry soil. Under these conditions *P. cinnamomi*, *P. citricola* and *A. luteobubalina* were killed after 1 hr at 45°C, 60°C and 50°C respectively.

Introduction

One of the most important ways in which fungal pathogens such as *Phytophthora* spp. and *Armillaria luteobubalina* Watling and Kile are spread in the jarrah forest in the south west of Western Australia, is by soil movement during logging and mining operations. Control measures designed to reduce the spread of *P. cinnamomi* Rands include washing down vehicles to remove adhering soil, and restricting access during wet weather. These measures are expensive and inconvenient. Some infestations are very small so that it may be possible to eradicate them with an appropriate technique.

Phytophthora spp. and other fungal pathogens are routinely eradicated from nursery soil using aerated steam to raise its temperature to 60-80°C for 30 min, or solarization

where temperatures of 50-60°C can be achieved in the top 5 cm of soil and maintained for many weeks (Handreck and Black 1984). In field soil these temperatures may be achieved at a depth of 80 cm through heat generated at the surface by a burning log pile, with more modest temperatures at greater depth and distance from the pile (Tunstall *et al.* 1976). This may have particular application in road construction in the jarrah forest as a disease hygiene measure. Although it is not possible to destroy propagules throughout the soil horizon, heat treatment prior to compaction of the road may effectively disinfest the road surface. This would eliminate or reduce the risk of machinery spreading propagules of *P. cinnamomi* into presently uninfested forest. Subsequent compaction may act as a barrier to reinfestation from the adjacent forest (Wright *et al.* 1988).

To test the efficacy of heating as a disease control measure, three significant root pathogens *Phytophthora cinnamomi, P. citricola* Sawada and *Armillaria luteobubalina* were subjected a range of temperatures for up to five days duration. In soil these fungi are likely to be present in colonised organic material, so for this reason, as well as ease of handling, trials were conducted using colonised millet seed and pine plugs to establish lethal temperatures *in vitro* for these species.

Materials and Methods

Isolates

Five isolates of *Armillaria luteobubalina* and four isolates each of *Phytophthora cinnamomi* and *P. citricola* were used for the experimental work (Table 1). *Phytophthora* isolates were maintained on corn meal agar (CMA), and *A. luteobubalina* on 3% malt agar (MA).

Inoculum for Laboratory Trials

1. Millet seed inoculum

   Millet seed inoculum was generated by allocating a ratio of 100 g of seed per 80 ml distilled water to flasks which were then plugged with non-absorbent cotton wool, covered with aluminium foil and autoclaved for 30 min at 121°C. Sterile

seed was inoculated with either 7-10 d old *Phytophthora* spp. grown on CMA and 30-40 d old *A. luteobubalina* grown on MA was used for inoculation. Colonisation by *Phytophthora* spp. took 14-21 d and *A. luteobubalina* 30-40 d at 22°C, as determined by visual examination and plating on to agar.

2. Pine plug inoculum

Pine plug inoculum was generated following the methods described by Butcher *et al.* (1983). Stems of *Pinus radiata* approximately 2 y old were pruned, stripped of bark and sectioned into 2 cm lengths. Sections in the diameter range 15-25 mm were selected and placed in distilled water. Plugs were transferred to 2 l or 3 l flasks, leaving enough room for agitation, 100 ml of distilled water was added, the flasks were plugged with non-absorbent cotton wool, covered with aluminium foil and autoclaved once at 121°C for 30 min.

Each flask was then inoculated with the respective fungus. These were 7-12 d old *Phytophthora spp.* grown on pea agar (200 g of frozen peas homogenised with 15 g agar and D.I. water to 1 ltr). Ten 1 cm^2 segments of the appropriate fungus were aseptically transferred to flasks which were then incubated at 22°C and shaken periodically. Full colonisation of the pine plugs by *Phytophthora* spp was achieved in 14-28 d as determined by visual examination and plating on to agar.

Heat Treatments

Colonised millet seed inoculum was tested at temperatures between 30°C and 60°C under dry and wet conditions, while pine plugs were tested under wet conditions.

1. Wet heat

Colonised millet seed was transferred aseptically to 10 ml of sterile distilled water in McCartney bottles. All isolates were incubated for 0, 15, 30, 45, and 60 min at temperature between 30°C and 60°C, and at 40°C and 45°C for 2, 3 and 5 hours. *Phytophthora spp.* were also subjected to a further 1, 2 or 3 days at 40°C and 45°C. A constant temperature water bath was used to manipulate and

maintain temperatures. Timing commenced when test bottles equilibrated with the water bath temperature (+/- 1°C) and the samples were staggered to allow processing. Survival was determined by plating 40 millet seeds onto CMA or MA for *Phytophthora* spp. and *A. luteobubalina* respectively. Plates were incubated for a minimum of 14 days for *Phytophthora* and 30 days for *A. luteobubalina.*

Pine plug inoculum was treated in a similar manner. Plugs were incubated at 0, 15, 30, 45 and 60 min at temperatures ranging from 40°C to 55°C. Ten pine plugs were placed in self sealing plastic bags and immersed in water for all temperature treatments, with timing commencing as the core of a test plug equilibrated with the water bath temperature, determined by an electronic temperature probe. After treatment the plugs were split into two pieces, surface sterilised in 70% ethanol for 10 seconds, and washed twice in distilled water before plating on to CMA and incubated for a minimum of 14 days.

2. Dry heat

Dry, heat-sterilized road gravel was used to simulate dry forest conductivity. An oven was used to maintain desired temperatures. Soil was placed in small plastic soil-drying containers, dry heat sterilised and then preheated to the test temperature before addition of colonised millet seed. Seed was allowed to equilibrate for 5 min, and then samples were removed at 0, 15, 30, 45 and 60 min.

Survival was determined by plating 40 millet seeds from each sample onto CMA or MA for *Phytophthora* spp. and *A. luteobubalina* isolates respectively. Plates were incubated for a minimum of 14 d for *Phytophthora* spp. and 30 d for *A. luteobubalina.*

Propagules

Colonised millet seed were macerated and pine plugs were sectioned and examined under a microscope to determine the propagules present.

Results

Different isolates of the same fungus behaved in similar manner so results for each species have been combined as a percentage. Results for individual isolates are presented in Appendix A. The minimum exposure times after which the test fungi could not be recovered from millet seed and pine plugs are summarised below.

Wet Heat Treatments

P. cinnamomi could not be recovered from either millet seed or pine plug incubated for 15 min at 45°C, 45 mins at 42.5°C (Tables 2 and 3) and could not be recovered from millet seed incubated for one day at 40°C (Table 4).

P. citricola tolerated higher temperatures than *P. cinnamomi*. It could not, however, be recovered from millet seed after 15 min at 52.5°C or from pine plugs after 15 min at 55°C (Tables 2 and 3). It did not survive for 5 hr at 45°C, but could still be recovered at a low level at 40°C after 5 days (Table 4).

A.luteobubalina tolerated higher temperatures than *P. cinnamomi*, but was not as heat tolerant as *P. citricola*. It could not be recovered from millet seed after 15 min at 47.5°C (Table 2) or 2 hr at 45°C (Table 4). There was a low level of recovery after 5 hr at 40°C (Table 4).

Dry Heat

P. cinnamomi was not recovered from millet seed incubated for 15 min at 60°C, or from millet incubated for 45 min at 45°C (Table 5).

P. citricola was more heat tolerant than *P. cinnamomi*, it could not be recovered from millet seed incubated for 45 min at 60°C (Table 5).

A. luteobubalina behaved in a similar way to *P. cinnamomi*. It did not survive for 15 min at 60°C, or 30 min at 50°C (Table 5).

Comparison of wet and dry heat treatments

The wet heat treatment was more effective than the dry treatment for all species at all temperatures. For *P. cinnamomi* and *A. luteobubalina* at similar exposure time lethal temperatures in the dry treatment were 2.5°C higher than the wet treatment. However to kill *P. citricola* in infested millet, 15 min at 52.5°C was required for wet treatment whereas the dry treatment required 45 min at 60°C (Tables 2 and 5).

Comparison of millet seed and pine plugs

No differences were found between the survival of *P. cinnamomi* on pine plug and millet inoculum. *P. citricola* had slightly lower survival on pine plug inoculum than on millet possibly due to the more vigorous colonisation of the millet substrate. *A. luteobubalina* was not tested on pine plug inoculum.

Propagules

Isolates of *Phytophthora* spp. were examined to determine the propagules present. The heterothallic *P. cinnamomi* was observed to produce sporanga and chlamydospores readily on millet seed, and chlamydospores occasionally on the outer surfaces of pine plugs.

All isolates of *P. citricola* formed sporanga on millet seed. Oospores were produced abundantly in both millet seed and pine plugs, on pine plugs however, oospores were produced only on the overmost cells.

Discussion

Our results with *P. cinnamomi* correlate closely with other work using single isolates. Unpublished work by Crane (1987) found 25 min at 42°C and 15 min at 45°C lethal to *P. cinnamomi*-colonised wood plugs immersed in water. Theron *et al.* (1982) using *P. cinnamomi* colonised wheat grains immersed in water found that this fungus could not be recovered after 20 min at 42°C and 5 min at 45°C. Benson (1978), however, found that two isolates of *P. cinnamomi* could not be recovered from V8 agar discs immersed in water at 39°C after 90 min and 44°C after 4.5 min. These temperatures are lower

and exposure times shorter than our results and may be due to the volume of, or thermal characteristics of the colonised tissue rather than an effect of substrate on the physiological tolerance of *P.* spp.

The wet heat treatments indicate heat would be most effective in killing *P.* spp and *A. luteobubalina* in soils at, or wetter than, field capacity. Where soils are very dry, heat is not as effective, and slightly higher temperatures were needed to eliminate fungi.

These temperatures are achievable in soils and may have practical application in treating small areas of infested forest.

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Table 1. *Phytophthora* and *Armillaria* isolates used in the experemental work.

Isolate	Isolated from	Locality
P. cinnamomi		
DCE50 (A1)[1](IMI 165644)[2]	Soil	Dog Rd
DCE60 (A2)	*Hibbertia subvaginata* (Steudel) F. Muell.	Ravenswood
DCE229 (A2)	*Eucalyptus marginata* Donn ex Sm.	Jarrahdale
DCE230 (A2)(DAR 53101)[3]	*E. marginata*	Jarrahdale
P. citricola		
1450(IMI 329674)	Soil	Walpole
1723(IMI 329676)	Soil	Jarrah forest
3237	*Banksia prionotes* Lindl.	Jurien
3253	*B. attenuata* R. Br .	Yanchep
A. luteobubalina		
DCE218	*E. wandoo* Blakely	Cooke Block
DCE459	*E. calophylla* R. Br ex Lindl.	Manjimup
DCE460	*E. diversicolor* F. Muell.	Manjimup
DCE460	*Acacia* sp.	Manjimup
DCE461	*Agonis flexuosa* (Spreng.) Schau.	Ludlow

1 A1/A2: Mating type

2 IMI : International Mycological Institute, U.K.

3 DAR : Department of Agriculture, N.S.W.

Table 2. WET HEAT. Percent survival at elevated temperature of millet seed inoculum for 0-1 hour, isolates averaged for each species.

Species	Temperature	Time (hr)				
	(°C)	0	0.25	0.5	0.75	1.0
P. cinnamomi	30	100	100	100	100	100
	40	100	100	100	99.4	99.4
	42.5	100	63.1	5.6	0	0
	45	100	0	0	0	0
	60	100	0	0	0	0
P. citricola	30	100	100	100	100	100
	40	100	100	100	100	100
	45	100	100	98.8	98.8	91.9
	47.5	100	100	93.1	60.6	36.9
	50	100	96.3	57.5	16.3	3.1
	52	100	0	0	0	0
	60	100	0	0	0	0
A. luteobubalina	35	100	99.5	100	98.5	98
	40	100	97.5	92.5	95	82.5
	42.5	100	13.5	6	0	0
	45	100	10.5	2	0.5	1
	47.5	100	0	0	0	0
	60	100	0	0	0	0

Table 3 WET HEAT. Percent survival at elevated temperatures of pine plug inoculum for 0-1 hours, isolates averaged for each species.

Species	Temperature	Time (hr)				
	(°C)	0	0.25	0.5	0.75	1.0
P. cinnamomi	40	100	100	100	100	97.5
	42.5	100	62.5	12.5	0	0
	45	100	0	0	0	0
	50	100	0	0	0	0
	55	100	0	0	0	0
P. citricola	40	100	100	100	100	100
	45	100	87.5	82.5	55	47.5
	47.5	100	100	62.5	47.5	12.5
	50	100	25	0	0	0
	55	100	0	0	0	0

Table 4 WET HEAT. Percent survival at elevated temperature of millet inoculum for 1-120 hours, isolates averaged for each species

Species	Temperature	Time (hr)					
	(°C)	2	3	5	24	48	120
P. cinnamomi	40	91.2	36.9	0.6	0	0	0
	45	0	0	0	0	0	0
P. citricola	40	100	100	100	77.5	20	6.9
	45	32.5	1.3	0	0	0	0
A. luteobubalina	40	21	2.5	1			
	45	0	0	0			

Table 5 DRY HEAT. Percent survival at elevated temperature of millet inoculum for 0-1 hour, isolates averaged for each species.

Species	Temperature (°C)	Time (hr) 0	0.25	0.5	0.75	1.0
P. cinnamomi	30	100	100	100	100	100
	40	100	100	100	100	95.6
	42.5	100	100	99.4	90.6	52.5
	45	100	75	1.9	0	0
	50	100	24.4	0	0	0
	60	100	0	0	0	0
P. citricola	30	100	100	100	100	100
	45	100	100	100	100	100
	47.5	100	93.8	100	95.6	65
	50	100	200	98.8	91.3	86.9
	55	100	58.1	25.6	6.3	1.9
	60	100	30.6	3.1	0	0
A. luteobubalina	50	100	4.5	0	0	0
	60	100	0	0	0	0

APPENDIX A

HEAT TOLERANCE - Millet inoculum in water

		Time (h)			
Temp (oC)	0	0.25	0.5	0.45	1
ISOLATE 30					
CIT 1450	30	30	30	30	30
CIT 1723	30	30	30	30	30
CIT 3237	30	30	30	30	30
CIT 3253	30	30	30	30	30
% survival	100	100	100	100	100
DCE 50	30	30	30	30	30
DCE 60	30	30	30	30	30
DCE 229	30	30	30	30	30
DCE 230	30	30	30	30	30
% survival	100	100	100	100	100
ISOLATE 40					
CIT 1450	40	40	40	40	40
CIT 1723	40	40	40	40	40
CIT 3237	40	40	40	40	40
CIT 3253	40	40	40	40	40
% survival	100	100	100	100	100
DCE 50	40	40	40	40	39
DCE 60	40	40	40	40	40
DCE 229	40	40	40	39	40
DCE 230	40	40	40	40	40
% survival	100	100	100	99.375	99.375
ISOLATE 42.5					
DCE 50	40	36	9	0	0
DCE 60	40	40	0	0	0
DCE 229	40	22	0	0	0
DCE 230	40	3	0	0	0
% survival	100	63.125	5.625	0	0
ISOLATE 0	0.25	0.5	0.45	1	
CIT 1450	40	40	40	40	40
CIT 1723	40	40	40	40	40
CIT 3237	40	40	40	40	29
CIT 3253	40	40	38	38	38
% survival	100	100	98.75	98.75	91.875
DCE 50	40	0	0	0	0
DCE 60	40	0	0	0	0
DCE 229	40	0	0	0	0
DCE 230	40	0	0	0	0
% survival	100	0	0	0	0

APPENDIX A (Cont)

HEAT TOLERANCE - Millet inoculum in water

		Time (h)				
	Temp (oC)	0	0.25	0.5	0.75	1
ISOLATE	47.5					
CIT 1450		40	40	38	12	6
CIT 1723		40	40	39	37	30
CIT 3237		40	40	39	34	22
CIT 3253		40	40	33	14	1
% survival		100	100	93.125	60.625	36.875
ISOLATE	50					
CIT 1450		40	40	9	8	0
CIT 1723		40	37	37	3	0
CIT 3237		40	40	23	10	0
CIT 3253		40	37	23	5	5
% survival		100	96.25	57.5	16.25	3.125
ISOLATE	52.5					
CIT 1450		40	0	0	0	0
CIT 1723		40	0	0	0	0
CIT 3237		40	0	0	0	0
CIT 3253		40	0	0	0	0
% survival		100	0	0	0	0
ISOLATE	60					
CIT 1450		40	0	0	0	0
CIT 1723		40	0	0	0	0
CIT 3237		40	0	0	0	0
CIT 3253		40	0	0	0	0
% survival		100	0	0	0	0
DCE 50		40	0	0	0	0
DCE 60		40	0	0	0	0
DCE 229		40	0	0	0	0
DCE 230		40	0	0	0	0
% survival		100	0	0	0	0

APPENDIX A (Cont)

HEAT TOLERANCE - Millet inoculum, dry heat

		Time (h)			
Temp (oC)	0	0.25	0.5	0.45	1
ISOLATE 30					
CIT 1450	30	30	30	30	30
CIT 1723	30	30	30	30	30
CIT 3237	30	30	30	30	30
CIT 3253	30	30	30	30	30
% survival	100	100	100	100	100
DCE 50	30	30	30	30	30
DCE 60	30	30	30	30	30
DCE 229	30	30	30	30	30
DCE 230	30	30	30	30	30
% survival	100	100	100	100	100
ISOLATE 40					
DCE 50	40	40	40	40	40
DCE 60	40	40	40	40	40
DCE 229	40	40	40	40	40
DCE 230	40	40	40	40	33
% survival	100	100	100	100	95.625
ISOLATE 42.5					
DCE 50	40	40	40	35	13
DCE 60	40	40	39	35	15
DCE 229	40	40	40	35	16
DCE 230	40	40	40	40	40
% survival	100	100	99.375	90.625	52.5
ISOLATE 45					
CIT 1450	40	40	40	40	40
CIT 1723	40	40	40	40	40
CIT 3237	40	40	40	40	40
CIT 3253	40	40	40	40	40
% survival	100	100	100	100	100
DCE 50	40	40	3	0	0
DCE 60	40	40	0	0	0
DCE 229	40	0	0	0	0
DCE 230	40	40	0	0	0
% survival	100	75	1.875	0	0

APPENDIX A (Cont)

HEAT TOLERANCE - Millet inoculum, dry heat

		Time (h)			
Temp (oC)	0	0.25	0.5	0.45	1
ISOLATE 47.5					
CIT 1450	40	40	40	40	38
CIT 1723	40	40	40	36	11
CIT 3237	40	30	40	38	39
CIT 3253	40	40	40	39	16
% survival	100	93.75	100	95.625	65
ISOLATE 50					
CIT 1450	40	40	40	40	40
CIT 1723	40	40	40	40	40
CIT 3237	40	40	40	39	33
CIT 3253	40	40	38	27	26
% survival	100	100	98.75	91.25	86.875
DCE 50	40	17	0	0	0
DCE 60	40	20	0	0	0
DCE 229	40	2	0	0	0
DCE 230	40	0	0	0	0
% survival	100	24.375	0	0	0
ISOLATE 55					
CIT 1450	40	34	20	8	1
CIT 1723	40	35	14	1	2
CIT 3237	40	10	3	1	0
CIT 3253	40	14	4	0	0
% survival	100	58.125	25.625	6.25	1.875
ISOLATE 60					
CIT 1450	40	14	0	0	0
CIT 1723	40	34	5	0	0
CIT 3237	40	1	0	0	0
CIT 3253	40	0	0	0	0
% survival	100	30.625	3.125	0	0
DCE 50	40	0	0	0	0
DCE 60	40	0	0	0	0
DCE 229	40	0	0	0	0
DCE 230	40	0	0	0	0
% survival	100	0	0	0	0

APPENDIX A (Cont)

HEAT TOLERANCE - millet inoculum wet/dry A. luteobubalina

MOIST

		Time(min)				
Temp (oC)		0	15	30	45	60
ISOLATE	35					
DCE 218		40	39	40	38	39
DCE 459		40	40	40	40	39
DCE 460		40	40	40	39	38
DCE 461		40	40	40	40	40
DCE 462		40	40	40	40	40
% survival		100	99.5	100	98.5	98
ISOLATE	40					
DCE 218		40	35	28	36	24
DCE 459		40	40	40	38	39
DCE 460		40	40	38	38	29
DCE 461		40	40	40	38	37
DCE 462		40	40	39	40	36
% survival		100	97.5	92.5	95	82.5
ISOLATE						
DCE 218	42.5	40	7	3	0	0
DCE 459		40	7	7	0	0
DCE 460		40	5	1	0	0
DCE 461		40	4	1	0	0
DCE 462		40	4	0	0	0
% survival		100	13.5	6	0	0
ISOLATE	45					
DCE 218		40	5	2	0	2
DCE 459		40	3	0	0	0
DCE 460		40	11	1	1	0
DCE 461		40	1	1	0	0
DCE 462		40	1	0	0	0
% survival		100	10.5	2	0.5	1
ISOLATE	47.5					
DCE 218		40	0	0	0	0
DCE 459		40	0	0	0	0
DCE 460		40	0	0	0	0
DCE 461		40	0	0	0	0
DCE 462		40	0	0	0	0
% survival		100	0	0	0	0
ISOLATE	50					
DCE 218		40	0	1	0	0
DCE 459		40	0	0	0	0
DCE 460		40	0	0	0	0
DCE 461		40	0	0	0	0
DCE 462		40	0	0	0	0
% survival		100	0	0.5	0	0

APPENDIX A (Cont)

		DRY Time(min)			
Temp (oC)	0	15	30	45	60
ISOLATE 50					
DCE 218	40	8	0	0	0
DCE 459	40	1	0	0	0
DCE 460	40	0	0	0	0
DCE 461	40	0	0	0	0
DCE 462	40	0	0	0	0
% survival	100	4.5	0	0	0
ISOLATE 60					
DCE 218	40	0	0	0	0
DCE 459	40	0	0	0	0
DCE 460	40	0	0	0	0
DCE 461	40	0	0	0	0
DCE 462	40	0	0	0	0
% survival	100	0	0	0	0

APPENDIX A (Cont)

HEAT TOLERANCE - Wood plug inoculum

		Time (h)				
	Temp (oC)	0	0.25	0.5	0.75	1
ISOLATE	40					
DCE 50		10	10	10	10	10
DCE 60		10	10	10	10	10
DCE 229		10	10	10	10	10
DCE 230		10	10	10	10	10
% survival		100	100	100	100	100
1450		10	10	10	10	10
1723		10	10	10	10	10
3237		10	10	10	10	9
3253		10	10	10	10	10
% survival		100	100	100	100	97.5
ISOLATE	42.5					
DCE 50		10	5	0	0	0
DCE 60		10	7	0	0	0
DCE 229		10	9	5	0	0
DCE 230		10	4	0	0	0
% survival		100	62.5	12.5	0	0
ISOLATE	45					
DCE 50		10	0	0	0	0
DCE 60		10	0	0	0	0
DCE 229		10	0	0	0	0
DCE 230		10	0	0	0	0
% survival		100	0	0	0	0
1450		10	10	10	3	7
1723		10	10	10	10	9
3237		10	5	3	8	3
3253		10	10	10	1	0
% survival		100	87.5	82.5	55	47.5
ISOLATE	47.5					
1450		10	10	9	6	5
1723		10	10	10	10	0
3237		10	10	0	0	0
3253		10	10	6	3	0
% survival		100	100	62.5	47.5	12.5
ISOLATE	50					
DCE 50		10	0	0	0	0
DCE 60						
DCE 229						
DCE 230						
% survival		100	0	0	0	0

APPENDIX A (Cont)

HEAT TOLERANCE - Wood plug inoculum

Temp (oC)		Time (h)				
ISOLATE	55	0	0.25	0.5	0.75	1
1450		10	0	0	0	0
1723		10	10	0	0	0
3237		10	0	0	0	0
3253		10	0	0	0	0
% survival		100	25	0	0	0
ISOLATE	55					
DCE 50		10	0	0	0	0
DCE 60						
DCE 229						
DCE 230						
% survival		100	0	0	0	0
1450		10	0	0	0	0
1723		10	6	0	0	0
3237		10	0	0	0	0
3253		10	0	0	0	0
% survival		100	15	0	0	0

APPENDIX A (Cont)

HEAT TOLERANCE - Millet inoculum in water

		Time (h)				
Temp (oC)		2	3	5	24	48
ISOLATE	40					
CIT 1450		40	40	40	39	2
CIT 1723		40	40	40	33	30
CIT 3237		40	40	40	13	0
CIT 3253		40	40	40	39	0
% survival		100	100	100	77.5	20
DCE 50		20	2	0	0	0
DCE 60		22	0	0	0	0
DCE 229		10	0	0	0	0
DCE 230		0	0	0	0	0
% survival		32.5	1.25	0	0	0
ISOLATE	45					
CIT 1450		40	12	0	0	0
CIT 1723		37	19	1	0	0
CIT 3237		33	5	0	0	0
CIT 3253		36	23	0	0	0
% survival		91.25	36.875	0.625	0	0
DCE 50		0	0	0	0	0
DCE 60		0	0	0	0	0
DCE 229		0	0	0	0	0
DCE 230		0	0	0	0	0
% survival		0	0	0	0	0