STUDIES ON SILVER LEAF DISEASE OF STONE AND POME FRUIT TREES

by

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TABLE OF CONTENTS

ERRATA

page 4	line 9	Brenchley
"	last line	saprophyte
13	Table	*Prunus institia*
14	"	*Escallonia*
20	line 9	*C. purpureum*
"	last line	*Peniophora gigantea*
21	line 3	*Poria carbonica*
"	" 11	Etheridge
22	" 24	infection
23	" 15	Leishman
27	" 18	*C. purpureum*
30	" 9	"an" for "on"
32	" 19 & 21	substitute "discharge" for "deposition"
34	" 3	Grosclaude
"	" 4	*C. purpureum*
"	" 25	successive
38	" 12	individual
46	" 10	Albersheim
48	" 36	should read "... not only due..."
52	" 9	should read "... was found associated with a twig dieback of mulberry..."
60	" 10	Carter & Ilsley (not Carter & Price)
63	'B'	*C. purpureum* not inhibited
65	line 10	pathogen's
69	" 28	Figure 14a
71	figure	Figure 14a
87	line 27	Pottle
94	" 3	Burkard
96	" 12	possess
99	ref. 15	*sambucinum*
104	"	Insert: D'Aeth, H.R.X. (1939) A survey of interaction between fungi. Biological Reviews 14, 105-131.
113	" 8	physiological
116	" 6	Wood

G.C. Bishop
14.11.78

SUMMARY

A comprehensive list of the hosts of *Chondrostereum purpureum* has been compiled from the literature and from field observations in South Australia. Thirty-nine plant species were recorded as hosts in South Australia and of these 11 had not previously been listed as hosts from any other country.

Basidiospores of *C. purpureum* from apple, broom and willow were found to be as effective as those from cherry in invading freshly pruned cherry sapwood. The isolates did, however, differ in the amount of silvering of the foliage that they caused. It was found that those isolates which caused little foliar silvering produced relatively little phytolysin, the substance that disrupts the leaf tissues and causes silvering symptoms.

Studies seeking a potential biological control agent for *C. purpureum* were initiated. *Fusarium lateritium,* which has been found to give significant protection against another vascular pathogen of woody plants, *Eutypa armeniacae,* was tested against *C. purpureum* both in the laboratory and in the field. *F. lateritium* was found to inhibit mycelial growth of *C. purpureum in vitro,* the size of the inhibition zone increasing with the age of the *F. lateritium* colony. The *invitro* inhibition of *C. purpureum* was due to the production of an antibiotic, enniatin, by *F. lateritium.*

A study of a natural population of *Fusarium lateritium* in a commercial apple orchard was carried out. The number of viable propagules of *F. lateritium* on apple tree bark was assessed at weekly intervals over a period of 30 months. The population of *F. lateritium* increased from autumn onwards, reaching a peak in spring and falling to a low level over summer. On several occasions during the growing season, temporary decreases in the population could be attributed to the application of fungicidal sprays, notably Difolitan and Dithane. When these fungicides were tested in the laboratory for toxicity to *F. lateritium* it was found that < 5 p.p.m. active ingredient of both Difolitan and Dithane would completely inhibit the germination of *F. lateritium* macroconidia, and 4 p.p.m. A.I. Difolitan would inhibit hyphal growth. The effect of rainfall on the population of *F. lateritium* appeared to be more complex than that of the fungicidal sprays. No significant correlations were found between spore count and rain that fell in 24 hour intervals up to 7 days prior to sampling.

When macroconidia of *F. lateritium* were applied to a freshly pruned surface, sporulation occurred within 7 days, and continued at a high level for at least 6 weeks. However, a suspension of *F. lateritium* macroconidia applied to wounds immediately after pruning afforded little protection against inocula of *C. purpureum* introduced on the pruned cherry sapwood either 1, 3 or 5 days after pruning. When only the pathogen, *C. purpureum,* was applied to pruning wounds all sites were invaded; but when *F. lateritium* was applied 5 days before inoculation

with *C. purpureum* only 68% of the sites became infected with the pathogen.

Several other fungi that were isolated from the surface of pruning wounds on fruit trees were tested in the laboratory against *C. purpureum* and some showed promise as possible biological control agents. Three fungi, a *Fusarium* sp. and two *Trichderma* spp. were found to prevent the invasion of cherry wood blocks by *C. purpureum.* Spores of all three germinated and started to grow well in advance of *C. purpureum,* and the Trichodermas sporulated readily on the pruned sapwood.

STATEMENT

This thesis contains no material which has been accepted for the award of any other degree in any university and to the best of my knowledge and belief contains no material previously published or written by another person, except where due reference is made in the text.

30.V.1978

G. C. Bishop

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FIGURE 1

Edouard Prillieux (1829-1915) who delivered the first recorded scientific paper on Silver Leaf Disease to a meeting of the Société National D'Agriculture de France held on July 22 1885.

1829-1915

I. GENERAL INTRODUCTION

The Disease

'Silver Leaf' is the name given to a disease of stone and pome fruit trees caused by the fungus *Chondrostereum purpureum* (Pers. ex Fr.) Pouzar. Pouzar (1959) proposed the genus *Chondrostereum* with *C. purpureum* (Pers. ex Fr.) Pouzar as type species. Its synonymy is extensive (Cunningham, 1963). The name most frequently used for this species is *Stereum purpureum* Pers. ex Fr.

The first scientific observations of this disease were made by Prillieux (1885) in France (Figure 1.) and published under the title of 'Le plomb des arbres fruitiers'. He considered the leaden colour of the leaves to be the result of the presence of large air spaces between the epidermal and palisade cells. In 1894 Aderhold (1895) referred to the 'Milchglanz' of fruit trees as occurring in Germany. He considered the disease to be of nonparasitic origin. The descriptive name, 'Silver Leaf Disease', was given in 1902 in England by Percival (1902). He established the parasitic origin of the condition by demonstrating the presence of fungal hyphae in the wood of silvered plum-trees and, by means of artificial inoculations, brought about the silvering symptoms.

C. purpureum is a wound parasite, that is, it gains entry to a tree through fresh pruning wounds or damaged limbs. Successful artificial inoculations have been made only during the autumn and winter months. Infection during summer is prevented by the rapid formation of 'gum barriers' through which the fungus is not able to grow (Brooks & Moore, 1926; Brooks, 1936; Mostafa, 1947; Dye, 1967). Pruning wounds remain susceptible to invasion by *C. purpureum* for only a couple of days after pruning. Exposed sapwood soon becomes inhabited by saprophytic bacteria and fungi, and their presence probably exerts an inhibiting or competitive influence (Brooks & Storey, 1923).

On arriving on a fresh pruning site, the spores of *C. purpureum* are taken into the wood vessels and there they germinate (Brooks & Moore, 1924; Grosclaude, 1973d). The fungus begins to spread rapidly in a tree only when air temperatures begin to rise in the spring. It is inactive over the summer period and resumes growing in the autumn. The two periods of active spread within the tree correspond to the periods of the year when the levels of nutrients

FIGURE 2

A. Silvered foliage on an apple tree *(Malus sylvestris).*

B. Fructifications of *C. purpureum* on the stump of an apple tree.


A


B

in the tree sap are highest (Dixon & Atkins, 1915; Mostafa, 1948; Beever, 1970). The margin of the fungus may grow forward as much as 18cm in plum trees in England over a period of 6 weeks during the spring (Brooks & Moore, 1926).

The first external indication of Silver Leaf Disease is the silver-grey appearance of the leaves on an affected limb (Figure 2.). This symptom probably does not appear until the tree has been diseased for one or more seasons (Grosclaude, 1960a). Mature leaves on a diseased limb show curling of the edges, browned margins and crinkled laminae. As the severity of the disease increases, leaf size is reduced and browning becomes more pronounced. Diseased leaves fall prematurely (Brooks & Benchley, 1929; Cunningham, 1931; Tetley, 1932; Williams & Cameron, 1956; Atkinson, 1971).

The silvering of the foliage results from separation of the epidermal cells from the palisade cells beneath (Figure 3.). Air cavities thus formed in the leaf affect light reflection and a silvering of the foliage is then apparent (Smolák, 1915; Bintner, 1919; Brooks & Storey, 1923; Tetley, 1932). The separation of the layers of cells is due to a 'toxin' which has been given the general name phytolysin (Naef-Roth, Kern & Toth, 1963), produced by the fungus and carried in the xylem vessels to the leaves where it has its effect. The exact chemical nature and mode of action of the substance(s) concerned is not known (Smolák, 1915; Brooks & Brenchley, 1929, 193lb; Naef-Roth, Kern & Toth, 1963; Wood, Ballio & Granti, 1972).

An affected limb or tree will die within 2-3 seasons (Figure 3.) after the foliar symptoms have first become apparent (Heyns, 1965). Seriously affected trees will fail to mature any fruit that is set (Figure 4.). A cross-section of a diseased branch reveals extensive brown staining in the wood (Figure 4.). In the final stage of the disease, the fungus grows through the sapwood and kills the cambium, and fructifications of the fungus form on the bark (Figure 2.).

Distribution

A disease of Portugal Laurel *Prunus lusitanica* which from the description given was Silver Leaf Disease, was reported from Kelso, Roxburghshire, Scotland in 1849. This appears to have been the first recording of this disease from Great Britain (K.,W.S., 1849). Other early recordings of the disease were from France (Prillieux, 1885), Germany (Aderhold, 1895) and New Zealand (Blackmore, 1909). Since these early reports, the disease and/or the causal fungus have been recorded throughout the temperate areas of the world (C.M.I., 1943, 1953; Pirozynski, 1968). The countries in which *C. purpureum* has been recorded are given in Table 1.

There are very few records of *C. purpureum* occurring in the tropics. Léveille described *C. purpureum* from Java in 1844-5 (C.M.I., 1943) and Bresadola & Roumeguère (1890) recorded it as a saprophytic in Saõ Thomé in the Gulf of Guinea.

FIGURE 3

A. A 45 year old cherry tree (*Prunus avium* cv. Waterloo) showing advanced symptoms of Silver Leaf Disease.
   a = limbs that have already died
   b = other half of tree showing severe silvering of the foliage

B. Transverse section of upper epidermis of a silvered plum leaf, showing intercellular spaces filled with air beneath the cuticle along the lines of union of the epidermal cells. The silver-grey colour of the leaves is due to these air-filled spaces, and not to any alteration in the chloroplasts (from Arnaud & Arnaud, 1931).

B

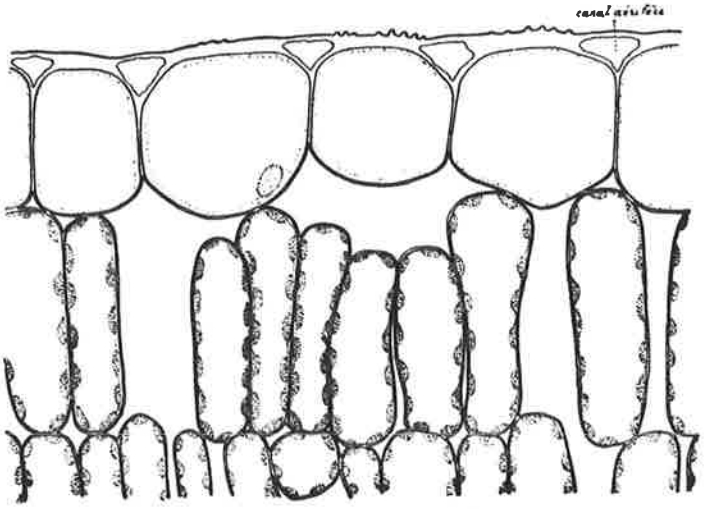


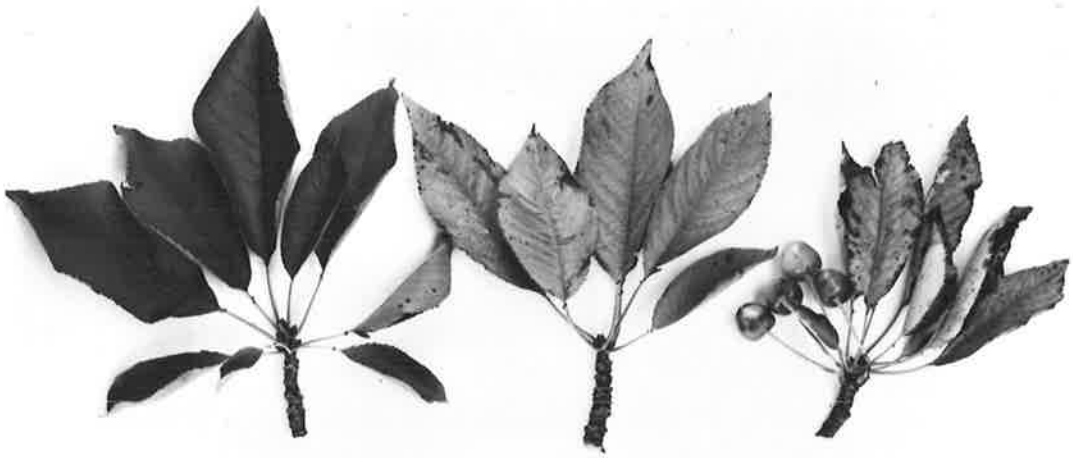
FIGURE 4

A. Foliar symptoms on cherry (*P. avium* cv. Napoleon)

a = healthy leaves
b = moderately silvered leaves with brown margins
c = leaves and fruit from a severely silvered tree

B. Cross and longitudinal sections of a diseased branch of a cherry tree (*Prunus avium* cv. Waterloo) showing brown staining of the wood (indicated by the white arrows) caused by *C. purpureum.*

A



a b c

TABLE 1

Countries in which *Chondrosterum purpureum* has been recorded

COUNTRY	REFERENCE
AFRICA	
Algeria	C.M.I. (1953)
Egypt	Mostafa (1947)
Morocco	Malençon (1952)
Republic of South Africa	Putterill (1923), Talbot (1954), Heyns (1965)
Saõ Thomé	Bresadola & Roumeguère (1890)
ASIA	
China	Wang (1942)
India – States of	
Almora	Singh (1943)
Bhowali	Singh (1943)
Darjeeling	C.M.I. (1953)
Himachal Pradesh	Gupta & Agarwala (1971)
Mashobra	Agarwala & Gupta (1968)
Mussoorie	C.M.I. (1953)
Raytal	Lentz (1955)
Rungling Pass	Lentz (1955)
Uttar Pradesh	Butler & Bisby (1931)
West Bengal	Butler & Bisby (1931)
Indonesia - Java	Léveille (1844 - 5)
Japan	Shirai & Hara (1927)
AUSTRALASIA	
Australia – States of	
New South Wales	McAlpine (1895)
Queensland	McAlpir e (1895)
South Australia	Cleland (1934), Moller & Talbot (1962), Talbot (1964)
Tasmania	Cooke (1892)
Victoria	McAlpine (1895), Anon. (1943)
Western Australia	Cooke (1892)
New Zealand	Blackmore (1909), Cunningham (1956), Dingley (1969)
EUROPE	
Austria	Wahl (1924)
Bulgaria	Christoff (1934)
Denmark	Ferdinandsen (1923)
Eire	Adams & Pethybridge (1910)
Finland	C.M.I. (1953)
France	Bourdot & Galzin (1928), Viennot-Bourgin (1949)
Germany	Burt (1920)
Great Britain –	
England	Moore (1959), Peace (1962)
Northern Ireland	Adams & Pethybridge (1910)
Scotland	Dennis & Foister (1942)
Wales	C.M.I. (1953)

COUNTRY	REFERENCE
Greece	Marie & Politis (1940)
Holland	Grosjean (1943)
Hungary	Husz (1947)
Italy	Bresadola & Cavara (1901)
Latvia	Smarods (1930)
Lithuania	C.M.I. (1953)
Norway	Robak (1942), Jørstad (1948)
Poland	Garbowski (1935)
Portugal	Pinto (1941)
Rumania	Săvulescu (1938)
Spain	Marie (1937)
Sweden	Björkman (1946)
Switzerland	Gäumann (1936)
Turkey	Lohwag (1959)
U.S.S.R. – Siberia	Pilát (1933) (in C.M.I., 1953)
Ukraine	Borggardt (1934)
Yugoslavia	Škorić (1946)
SOUTH AMERICA	
Argentina	Spegazzini (1926), Lentz (1955)
Chile	English, Moller & Nome (1967)
Paraguay	C.M.I. (1953)
Uruguay	Burt (1920)
NORTH AMERICA	
Canada – general	Güssow (1911), Connors (1967)
British Columbia	C.M.I. (1953)
Manitoba	C.M.I. (1953)
New Brunswick	C.M.I. (1953)
Newfoundland	Burt (1920)
Nova Scotia	Wehmeyer (1940)
Ontario	Burt (1920)
Prince Edward Island	C.M.I. (1953)
Quebec	C.M.I. (1953)
Vancouver Island	Lentz (1955)
United States of America –	
California	Chaney, Moller & Gotan (1973)
Connecticut	Burt (1920)
Delaware	C.M.I. (1953)
Idaho	Lentz (1955)
Indiana	Lentz (1955)
Iowa	Lentz (1955)
Maryland	Lentz (1955)
Massachusetts	C.M.I. (1953)
Maine	C.M.I. (1953)
Michigan	C.M.I. (1953)
Minnesota	Lentz (1955)
Montana	Lentz (1955)
New Jersey	C.M.I. (1953)
New York	Orton & Wood (1924)
Ohio	C.M.I. (1953)
Oklahoma	C.M.I. (1953)
Oregon	Williams & Cameron (1956)
Pennsylvania	Overholts (1939)
Virginia	Lentz (1955)
Vermont	C.M.I. (1953)
Washington	Orton & Wood (1924)
Wisconsin	Setliff & Wade (1974)
Wyoming	C.M.I. (1953)

Hosts of *C. purpureum*

Over 180 plant species are recorded in the literature as being hosts of *C. purpureum.* They belong to 73 genera in 26 families (Table 2).

The families with the largest number of hosts are Rosaceae (58 species), Salicaceae (20 species) and Leguminosae (14 species). Silver Leaf Disease is probably of greatest economic importance in the Rosaceae which includes the genera *Prunus* (apricot, cherry, plum, peach), *Malus* (apple), *Pyrus* (pear) and *Cydonia* (quince).

C. purpureum appears to be parasitic on 63 of the recorded hosts and is an unconfirmed parasite of a further 9 species. The silvering of the leaves which is characteristic of the disease, is mainly confined to hosts in the family Rosaceae, but silvering has also been recorded in New Zealand on Gold Willow (*Salix alba* var. *vitellina*) (Gilmour, 1966) and in Great Britain on *Salix* sp. (Rishbeth, personal communication) and *Ribes cereum* (Bintner, 1919).

In the Northern Hemisphere, *C. purpureum* is well known as a saprophyte on stored timber and forest slash of softwoods, and as such causes considerable damage (Björkman, 1946; Connors, 1967; Jacquiot, 1968; Lehmann & Schieble, 1923; Lohwag, 1959; Weir, 1914). It has been suggested that it may be weakly parasitic on many forest tree species (Anderson, 1956).

TABLE 2

A list of plant hosts of *Chondrostereum purpureum*

	Botanical Name	Common Name	P/S*)	Reference (*)
	ACERACEAE			
	Acer sp			5,14
*	*A. negundo*	Box-Elder	(P) S	36
	A. platanoides	Norway Maple	P	14,33,46
	A. pseudoplatanus	Sycamore		12,43
*	*A. rubrum*	Red Maple	(P) S	36
	A. saccharinum	Silver Maple	(P) S	5,36
	A. saccharophorum	Sugar Maple	(P) S	36
	BETULACEAE			
	Alnus sp.		S	36
	A. rubra	Red Alder	S	5
	A. sinuata	Sitka Alder	S	5
	A. tenuifolia	Mountain Alder	S	5
	Betula sp.		S	12,14
	B. alba			8
	B. lutea (syn. alleghaniensis)	Yellow Birch	(P) S	36,37,38
	B. occidentalis	Merisier Birch	S	5
	B. papyrifera	Paper Birch	S	5,38
*	*B. pendula*	Silver Birch	S	6
	B. populifolia	Bouleau Birch		5
	B. verrucosa	Silver Birch	S	10,11,33
	Corylus sp.	Filbert, Hazel Nut	S	12
	CAPRIFOLIACEAE			
	Lonicera sp.	Honeysuckle		12,20
	L. nitida		P	12
	Sambucus nigra	Elder Tree	P	41
	Viburnum tinus	Laurustinus		16
	Weigela sp.			10
	CELASTRACEAE			
	Euonymus europaeus	Spindle Tree	P	16
	E. japonicus	Japanese Spindle Tree		10,41
	ERICACEAE			
	Pernettya micronata	Prickly Heath	P	2,12
	Rhododendron sp.			14
	R. arboreum		(P)	12,18
	R. barbatum		P	12,18
	R. griffithianum		P	12,18

*) P or S – recorded as a parasite (or saprophyte) on this species
(P) – probably a parasite on this species
Blank – no details given in the literature

* recorded on this species in South Australia

** new host record from South Australia

(*) References – for key to the references see Table 2a.

TABLE 2 continued

	Botanical Name	Common Name	P/S	Reference
	FAGACEAE			
	Castanea spp.	American and European Chestnuts	S	16,36
	Fagus sp.		S	13,14,36
	F. grandifolia	American Beech	S	5
	F. sylvatica	Beech	S	8,12
	Nothofagus solandri var. cliffortioides	Southern Mountain Beech	S	8,14
	Quercus sp.			
	Q. garryana	Oregon Oak	S	17
	Q. ilex	Holm Oak	S	47
	FLACOURTIACEAE			
	Azara microphylla			12
	GUTTIFERAE			
	Hypericum calycinum	St. John's Wort		16
	HIPPOCASTANACEAE			
	Aesculus hippocastanum	Horse-Chestnut	SP	2,5,12,34
	A. carnea	Red Horse-Chestnut	SP	2,12
	JUGLANDACEAE			
	Juglans sp.	Walnut	(P) S	12,14,30
	LEGUMINOSAE			
	Acacia sp.			14
	Cercis siliquastrum	Judas Tree		20
*	*Chamaecytisus proliferus*	Tagasate, Tree Lucerne	P	14,16
*	*Cytisus scoparius*	Common Broom	PS	6,7,8,14,16
	Genista sp.			12
	Laburnum sp.			3,10,14
	L. alpinum	Scottish Laburnum		2,12
	L. anagyroides (syn.L. vulgare)	Golden Chain	PS	12,13
	L. vulgare		PS	2,35
	Lupinus arboreus	Tree Lupin		8,12
	Robinia pseudoacacia	Black Locust	(P) S	8,10
**	*Teline monspessulana*	Small-flowered Broom	PS	
	Ulex europaeus	Gorse, Furze	PS	8,10,14
**	*Wisteria floribunda*	Wisteria	S	
	MELASTOMATACEAE			
**	*Tibouchina semidecandra*	Lasiandra	P	
	MYRTACEAE			
	Eucalyptus ficifolia	Flowering Gum	(P) S	10
	E. gunnii		S	12,20
	Leptospermum scoparium	South Sea Myrtle, Manuka	PS	10,11

TABLE 2 continued

	Botanical Name	Common Name	P/S	Reference
	OLEACEAE			
	Fraxinus sp.	Ash		34
	F. excelsior			10
	Syringa sp.			2,45
*	*S. vulgaris*	Lilac		2,6,14,32
	PINACEAE			
	Abies sp.			
	A. balsamea	Balsam Fir	S	5
	A. lasiocarpa	Alpine Fir	S	5
	Cedrus sp.	Cedar		12
	Cupressus macrocarpa	Monterey Cypress	S	8,14
	Larix decidua	European Larch	S	12
	L. occidentallis		S	19
	Picea glauca	White Spruce	S	5
	Pinus sp.			12
	Pseudotsuga menziesii	Douglas Fir	S	5
	Thuja plicata	Cedre	S	5
	PITTOSPORACEAE			
	Pittosporum crassifolium			10
	P. tenuifolium	Kohuhu	PS	8,10,11
	PLATANACEAE			
	Platanus sp.			3,12
	P. occidentalis	American Plane Tree	S	36
	PODOCARPACEAE			
	Dacrydium cupressinum	Rimu, Red Pine	S	11
	RHAMNACEAE			
	Rhamnus carthartica			16
	R. frangula	Buckthorn		14
**	*Ceanothus thyrsiflorus*	Californian Lilac		
	ROSACEAE			
	Cotoneaster sp.			14
	C. franchietti		S	20
	C. horizontalis			12,13
	C. melanocarpa			39
	C. vulgaris		S	8
	Crataegus sp.			
	C. monogyna			2
	C. oxycantha	Common Hawthorn	S	8,12,14
	C. sanguinea			39
*	*Cydonia oblonga*	Quince	PS	12,14

TABLE 2 continued

	Botanical Name	Common Name	P/S	Reference
	Eriobotrya japonica	Loquat		9,10
	Exochorda sp.	Pearl Bush	P	2,12
	Kerria japonica	Jew's Marrow	S	5
*	*Malus x adstringens*	Crab-apple	PS	20
	M. angustifolia			10
	M. baccata	Siberian Crab-apple		5
	M. prunifolia		PS	2,44
*	*M. sylvestris*	Apple	PS	5,8,12,14,15
	Mespilus germanica	Medlar	PS	1,10,12,34
	Neviusia alabamensis	Alabama Snow Wreath	P	2,12
	Prunus sp.			
*	*Amygdalus communis*	Almond	PS	1,12,14
	Prunus acida		P	2
*	*P. armeniaca*	Apricot	PS	1,12,14,36
*	*P. avium*	Sweet Cherry	PS	12,13,14,21,40
*	*P. cerasus*	Sour Cherry	PS	2,5,17
*	*P. cerasus var. austera*	Morello	PS	12,19,36
*	*P. cerasifera*	Cherry Plum	P	
**	*P. cerasifera var. atropurpurea*		PS	
	P. communis	Wild Plum	PS	
*	*P. domestica*	Plum	PS	1,2,12,14,20,28
	P. emarginata	Bitter Cherry	PS	5
	P. insitita	Damson		14
	P. japonica	Flowering Cherry	P	2
	P. laurocerasus	Cherry Laurel	PS	5,12,13
	P. lusitanica	Portugal Laurel	PS	3,12,26,43
	P. mahaleb	Mahaleb	PS	13,34
	P. mariana		P	31
	P. myrobolanus	Myrobella Stock	P	2
	P. padus	European Bird Cherry	PS	5,33
*	*P. persica*	Peach	PS	12,14,25
*	*P. persica var. nectarina*	Nectarine	PS	12,14
	P. pumila	Sand Cherry	PS	5
*	*P. salicina*	Japanese Plum	PS	10
	P. spinosa	Sloe, Buckthorn	PS	3,12,13,34,44
	P. triloba		PS	2,12,13,34,41
*	*Pyrus communis*	Pear	P	33
	P. americana nana		P	12,14,20,32,36
*	*Rosa sp. cult.*		P	12,14,34
	R. rugosa			28
	R. sericera var. pterocantha			28
	Rubus idaeus	Raspberry	PS	8,12,14,24,27
	Sorbus aucuparia	Rowan		12
**	*S. aucuparia cv. Xanthocarpa*		P	
	S. hybrida			39
	Spirea sp.			12,43
*	*S. arguta*			2,12
	S. japonica var. glabrata		P	2

TABLE 2 continued

	Botanical Name	Common Name	P/S	Reference
	SALICACEAE			
	Populus sp.			3,14
*	*P. alba*	Silver Poplar		42
	P. candicans	Balm of Gilead		29
**	*P. candicans var. aurea*	Golden Poplar	S	
	P. deltoides	Cottonwood		14
	P. nigra	Black Poplar	PS	10
*	*P. nigra var. italica*	Lombardy Poplar	PS	5,8
	P. tacamahaca	Balsam Poplar	S	5,36
	P. tremula	Aspen	PS	8,14,23
	P. tremuloides	Trembling Poplar		5
	P. trichocarpa	Black Cottonwood	S	5
	P. x euramericana var. serotina			10,11
	Salix sp.		PS	2,44
	S. alba var. vitellina	Golden Willow	PS	12,14
**	*S. alba var. vitellina pendula*	Golden Weeping Willow	S	
*	*S. babylonica*	Weeping Willow	PS	10,11
*	*S. caerulea*	Cricket Bat Willow	S	5
	S. capraea			10
**	*S. discolor*	Pussy Willow	PS	
	S. fragilis	Crack Willow	S	10
	S. viminalis	Osier Willow		14,49
**	*S. x rubeus*		S	
	(S. alba x S. fragilis)			
	SAPINDACEAE			
	Xanthoceras sorbifolia			16
	SAXIFRAGACEAE			
	Escallaria exoniensis			12
	E. macrantha			10,14,23
	E. vulgaris		S	8, 9
	Hydrangea hortensis			41
**	*H. macrophylla*		S	
	Philadelphus sp.	Mock Orange		2,12,16,43
	Ribes cereum		PS	2
	R. grossularia	Gooseberry		12,14,20
	R. nigrum	Black Currant		12,14,20,27
	R. sativum	Red Currant		1,12,14,27
	R. sylvestre			10
	R. uva-crispa var. sativum			10
	SOLANACEAE			
	Physalis peruviana	Cape Gooseberry		10
	ULMACEAE			
*	*Ulmus sp.*		S	12,28,34
	U. americana	American Elm	S	36
	Celtis laevigata	Southern Hackberry	S	36

TABLE 2 continued

Botanical Name	Common Name	P/S	Reference
VITACEAE			
* *Vitis amurensis*	Glory Vine	S	
V. labrusca	Fox Grape	S	36
V. vinifera	European Grape	(P) S	14,16,22,48

TABLE 2a

List of authors cited in host list (Table 2).

Note: the author citations refer to the main bibliography.

Reference Number	Author	Reference Number	Author
1	Ainsworth (1937)	25	Chaney, Moller & Gotan (1942)
2	Bintner (1919)	26	K., W.S. (1849)
3	Brooks & Bailey (1919)	27	Anon. (1961)
4	Brooks & Moore (1926)	28	Brooks & Storey(1923)
5	Connors (1967)	29	Grosjean (1943)
6	Cunningham (1925)	30	Wormald & Harris (1939)
7	Cunningham (1931)	31	Grosclaude (1971)
8	Cunningham (1956)	32	Moller & Talbot (1962)
9	Cunningham (1963)	33	Robak (1942)
10	Dingley (1969)	34	Gram & Weber (1952)
11	Gilmour (1966)	35	Gussow (1911)
12	Moore (1959)	36	U.S. Dept. Agric. (1960)
13	Peace (1962)	37	Shields & Atwell (1963)
14	Rev. Appl. Mycol. (1968)	38	Hepting (1971)
15	Setliff & Wade (1973)	39	Jørstad (1948)
16	Viennot-Bourgin (1949)	40	Wehmeyer (1950)
17	Williams & Cameron (1956)	41	Arnaud & Arnaud (1931)
18	Cotton (1925)	42	Putterill (1923)
19	Weir (1914)	43	Brooks (1911)
20	Min. Agric. Fish & Food (1972)	44	Brooks (1913)
21	Anon. (1958)	45	Laubert (1931)
22	Branas (1960)	46	Robak (1936)
23	Brien (1946)	47	Malençon (1952)
24	Brien & Atkinson (1942)	48	Bolay & Moller (1977)
		49	Anon. (1951)

Economic Importance

Silver Leaf Disease is of widespread importance in stone and pome fruit orchards. Economic loss results from reduction in yield and quality of fruit harvested, from costs of implementing control measures and from the death of infected trees.

After a survey in 1953, Watts (1954) found that Silver Leaf was the most serious disease of stone fruit trees in New Zealand. He found that 5.4% of bearing peach trees and 4.3% of bearing nectarine trees had the disease. At Hastings, 15% of bearing peach trees were affected. In the same country, Dye & Foxton (1968) estimated that losses from Silver Leaf Disease in an apple tree nursery at Levin were 2.5 and 8.0% per annum depending on seasonal conditions.

The disease is regarded as important in France (Grosclaude & Delmas, 1958; Grosclaude, 1960a) and in South Africa (Heyns, 1965), but no figures have been put on losses due to it.

Severe infections have been recorded in the United Kingdom. For example, in the Brentford district of Middlesex in 1918, 80 - 90% of Victoria plum trees, 5 - 30 years old, were so severely affected that they had to be removed by late summer (Bintner, 1919). An attempt was made to control its spread under the Silver Leaf Order of 1923 (Anon., 1923). In 1958 cherries were included under the Order (Anon., 1958). In recent years, ground mapping has been used to study an outbreak of the disease in plantings of perry pear cv. Hendre Huffcap (Corke, 1969).

Very little has been published on the importance of the disease in the Americas. It is of economic importance on cherry and apple in Wisconsin (Setliff & Wade, 1973). They reported the death of nearly an entire orchard containing 14 cultivars of apple within 30 months of planting in a marginal apple growing area of Lincoln County, Wisconsin. In Oregon, where Silver Leaf Disease has been known since 1926, Williams & Cameron (1956) surveyed an orchard of sour cherry and found that 14% (63/448) were affected.

FIGURE 5

Fructifications of *C. purpureum* on the stump of a dead cherry tree in an orchard at Basket Range, in the Adelaide Hills.

Control Measures

Attempts have been made to control Silver Leaf Disease in a variety of ways. These include the modification of cultural practices in the orchard, chemotherapy and the development of biological means of control.

The control of Silver Leaf Disease by the modification of cultural practices is discussed in detail by Dye (1972) and Grosclaude (1960a, 1963). These and other authors consider the following aspects:

(a) Orchard sanitation

This involves the elimination of sources of inoculum within and near to orchards. Hence, newly dead wood (prunings, stumps, logs) particularly from infected fruit trees should be burnt or buried before sporophores are formed (Figure 5.). Such action for the control of Silver Leaf was legislated for in South Australia under the Vine, Fruit and Vegetable Protection Acts, 1885 and 1910 in 1936 (South Australian Government Gazette, 1936).

(b) Pruning practices

Trees should be pruned in summer shortly after harvest. Trees are much less susceptible to invasion by *C. purpureum* in summer than they are in winter-spring (Brooks & Storey, 1923; Mostafa, 1947). In addition, spore release occurs far more frequently in winter-spring than during the summer (Dye, 1974).

(c) Wound dressings

If trees are pruned in the winter or spring, this should be done in fine weather and an effective wound dressing applied immediately afterwards. Many wound protectants have been tested over the years but few have been found to be effective against *C. purpureum* (Brooks & Storey, 1923; Padfield, 1955; Grosclaude, 1966b; Dye & Wheeler, 1968). Grosclaude (1966a, 1966b, 1968) found that the use of some wound dressings resulted in a higher percentage of trees becoming infected than in the unprotected controls. He suggested that the materials involved are not toxic to *C. purpureum* but are so to many of the saprophytic organisms that colonize pruning wounds and help to exclude *C. purpureum.*

In recent years, several dressings have been developed that are effective against *C. purpureum* in the orchard (Grosclaude, 1968; Dye & Wheeler, 1968; Dye & Foxton, 1968; Dye, 1971). These preparations contain mercuric compounds as active ingredients (Grosclaude, 1968; Dye, 1971).

Chemotherapy is the treatment of an existing disease by means of chemicals, in particular,

ones introduced into the plant (Stoddard & Dimond, 1949). Treatments involving the use of artificial manures applied to the ground, or compounds sprayed on the tree, have not proven at all successful in ameliorating the disease (Bedford & Pickering, 1910; Brooks, 1911; Brooks & Bailey, 1919; Brooks & Storey, 1928; Brooks & Moore, 1926; Brooks & Brenchley, 1931; Reitsma & Zwijns, 1966).

Early attempts at the chemotherapy of Silver Leaf Disease also met with little success (Brooks, 1911; Brooks & Bailey, 1919; Brooks & Storey, 1923). Of the compounds tested, 8 - hydroxyquinoline potassium sulphate ('Superol') was the most promising as a chemotherapeutic agent against *C. Purpureum* (Brooks & Storey, 1923). This substance was further investigated by Roach (1938, 1939). Grosjean (1951) investigating the possibility of controlling Silver Leaf Disease by using chemicals introduced into the tree by bore-holes, found that solid substances gave better results than liquids. However, only with 'Superol' in the solid state was a significant recovery rate obtained, and in only 9% of cases was complete recovery recorded.

Bennett (1962b) injected plum trees with 'Superol' at different times during the growing season. She found that injections made in June, the period of rapid extension growth of the tree, had the most marked effect in checking the spread of the pathogen. In an experiment extending over 4 years, it was found that symptoms were less severe in the 2 years following injection but thereafter increased in severity as compared to the controls. This suggests that 'Superol' has no permanent effects on the development of *C. purpureum* in the vascular system. Bennett suggested that chemotherapeutic treatment of Silver Leaf Disease by injection into the trunks of diseased trees is unlikely to have any practical value unless substances of greater antifungal activity can be found.

In the absence of any effective curative method of treatment (Bennett, 1962; Grosjean, 1951, 1960), and the low degree of success achieved by covering pruning wounds with fungicidal paints, some of which were based on mercuric compounds (Dye, 1971; Dye & Wheeler, 1968; Grosclaude, 1968), Grosclaude & Ricard (1970) considered the search for a biological means of control to be justified.

Baker & Cook (1974) defined biological control as 'the reduction of inoculum density or disease-producing activities of a pathogen or parasite in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, host, or antagonist, or by mass introduction of one or more antagonists.'

The biological control of *C. purpureum* would involve protecting the infection court, that is, a freshly made pruning wound, by the action of a micro-organism in or on the infection court, capable of slowing or preventing infection by the pathogen. This may involve competition for nutrients on the pruned surface or the production of an antibiotic or other substance that supresses germination or growth of the pathogen, or injures it so that pathogenesis is inhibited (Cook, 1977).

The approach of protecting the infection court was that taken by Rishbeth in his application of *Peniophoragigantea* to stumps of forest trees to protect them against invasion by *Fomes*

annosus (Rishbeth, 1963; 1975), and by Ricard & Bollen (1968) in inoculating Douglas fir poles with *Scytalidium lignicola* to protect them against invasion by the wood-rotting basidiomycete *Poria carbonicum.*

It was first noted in the early 1920s that *C. purpureum* has difficulty in becoming established in wood that has already been invaded by saprophytic micro-organisms (Brooks & Storey, 1923; Brooks, 1936; Etheridge, 1971). It was later found that a host reaction as well as antagonism was involved (Mostafa, 1947; Swarbrick, 1926).

Michno-Zatorska (1960), in Poland, recorded antagonism *in vitro* between *Trichoderma viride* and *C. purpureum.* Grosclaude (1960b) found antagonism *in vitro* between *Cytospora leucostoma* and *C. purpureum. C. leucostoma* was one of a number of fungi isolated from pruning wounds, but this fungus is considered to be weakly pathogenic on fruit trees (Theridge, 1971). Shields & Atwell (1963) in investigating the effect of *Trichoderma viride* on decay of yellow birch logs *(Betula lutea)* by four storage rot fungi (3 species of *Polyporus* and *C. purpureum*) found that *T. viride* whether applied to birch sapwood before or after *C. purpureum* had little effect on reducing decay caused by *C. purpureum.*

More promising work on the possibility of controlling *C. purpureum* using *T. viride* has been done by Grosclaude and his co-workers in France. Grosclaude (1970) demonstrated antagonism *in vitro* between *C. purpureum* and *T. viride* in crossplating experiments and went on to investigate this phenomenon in the field. Grosclaude found that plum trees inoculated on Day 1 with *T. viride* and on Day 2 with *C. purpureum* basidiospores remained healthy, but if *C. purpureum* were introduced first, the trees became diseased. It was concluded that the antagonistic effect of *T. viride* only develops fully if it becomes established before the pathogen (Grosclaude, 1970; Grosclaude, Detienne & Roux, 1970).

Subsequent work investigated the possibility of using modified pruning secateurs to apply spores of *T. viride* to the pruning wounds during pruning (Grosclaude, 1973a; Grosclaude, Ricard & Dubos, 1973; Grosclaude & Ricard, 1971). It was found that the application of *T. viride* 48 hours before *C. purpureum* gave complete protection. Simultaneous application of *T. viride* and *C. purpureum* was not as effective as separate applications. The use of the secateurs proved to be as effective as a brush in the application of *T. viride* spores. No pathogenic symptoms were observed when *T. viride* was applied alone.

Grosclaude (1973a) suggested several possible modes of action for *T. viride*:

(a) trophic competition favourable to the organism introduced first and growing more rapidly than the pathogen,
(b) release by the spores or mycelium of *T. viride* of a substance which inhibits the growth of *C. purpureum,* or
(c) surface action of ungerminated *T. viride* spores.

Grosclaude (1973b) has shown that fresh wounds will absorb large amounts of water, and

where *T. viride* is applied first, its spores penetrate deeply. If *C. purpureum* is subsequently applied, it penetrates to a shallow depth only and its growth is stopped by the presence of the antagonist.

It has been found that ungerminated *T. viride* spores will inhibit the growth of *C. purpureum* (Grosclaude, Dubos & Ricard, 1974). Inhibition was shown to begin at concentrations of 1.0×10^4 - 1.0×10^5 *T. viride* spores per ml and to be total at spore concentrations of 5.2×10^7 to 2.6×10^8 per ml. The necessity for this high spore concentration could be explained if the antagonism were due mainly to a surface action of the spores requiring actual contact with *C. purpureum* spores or hyphae for full effect.

Curative attempts have been made by injecting diseased trees with suspensions of *T. viride* spores. Corke (1974) has termed this type of treatment biotherapy, that is, the use of selected micro-organisms for the remedial treatment of diseased plant tissues. Initial attempts at biotherapy of peach trees have proven fairly promising. Dubos & Ricard (1974) reported a significant improvement in a group of peach trees with Silver Leaf Disease that were injected with a suspension of *T. viride* spores.

Attempts at the biotherapy of pear trees in England have also resulted in a degree of success. Perry pear trees (cv. Hendre Huffcap) showing severe foliar silvering were inoculated with *T. viride* by placing propagules of the fungus into holes drilled in the trunk about 0.75m above the ground. A total of 48 moderately affected trees were treated in two separate experiments. In the first experiment the severity of symptoms was markedly reduced in 76% (13/17) of the trees as compared with untreated controls; in the second experiment the figure was 52% (16/31).

Objectives of the studies presented in this thesis

The principal aims of this work were to study the injection of pruned sapwood of stone fruit trees by *Chondrostereum purpureum*, and to study potential biological control agents for this pathogen. Initially, because of its effectiveness in the control of Dieback (or Gummosis) of apricot caused by the wound pathogen, *Eutypa armeniacae* (Carter & Price, 1974), the effect of *Fusarium lateritium* on *C. purpureum* both *in vitro* and in the field was studied. A natural population of *F. lateritium* in a commercial orchard and how climatic factors and cultural practices affected this population was also investigated. Other micro-organisms were also isolated from trees in an orchard and tested in the laboratory for possible use against *C. purpureum* as biological control agents.

II. SILVER LEAF DISEASE IN SOUTH AUSTRALIA

Incidence

The main fruit-growing districts of South Australia, average annual rainfall for the areas and the main stone and pome fruits grown are given in Table 3. Most of the rainfall occurs in winter. The Upper and Lower Murray districts are on the River Murray and orchards in these areas are irrigated from the river. Supplementary irrigation is applied in summer where water supplies allow in the other districts, notably in the Adelaide Hills and at Coromandel Valley and Ashbourne in the Southern Districts.

The first recorded occurrence of Silver Leaf Disease in South Australia was in 1925 in an apple orchard at Balhannah. The fungus was not isolated in culture (M.V. Carter, unpublished notes). The first authentic record of *C. purpureum* as a parasite was in December, 1925 when fructifications were found on an apricot tree at Clarendon (M.V. Carter, unpublished notes; Cleland, 1934-5; Herb. A.D.W. No. 2363).

By the early 1930s the disease was gradually increasing in orchards in the Adelaide Hills (Garrett & Leishmann, 1931). Since then it has been recorded in all fruit-growing districts of the State (Figure 6 and Table 3). Its importance varies considerably between districts. If the Adelaide Hills are given an importance-severity rating of 10, the other districts could be rated as follows:

Ashbourne	8
Barossa Valley	3-4
Clare	3-4
Lower Murray	3-4
Southern Districts	2
Upper Murray	2
Midnorth	1

(D.T. Kilpatrick & R.L. Wishart, South Australian Department of Agriculture, personal communication).

TABLE 3

Fruit-growing districts of South Australia, annual average rainfall (mm) and main stone and pome fruits grown.

District	Annual average rainfall (mm) *	Main stone and pome fruits grown
Adelaide Hills	800 - 1000	apples, pears, cherries, peaches, nectarines, plums
Barossa Valley	500 - 550	apricots, plums, peaches, apples
Clare	600	apricots, plums, peaches, apples
Mid North	500	peaches, nectarines
Southern Districts	600	apples, pears, almonds
Upper Murray	250	apricots, peaches, apples, pears, nectarines
Lower Murray	250	apricots, almonds, peaches

* Source: Year Book of South Australia 1975, Bureau of Statistics, Adelaide.

FIGURE 6

Distribution of Silver Leaf Disease *(Chondrostereum purpureum)* in South Australia.

- ● silvered foliage and *C. purpureum* recorded
- ◆ silvered foliage only recorded

The occurrence of Silver Leaf Disease is recorded by townships on this map and not by individual recordings. The list of townships in the various fruit-growing districts (designated A to H on the map) is given in Table 4.

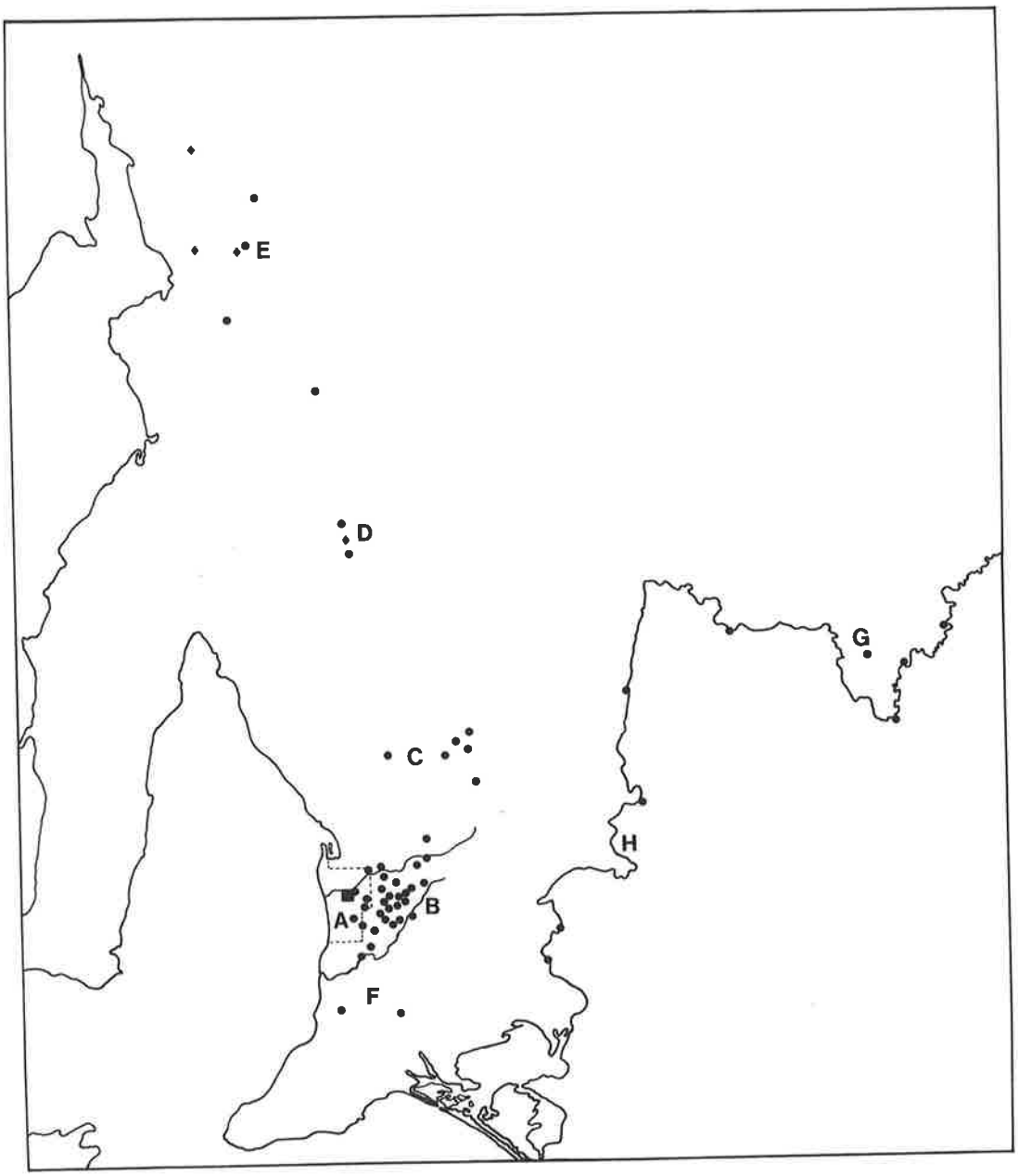

E
D
C
G
H
A
B
F

TABLE 4

Townships in fruit-growing districts of South Australia where Silver Leaf Disease has been recorded.

A. ADELAIDE
- Beaumont
- Belair
- Glen Osmond
- Hackney
- Hope Valley
- Marion

B. ADELAIDE HILLS
- Aldgate
- Ashton
- Balhannah
- Basket Range
- Bridgewater
- Carey's Gully
- Cherryville
- Crafers
- Cudlee Creek
- Forest Range
- Forreston
- Gumeracha
- Lenswood
- Lobethal
- Montacute
- Norton Summit
- Paracombe
- Piccadilly
- Stirling
- Summertown
- Uraidla

C. BAROSSA VALLEY
- Angaston
- Light Pass
- Mount McKenzie
- Nuriootpa
- Roseworthy
- Tanunda

D. CLARE
- Clare
- Penwortham
- ◆ Sevenhill

E. MID-NORTH
- ◆ Alligator Gorge
- Beetaloo
- Booleroo Centre
- Bundaleer Forest
- ◆ Wirrabara
- ◆ Wirrabara Forest
- Wongarra

F. SOUTHERN DISTRICTS
- Ashbourne
- Clarendon
- Cherry Gardens
- Coromandel Valley
- Willunga

G. UPPER MURRAY
- Barmera
- Berri
- Loxton
- Renmark
- Waikerie

H. LOWER MURRAY
- Blanchetown
- LanghorneCreek
- Mypolonga
- Nildottie
- Swanport

The Adelaide Hills and Ashbourne have wet, cool winters with mild conditions prevailing in spring and autumn and an annual rainfall of approximately 800 to 1000 mm. Such conditions would be more favourable to the formation of sporophores of *C. purpureum* and the release of basidiospores, than the hotter and drier climates in the other fruit-growing districts of South Australia.

During this study, Silver Leaf Disease was encountered in the field at Clare, Mypolonga, Ashbourne, Nildottie and extensively in the Adelaide Hills. *C. purpureum* was isolated in culture from almond trees at Nildottie, an infrequent host in South Australia. Silvering symptoms were seen in the Midnorth on peaches at Wirrabara and on almonds near Alligator Gorge, but *C. purpureum* could not be isolated in culture. Sporophores of *C. purpureum* were recorded some years ago in orchards near Wirrabara, at Wongarra and Beetaloo (R.L. Wishart, South Australian Department of Agriculture, personal communication).

Hosts of *Chondrosterum purpurem*

Thirty-nine plant species were recorded as hosts of *C. purpureum* in South Australia (indicated * in Table 2). Of these, 18 are members of the family Rosacaea, 8 of Salicaceae and 4 of Leguminosae. Silver Leaf Disease has been recorded on all members of the genera *Prunus* and *Malus* that are cultivated in South Australia (Moller & Talbot, 1962; Talbot, 1964; D.T. Kilpatrick, personal communication). *C. Purpureum* has not been recorded on any species native to Australia in Australia (Cooke, 1892; Garrett & Leishmann, 1931; Cleland, 1934-5; Noble, 1935, 1937; Adam, 1937a, 1937b; Talbot, 1964; Simmonds, 1966). It has however, been recorded in England on *Eucalyptus gunnii* (Min. Agric. Fish & Food, 1972) and in New Zealand on *Eucalyptus ficifolia* (Dingley, 1969) both of which are native to Australia.

In this study, 11 plant hosts of *C. purpureum* that had previously not been recorded from any other country were discovered in South Australia (indicated ** in Table 2). The new records are as follows:

Teline monspessulana (L.) C. Koch
(syn. *Genista monspessulana* (L.) L.A.S. Johnson)
Wisteria floribunda (Willd.) DC.
Tibouchina semidecandra Cogn.
Ceanothus thyrsiflorus Esch.
Prunus cerasifera Ehrh. var. *Atropurpurea* Jaeg.
Sorbus aucuparia L. cv. Xanthocarpa
Salix discolor Muhlenb.
Salix alba L. var. *vitellina pendula* Rehd.
Salix x rubens Schrank

FIGURE 7

New hosts of *C. purpureum* recorded from South Australia.

A. Fructifications on *Teline monspessulana.*

B. Symptoms – small, discoloured leaves and die-back – on Lasiandra *(Tibouchina semidecandra).*


A


B

Hydrangea macrophylla (Thunb.) DC.
Vitis amurensis Rupr.

Collections of several of these were deposited in The Herbarium at the Waite Agricultural Research Institute. These were *Teline monspessulana* (A.D.W. No. 16522), *Tibouchina semidecandra* (A.D.W. No. 16523) and *Salix alba* var. *vitellina pendula* (A.D.W. No. 16527).

All the new hosts were found in the Adelaide Hills, some were single recordings and others occurred more widely. In all cases sporophores of *C. purpureum* were found on plants that had recently died and in three cases the pathogen was successfully isolated from living plants. These were *Tibouchina semidecandra, Prunus cerasifera* var. *atropurpurea* and *Sorbus aucuparia* cv. Xanthocarpa. Single recordings were on the garden plants *Wisteria floribunda, Sorbus aucuparia, Hydrangea macrophylla, Vitis amurensis* and *Ceanothus thyrsiflorus.* In all cases the plant concerned had been severely pruned and sporophores of *C. purpureum* had formed within 2 years of pruning. *Sorbus aucuparia* showed marked silvering of the foliage.

The other six hosts were encountered more regularly and could constitute important sources of inoculum since *Teline monspessulana, Salix alba* var. *vitellina pendula* and *S. x rubens* are often found growing near orchards. No external symptoms are seen on any of these hosts. With *Teline monspessulana,* an extensive hymenial surface forms once a plant has died (see Figure 7). *T. monspessulana* and another broom, *Cytisus scoparius* are garden escapes that have become well established throughout the high rainfall areas of the Adelaide Hills.

Bushes of *Tibouchina semidecandra* (= *Lasiandra semidecandra*) infected with *C. purpureum* showed branch dieback and small, often discoloured leaves (dull green with orange markings). In the later stages of the disease, the bushes displayed split, papery bark. This latter symptom has also been recorded in apple trees with Silver Leaf Disease (Wormald, 1943; Montgomery & Wormald, 1944). *T. semidecandra,* a garden shrub which is grown for its large mauve flowers, is pruned quite severely each year in late winter. This would create a large number of potential sites of entry for *C. purpureum.* A diseased specimen of *T. semidecandra* is shown in Figure 7.

Economic Importance

No assessment has ever been made of the importance of Silver Leaf Disease in orchards in South Australia. In the fruit-growing districts of the Adelaide Hills, it is probably the most important fungal vascular disease of apple and cherry trees. The only other fungal vascular pathogen of any importance is *Coriolus versicolor* (L. ex Fr.) Quél. (= *Trametes versicolor* (L. ex Fr.) Lloyd) which has been recognised as an important pathogen of apple trees in Tasmania (Kile & Wade, 1974). It appears that Silver Leaf Disease is more serious in older fruit trees, that is those in excess of 35 years old. Two plantings of cherries were surveyed for the occurrence of Silver Leaf Disease in an orchard at Basket Range. The first was planted in 1933 to the cultivars Waterloo, Napoleon and St. Margaret. Of these, 48% (18/37) showed foliar silvering or had died of the disease within the last 5 years. In addition to the dead trees, there

were 5 replants in the orchard. The second planting dated from 1954; cultivars Lustre, Bedford's Prolific, St. Margaret and William's Favourite. Only 4% (3/76) of the trees showed silvering symptoms or had died of the disease within the last 5 years. There were 5 replants in the total planting of 81 trees. *Armillaria* root rot is a problem in part of this orchard, and occurs in patches in most orchards in the Adelaide Hills.

Kile & Wade (1974) found a similar situation with *Coriolus versicolor,* that is, old trees were more susceptible to infection than young trees. It has been suggested that the resistance of sapwood to invasion by fungi depends on the physiological activity of the living cells and their ability to elaborate on effective inhibitory barrier against the pathogen (Brooks & Moore, 1926; Brooks & Brenchley, 1936; Brooks, 1936). With ageing of sapwood there is a decline in cellular physiological activity and in consequence a decline in host resistance (Kile & Wade, 1975).

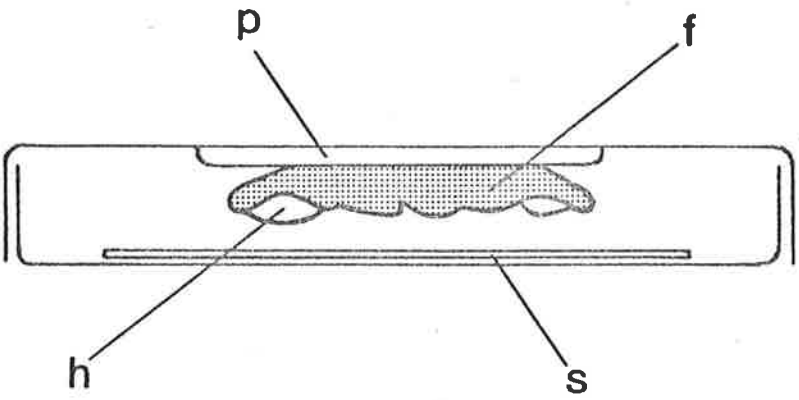


FIGURE 8

Method of spore collection (after Grosclaude, 1969b).

f = fructification (basidiocarp)
h = hymenial surface
p = plasticine
s = glass microscope slide (optional)

III COLLECTION AND CULTURE OF *Chondrostereum purpureum*

Collection of basidiocarps and basidiospores

Newly formed basidiocarps of *C. purpureum* were collected from the bark of host plants from about April to July. Collections were also made at other times of the year when weather conditions were favourable for enlargement of existing basidiocarps. All basidiocarps used for experiments in this study were collected at Basket Range in the Adelaide Hills.

Basidiospores were collected by the method first suggested for this fungus by Marshall Ward (1897) and later modified by Bennett (1962a) and Grosclaude 1969b). A dry basidiocarp was attached by a piece of plasticine to the underside of the lid of a sterile petri dish (Figure 8) and was soaked in sterile distilled water for about 30 minutes in a laminar flow cabinet. After this time, the water was poured off and any excess moisture on the sporophore allowed to evaporate. The closed plate was then placed in the dark at 20°C for 12 to 18 hours. Basidiospores were collected either on the bottom of the petri dish or on a glass microscope slide placed in the dish.

Fungal contaminants were often found to be a problem in spore deposits. This could be minimized if newly formed basidiocarps were collected when fairly dry, hence not actively discharging, and were removed from the bark on which they were growing before being rewetted for the collection of spores.

Under field conditions in New Zealand, spore deposition occurs with air temperatures ranging from 11 to 19°C (Dye, 1974). Grosclaude (1969a) with sporophores collected from poplar trees in France found that under laboratory conditions some spore deposition occurred from 3°C to 25°C but maximum sporulation occurred at 20°C. Buller (1909) suggested that light may be necessary in Hymenomycetes in general for the development of the hymenium but not for spore discharge. Grosclaude (1969a, b) reported the existence of a circadian rhythm, spore discharge being at a maximum at night, and that the alternation of light and dark periods increased spore discharge during the dark phase. He considered that this was due to a time lag in the stimulatory effect of light. Dye (1974) confirmed the nocturnal maximum, but not the existence of a circadian rhythm.

Storage of basidiospores

As *C. purpureum* forms sporophores only at certain times of the year, it would be desirable to be able to store spores collected during these periods for use at a later date when fresh sporophores are not available. Initially spore collections were stored in petri dishes in the dark at room temperature. However, these spores were found to lose viability quite quickly.

TABLE 5

Percent germination of *C. purpureum* basidiospores following storage at different temperatures at 4-weekly intervals up to 20 weeks

Storage Period (weeks)	Spores from willow					Spores from apple				
	2°C	10°C	15°C	20°C	RT *	2°C	10°C	15°C	20°C	RT *
4	100**	35	30	14	6	30	61	13	18	1
8	29	19	24	19	3	95	94	24	18	0
12	42	0	19	8	0	48	95	8	0	0
16	45	5	9	0	0	52	25	5	0	0
20	40	0.5	0.5	0	0	50	9	0	0	0

*RT = room temperature

** figures are the germination count (in percent) after 48 hr at 20°C

As a result, a test was designed to determine what are the optimal conditions for spore storage. The test concentrated on storage in the dark because it has been found that basidiospores of *C. purpureum* rapidly lose their viability when left in sunlight (Groscalude, 1969a).

C. Purpureum basidiospores were collected from newly formed sporophores on apple *(Malus sylvestris)* and willow *(Salix babylonica).* Collections of spores were made on successive days from each sporophore, the spores being collected on the bottom of a sterile petri dish. The sporophores were rewetted as thought necessary. The viability of the spores in all collections were tested before they were placed under different storage conditions: all collections used gave a germination rate of 90-100% after being incubated for 24 hrs. at 20°C.

Petri dishes containing the spore collections were stored in brown paper bags in the dark at 2, 10, 15 and 20°C and at room temperature (18 - 22°C). At 4-weekly intervals a portion of the spore deposit on each plate was suspended in sterile distilled water and 5 drops of the suspension put on the surface of 2% distilled water agar (Difco Bacto). Plates were incubated at 20°C for 24 hr and then a germination count was made by scoring 150-200 basidiospores in each of the drops. This was reviewed after 48 hr.

The germination counts after 48 hr are given in Table 5. The results showed that basidiospores stored at 2°C in the dark retained 40-50% viability for at least 20 weeks even though germination was variable during the test period. The germination rate of spores stored at temperatures above 10°C dropped off very rapidly after about 8-12 weeks. The spores from apple retained 25% viability up to week 16 when stored at 10°C.

The percent germination of most of the spore collections in this test varied greatly over the storage period. This is possibly due to variability among spores in any one collection. Spores at one end of a deposit may be different from those at the other end because of their origin from a different part of the sporophore. Such variations might well account for the erratic germination counts in seccessive tests. It has been found that basidiospores of *Polyporus tomentosus* collected from expanding sporophores had a higher germination rate than spores from older sporophores (Whitney, 1966).

Basidiospores stored at 10°C or higher lost their viability quite rapidly compared with those stored at 2°C, probably because of a more rapid rate of respiration and dehydration at the higher temperatures. The viability of *C. purpureum* spores may well be prolonged even further by storage at temperatures below 0°C. Whitney (1966) found that the rate of germination of basidiospores of *Polyporus tomentosus* was still high after 3 months' storage at -18°C, and some spores survived up to 20 months at -18°C. Spores of other fungi that have been found to retain their viability when kept at temperatures below 0°C include *Uromyces phaseoli* (Harter & Zaumeyer, 1941), *Puccinia graminis* (Melander, 1935) and *Botrytis* sp. (Deverall, 1965). This is not to say that spores of all fungi will retain their viability for longer if stored at low temperatures. For example, Forbes (1939) found that spores of *Puccinia coronata* did not survive exposure to -18°C although they retained their viability when kept at 0°C.

Taking into account the huge number of basidiospores deposited by a sporophore of *C. purpureum* (Grosclaude, 1969a) storage at 2°C (50% still viable after 5 months) was considered to be adequate for the purposes for which spores were required in this work.

Growth on artificial media

Most workers have used malt agar for laboratory culturing of *C. purpureum* (Robak, 1942; van der Westhuizen, 1958; Bennett, 1962a, b; Dye & Wheeler, 1968; Beever, 1969; Dye, 1971), although Robak (1942) showed that growth was just as rapid on potato dextrose and Czapek-Dox agars. Robak (1942) found that growth was poor with galactose and lactose as carbon sources, but was good with xylose, laevulose and maltose. Good nitrogen sources were asparagine, peptone, urea and sodium nitrate. Beever (1970) found that growth with sucrose and sorbitol was poor, but was good with glucose and fructose as carbon sources. Good nitrogen sources included glutamine and asparagine.

C. purpureum will grow *in vitro* over a wide range of temperatures, 3 to 30°C, with a maximum rate of growth at 25-27°C (Cartwright & Findley, 1934). Robak (1942) found that the rate of growth accelerated rapidly between 15 and 20°C on all of the media that he tested. All isolates had a maximum rate of growth at 23-26°C and all had ceased to grow at 32°C (Robak, 1942).

At the start of this study, malt agar was used when isolating *C. purpureum* from infected wood or when starting new cultures from basidiospores. Although the fast rate of growth on malt agar was often advantageous when subculturing from cultures started from spores, this medium was not always useful when attempting to reisolate *C. purpureum* from artificially inoculated pruning wounds. This was because fungal contaminants were often present and grew just as fast as *C. purpureum* on malt agar. Consequently it was desirable to find a medium on which *C. purpureum* grew reasonably fast and yet could be readily identified by macroscopic features without having to regularly resort to microscopic examination of hyphae. A test was designed to investigate the rate of growth of a number of South Australian isolates of *C. purpureum* from different host plants on several artificial growth media.

Discs 7 mm in diameter were cut from a 7-day-old colony of each of the isolates of *C. purpureum* growing on malt agar. The isolates were as follow:

Isolate	Plant Host
CPC12	*Prunus avium* cv. Black Tartarian
* CPL2	*Tibouchina semidecandra*
CPA10	*Malus sylvestris* cv. Jonathon
CPB1	*Teline monspessulana*
* CPR1	*Rose* sp. cult.
* CPN1	*Prunus persica* var. *nectarina*

TABLE 6

Average rate of growth of *C. purpureum* isolates on different media (mm/day).

Medium		*C. purpureum* Isolate					
		CPC12	CPL2	CPA10	CPB1	CPR1	CPN1
Glucose Peptone (GP)	Av.**	9.1	8.2	9.5	10.4	4.6	10.5
	Max.**	10.8	9.0	10.9	11.3	5.0	12.0
GP/20	Av.	7.6	5.9	5.5	8.5	3.2	9.3
	Max.	8.9	7.5	7.0	10.4	4.0	10.4
Brown's	Av.	10.7	7.6	8.6	14.2	5.1	12.4
	Max.	12.2	8.4	10.7	15.8	5.9	13.8
Cherry	Av.	9.9	10.4	8.0	9.7	10.7	11.3
	Max.	10.4	11.8	13.0	11.8	11.7	12.8
Malt	Av.	12.1	13.6	13.7	13.5	14.2	14.9
	Max.	13.4	15.6	15.8	14.2	15.8	16.4
NDY	Av.	10.1	10.1	10.6	14.5	12.1	13.0
	Max.	11.2	11.2	12.8	15.8	13.4	15.0

** Average (or maximum) rate of growth (mm/day), readings taken daily for 9 days or until the plates were entirely covered.

The isolates marked * were isolated from wood of host plants and cultures of the other isolates were started from basidiospores.

The discs were placed at the centre of 90 mm diameter petri dishes containing 15 ml of Brown's, Cherry, Glucose Peptone (GP), GP/20, Malt or NDY agars.* The plates, three of each agar medium, were incubated at 25°C and the average rate of growth was calculated from measurements of the diameter of the colony taken daily over a 9 day period from the time of inoculation.

The results are given in Table 6. The rate of growth of all isolates (except CPR1) was reasonably uniform on all media tested, with a maximum average rate of growth for most isolates occurring on Malt agar. Isolate CPR grew at half the rate of the other isolates on GP, GP/20 and Brown's agars, but its rate of growth was equivalent to the other isolates on Cherry, Malt and NDY agars. The maximum rate recorded was 16.4 mm/day on Malt agar with isolate CPN1. For most isolates the rate of growth on GP/20 was quite similar to that on the full-strength medium (GP) but on this and on Cherry agar the colony formed was less dense than on Malt or NDY.

The absence of any correlation between the spread of a mycelial front on a solid surface and the total amount of fungus produced, as is seen in this instance, was recognised by Mandels (1965) as the one fundamental limitation to the use of linear measurements for quantifying growth rates. Thus, as was seen here, linear extension on agar lacking an available carbon source can be as rapid or even more rapid than on a complete medium, viz. GP and GP/20.

C. purpureum is readily recognised on GP/20 agar. The advancing margin of the colony is markedly uneven to bayed, and the mycelia are white, presenting a curled appearance. On NDY and Brown's agars the appearance of *C. purpureum* has few distinguishing features and is very similar to that on Malt agar, namely a thick luxuriant growth with a cottony appearance and a fairly uniform advancing margin.

Maintenance of cultures

All isolates of *C. purpureum* were maintained on 15 ml slopes of NDY agar (see Appendix II). Isolates were transferred to new slopes as required, usually at intervals of 4-6 months. Cultures were kept at 10°C.

* For details of the growth media used here and elsewhere in this thesis, see Appendix II (p. 119).

IV STUDIES ON INFECTION OF FRUIT TREES BY *Chondrostereum purpureum*

Quantity of inoculum required to induce infection by *Chondrostereum purpureum* in freshly pruned cherry sapwood.

Early workers with Silver Leaf Disease inoculated branches of plum trees with blocks of agar containing *C. purpureum* hyphae in attempts to induce the disease (Bedford & Pickering, 1910; Brooks & Bailey, 1919; Brooks & Storey, 1923). Later workers have used large spore doses when trying to induce the disease (for example, Grosclaude, 1973a). Neither method is very representative of the field situation.

Carter & Moller (1971) in studies on the quantity of inoculum needed to infect *Prunus* sp. with *Eutypa armeniacae* in the winter months, found that when trees were inoculated on the day of pruning, 10 ascospores per wound surface were sufficient to give an infection rate of 45%. With 100 spores per wound surface this is nearly doubled to 83%. Moller & Carter (1970) had found a natural infection rate of 10-15% after pruning trees in June (wounds left unprotected). Thus they concluded that quantities of ascospores exceeding 10 are rarely, or never, deposited naturally on idividual wounds and that this spore load is compatible with known concentrations of airborne ascospores in orchards and to the deposition processes that must take place before ascospores finally reach their infection courts.

The only spore dosage experiment using *C. purpureum* was done in France by Grosclaude (1973d). Grosclaude artificially inoculated plum trees in late winter with *C. purpureum* spore doses of 2-4, 22-44, 4.38×10^2, 4.38×10^3, 4.38×10^4 and 3.92×10^5 spores per pruned surface. Using 14 pruned sites per treatment, he found that the greatest number of silvered trees and the most severe silvering symptoms were with the 22-44 and 4.38×10^2 spore doses. Both of these gave an infection rate of approximately 80%. The other treatments gave infection rates of less than 10%. He offers no explanation for why the high spore loads were unsuccessful.

A study was made to determine the quantity of inoculum required to artificially infect cherry trees with *C. purpureum* under South Australian conditions. Concurrent with this experiment was an investigation into the survival of infections over an 18 month period from the time of inoculation. Putterill (1923) reported that in the west of Cape Province, South Africa, a high proportion of new infections do not survive the first summer following inoculation. Because of similar climatic conditions, one might expect a similar situation to exist in South Australia.

Materials and Methods

Selected branches on cherry trees (*Prunus avium* cv. Lustre) were pruned on July 9, 1975 just above a dormant bud to give a wound surface of 1.0-1.5 cm in diameter. The pruned surface was inoculated immediately after being pruned, with a measured dose of *C. purpureum* basidiospores in 10 µl water using a Burkard microapplicator. A total of 40 sites were inoculated for each of the spore dosages and for each of the 3 intended harvests, giving a total of 480 sites inoculated. The spore doses used were 0, 5, 100 and 1000 viable spores per pruned surface.

A suspension of the basidiospores to be used was made and drops of this placed on the surface of distilled water agar and a germination count made after 24 hr at 20°C. The spore dosage used was subsequently adjusted to include the required number of viable spores. The treatments were assigned at random to the pruned surfaces and labelled with coloured tapes for future identification. As the trees used were once a stool bed, they did not readily lend themselves to an experimental design that could be statistically tested.

The experiment was harvested after periods of 5, 10 and 18 months, that is, in December 1975, May 1976 and February 1977. Those sites harvested after 18 months would therefore have been through two summers.

The number of sites infected by *C. purpureum* in each treatment was determined by visual assessment of internal symptoms and by attempting to reisolate the fungus from the wood. Chips of wood were taken at 0.5 cm intervals below the inoculated surface and plated on GP/20 agar to which had been added 100 ppm streptomycin and 250 ppm guaiacol (o-methoxyphenol, B.D.H. Chemicals, Poole, England) after the agar had been melted. Guaiacol has been used for some years to detect the presence of white wood-rot fungi (Boidin, 1951; Nobles, 1965; Grosclaude & Roux, 1971). In GP/20 agar, guaiacol turns orange-brown in the presence of *C. purpureum* (Figure 9). Guaiacol was found to be especially useful in cases where fast-growing contaminant fungi were present.

The fungi *Coriolus versicolor* (L. ex Fr.) Quél. and *Schizophyllum commune* (L. ex Fr.) Fr. are commonly found growing in dead fruit-tree wood in the orchard and could conceivably have invaded the pruning wounds made in this experiment. These two fungi were grown on GP/20 agar containing guaiacol and it was found that both gave the same colour reaction to guaiacol as was seen with *C. purpureum*. This was in agreement with the results of Boidin (1951). With experience, however, these fungi can be readily distinguished from *C. purpureum* in culture because of their differing growth forms.

Results and Discussion

The results are given in Table 7. In Harvest I there was no significant difference between the different treatments and the control. That is, in none of the treatments did the application

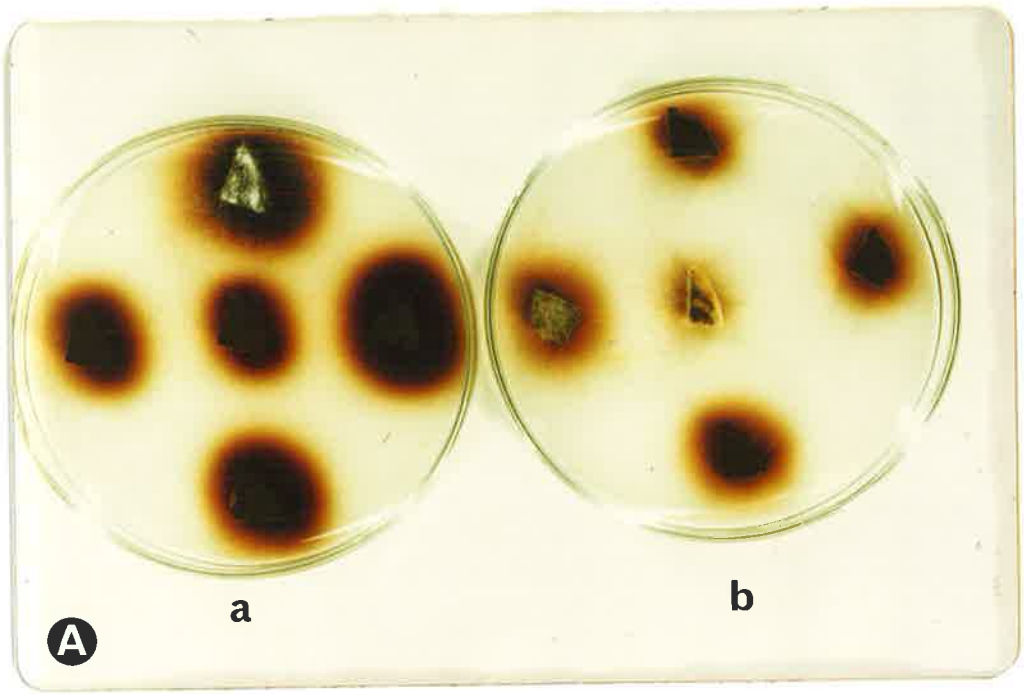
FIGURE 9

A. Plates of agar to which had been added 250 p.p.m. guaiacol (O-methoxyphenol) were used in the reisolation of *C. purpureum* from cherry wood.

a = Malt agar plus 250 p.p.m. guaiacol

b = GP/20 agar plus 250 p.p.m. guaiacol

B. Examples of internal discoloration of cherry wood caused by *C. purpureum.* At extreme right is an uninoculated control. (Wood is approximately three quarters of actual size.)


a
b
A


B

of *C. purpureum* spores result in a level of infection that was greater than the natural infection level. The results do however, suggest that the initial number of infections decline with time (Harvest I to Harvest III). In most cases the decline in the number of infections was of the order of 50% rising to a maximum of 85% in one case. Desiccation of the wood beneath the pruning wound and the formation of gum barriers in the wood in response to invasion by *C. purpureum* (Brooks & Moore, 1926; Wormald, 1926) would tend to account for the decline in the number of infections.

The high level of natural infection found in this experiment (35%) is probably accounted for by the fact that rain in excess of 0.5mm fell on every day from July 10 to July 22 1975, (see Appendix III) with 7mm and 26mm on the two days immediately following pruning. Thus, one would expect spore release throughout the first 14 days after the trees were pruned.

As to why the recovery of *C. purpureum* was no higher from inoculated than from uninoculated sites, it could be suggested that the basidiospores failed to germinate when applied to a pruning wound. However, the spores used germinated when tested before a suspension was prepared and also germinated when drops from the microapplicator were put on GP/20 agar plates. On being applied to the wound the drops were drawn in and would therefore not be subjected to dessication as they would have been had they remained on the surface of the wound.

Although this experiment was inconclusive, it was not repeated, partly because of a lack of trees available for experimental purposes and partly because several of the points raised were covered in the next experiment.

Susceptibility of cherry wood to different isolates of *Chondrosterum purpureum*

Early investigations using *C. purpureum* mycelium to inoculate wounds showed that different isolates of the fungus were not host specific (Bedford & Pickering, 1906; Bintner, 1919; Brooks, 1913). Brooks & Bailey (1919) inoculated a small number of plum trees with spores from isolates from plum, beech, willow and cherry, and found that there was no difference in infectivity between the different *C. purpureum* isolates. *C. purpureum* spores from poplar isolates have also been found to be capable of inducing Silver Leaf Disease in plum trees (Brooks & Storey, 1923; Grosclaude, 1973d).

The only recent experiments in this area are those by Bennett (1962a). These involved the placing of mycelial discs of *C. purpureum* from plum, poplar and sycamore into the trunks of plum trees. Bennett estimated silvering of the foliage and also the volume of discoloured wood in the branch in order to obtain a measure of virulence. She found that trees inoculated with the plum isolate showed twice the number of branches with silvered foliage as those trees inoculated with isolates from poplar and sycamore. Considerable progress was seen in all

TABLE 7

Effect of different dosages of *C. purpureum* basidiospores on the number of sites infected at 3 harvest dates (5, 10 and 18 months) after inoculation.

Harvest	Number of sites infected with *C. purpureum* (%)			
	Number of spores per inoculated sites			
	0	50	100	1000
1.	35	42	32	30
2.	20	21	17	23
3.	16	13	4*	18

* significantly different at P = 0.05

cases with respect to wood invasion, but in the trees inoculated with the plum isolate, the volume of wood invaded was twice that of trees inoculated with the poplar or sycamore isolate.

Although we may conclude from Bennett's work that there was no difference in infectivity between the poplar and sycamore isolates, and that both were less virulent than the plum isolate, these results were obtained using an unnatural method of inoculation. What is important in the field is whether airborne spores from different hosts are capable of invading fresh wounds, and whether different isolates vary in their virulence.

Commercial apple and cherry orchards in the Adelaide Hills are very often surrounded by non-commercial hosts of *C. purpureum*, such as poplars, willows, *Prunus* sp. and common broom (*Teline monspessulana*). It has been assumed that spores from *C. purpureum* on these hosts are just as important in the spread of the disease as inoculum from within the orchard (Kilpatrick, 1961). In the light of Bennett's work and the above assumption it would be of value to know if *C. purpureum* spores from these hosts are capable of invading pruning wounds on cherry trees and whether these isolates are as virulent as those from cherry itself.

Materials and Methods

Suitable branches on cherry trees (*Prunus avium* cv. Waterloo) of diameter 1.0 - 1.5cm were labelled and pruned above a dormant bud on September 3 1976. The pruned surface was inoculated immediately by means of a Burkard microapplicator with five 2 μl drops of a suspension of *C. purpureum* basidiospores containing 1.0×10^4 spores per ml. that is, 100 spores per site. As the previous experiment was inconclusive as to the effect of different inoculum dosages, 100 basidiospores per site was arbitrarily chosen for use in this experiment. Viability was tested and found to be nearly 100%. A total of 25 sites were inoculated per treatment. The treatments, which are set out below, were assigned at random to the sites.

Treatment

1 Control A – not inoculated, covered
2 Control B – not inoculated, not covered
3 Spores from willow *(Salix babylonica)*, covered
4 Spores from apple *(Malus sylvestris)*, covered
5 Spores from cherry *(Prunus avium)*, covered
6 Spores from broom *(Teline monspessulana)*, covered

In order to eliminate possible interference from a high natural infection rate as occurred in the previous experiment all pruned sites were covered with sterile aluminium foil for 14 days

following inoculation. The branches were harvested after 4 months and the presence of the pathogen was assessed by culturing from chips of wood taken from the discoloured zone and plated on GP/20 agar to which had been added 100 p.p.m. streptomycin and 250 p.p.m. guaiacol.

An attempt was made to assess severity by recording foliar silvering and the extent of internal discoloration (Figure 9, p. 40). No attempt was made to rate the severity of silvering symptoms as has been done by Grosclaude (1973a, d), as it was considered to be too subjective to be of much value.

Results and Discussion

The results are set out in Table 8. As *C. purpureum* was not recovered from any of the sites in Control A, it appears that covering the pruning wounds with aluminium foil was effective in preventing invasion by *C. purpureum* inoculum occurring naturally in the orchard. On this basis, it can be considered that the results obtained in the other treatments are as a result of the particular inoculum applied, without interference from background inoculum.

Between 90 and 100 percent of pruning wounds on the cherry trees inoculated with spores from apple, cherry and broom became invaded by *C. purpureum.* The percentage was somewhat lower (76%) in the case of spores from willow. Not only was the overall number invaded by the willow isolate lower, but the extent of internal discoloration which indicates the progress of the fungus in the wood (Bennett, 1962a) was also significantly less than with the other three isolates. The apple and cherry isolates both penetrated to an average depth of approximately 80mm over the period of the experiment.

Silvered foliage was recorded in a small number of the branches from which *C. purpureum* was successfully reisolated (See Table 8). No silvered foliage was recorded where sites were inoculated with spores from broom.

These results suggest that *C. purpureum* spores from sources other than cherry are equally effective in invading pruned cherry sapwood. This result differs from the work done in England where an isolate from plum was found to be more virulent on plum trees than isolates from poplar and sycamore (Bennett, 1962a). Of the isolates tested, the one from willow gave a 76% infection rate and grew significantly slower in cherry wood than the other isolates. This may have significance in an orchard in that this isolate would be more susceptible to desiccation than an isolate that penetrated deeper into the wood prior to the onset of summer.

The lack of silvered foliage in those trees inoculated with the isolate from broom may suggest either that this isolate does not produce the toxin phytolysin that causes the partial disorganization of leaf tissue (Brooks & Brenchley, 1929) or only produces toxin in relatively small amounts and hence takes longer to have an effect on the leaves. Toxin production by

TABLE 8

Effect of inoculating cherry trees with different isolates of *C. purpureum*

	No. yielding *C. purpureum* (total 25)	Number silvered[1]	Depth of internal discoloration[2] (mm)	Maximum depth of discoloration (mm)
1. Control A	0	-	-	17 *
2. Control B	5	$^{2}/_{5}$	75	35 *
3. Willow	19	$^{3}/_{19}$	46 *	65 *
4. Apple	25	$^{10}/_{25}$	80	170
5. Cherry	25	$^{6}/_{25}$	78	130
6. Broom	23	$^{0}/_{23}$	63	165

(1) number out of those that yielded *C. purpureum* showing silvered foliage.

(2) average maximum depth of internal discoloration in those samples from which *C. purpureum* was reisolated.

* significantly different at P = 0.05

the different isolates is investigated in the next section.

In conclusion, the results obtained here stress the need, as a control measure, to eliminate as much as is practical, dead host material from both within and surrounding orchards.

Phytolysin production by isolates of *Chondrostereum purpureum*

Phytolysin is the name that has been given to the compound produced by *C. purpureum* which when injected into the stem of plum trees will induce silvering similar to the symptoms seen when a tree is infected with *C. purpureum* (Naef-Roth, Kern & Toth, 1963; Wood, Ballio & Graniti, 1972). Phytolysin production is not restricted to *C. purpureum.* It has also been isolated from *Leucostoma persoonii* (Kern, 1961) and *Dothidea ribesia* (Naef-Roth, Gäumann & Albershein, 1961).

Early attempts to isolate the toxin from *C. purpureum* were not very successful. Brooks and Brenchley (1929, 1931) obtained an extract from culture filtrates of *C. purpureum* by means of simple water extraction or by means of precipitation with alcohol. Although they could not achieve a complete separation of the two constituents of this extract, they were able to identify one as the agent responsible for the silvering of leaves and the other for the browning of leaf margins. In their opinion neither agent was enzymatic in nature (Brooks & Brenchley, 1931). Very little work has been done on phytolysin since that of Brooks and Brenchley. Consequently, its exact chemical nature and mode of action remains largely unknown (Kern, 1961; Naef-Roth, Gäumann & Albersheim, 1961; Naef-Roth, Kern & Toth, 1963).

In the previous section it was found that cherry trees inoculated with an isolate of *C. purpureum* from broom *(Teline monspessulana)* failed to develop silvering symptoms after 3.5 months, whereas those inoculated with *C. purpureum* from other sources had done so. It was decided to investigate phytolysin production by this and other isolates.

Materials and Methods

Five isolates of *C. purpureum* were tested for production of phytolysin. The isolates and the plant hosts from which they came are given in Table 9.

The method used was similar to that of Naef-Roth, Kern & Toth (1963). Ten 8mm diameter discs were cut from 10-day-old cultures of the *C. purpureum* isolates grown on malt agar, and placed in 1000 ml Erlenmeyer flasks containing 250 ml of Modified Fries Medium (see Appendix II). Two flasks were inoculated per isolate. The flasks were incubated at 25^{o}C under continuous fluorescent lighting for 28 days.

The liquid cultures were filtered through Whatman No. 4 and then No. 2 filter papers. Half

TABLE 9

Isolates of *C. purpureum,* and their origin, tested for production of phytolysin.

Isolate	Host Plant
* CPB 1	*Teline monspessulana*
CPPa	*Populus alba*
CPL 2	*Tibouchina semidecandra*
* CPC 12	*Prunus avium* cv. Black Tartarian
* CPW 1	*Salix babylonica*

* Culture started from the same spore collection as used to inoculate trees in the previous section (p. 32).

of the cell-free filtrate was refrigerated at 2°C until tested and the remainder extracted with ethyl acetate. The organic phase was evaporated to dryness under reduced pressure at 25°C. Phytolysin slowly loses activity at or above 30°C and is immediately destroyed at 50°C (Naef-Roth, Gäumann & Albersheim, 1961).

Extracts were tested for phytolysin production by:

(a) The crude extract of phytolysin was dissolved in 2 ml of 2.5% acetone at a concentration of 1.5 or 2.5 mg/ml (after Naef-Roth, Kern & Toth, 1963). Mature nectarine leaves were placed in each solution in a 10 ml beaker, and incubated in the light at 25°C with observations being made at 3, 6, 9, 12, 15 and 21 hours. The controls were distilled water, 10% acetone and 2.5% acetone.

(b) Nectarine leaves were placed in a beaker containing 10 ml of culture filtrate, and incubated at 25°C in the light for 48 hours. The controls were distilled water, Modified Fries liquid medium, 0.5% ammonium tartrate solution, 0.05% $MgSO_4 . 7H_20$ solution and 0.05% KH_2P0_4 solution. The last three controls were the major components in Modified Fries liquid medium and were included because in an exploratory experiment it was found that the liquid medium was causing discoloration of the leaves. By including the individual components it was hoped to be able to identify which was responsible for the discoloration observed with the liquid medium. The experiment was performed on two separate occasions, using three nectarine leaves in each of two replicates of each treatment on each occasion.

Results and Discussion.

The ethyl acetate extract of the culture filtrate of all isolates of *C. purpureum* caused severe browning of the leaf tissue within 21 hours with both concentrations of the extract tested. Those leaves in water, 2.5% acetone and 10% acetone remained unaltered. The results obtained with the culture filtrates themselves are given in Table 10. They are expressed as an estimated percentage of the leaf surface showing discoloration after 24 and 48 hours. The figures in the table are the average of the measurements made on the two occasions that the experiment was performed. The symptoms displayed were interveinal discoloration, chlorosis or an overall silvered effect. In some cases browning of the petiole and veins was apparent by 48 hours. The visual symptoms are indicated in Table 10.

Discoloration increased in all treatments over the period 24 to 48 hours. Part of this increase could have been due to the growth medium, as symptoms with both ammonium tartrate solution and Modified Fries liquid medium increased over this period. With *C. purpureum* isolates CPPa, CPL2 and CPW1 the increase with time was quite substantial whereas with isolates CPC 12 and CPB 1 it was relatively small. This could suggest that the increase in discoloration from 24 to 48 hours was not due to the growth medium, but reflects the production of different amounts of phytolysin by the isolates. Ammonium tartrate was the only one of the components of Modified Fries medium tested that had any effect on nectarine leaves. Brown-

TABLE 10

Visible symptoms and percentage of nectarine leaves discoloured after incubation in culture filtrates of isolates of *C. purpureum* for 24 and 48 hours at 25°C.

	Time after treatment applied			
	24 hr		48 hr	
Treatment	Visible Symptoms	% of leaf Area Affected	Visible Symptoms	% of leaf Area Affected
Distilled Water	-	0	-	0
$MgSO_4$	-	0	-	0
KH_2PO_4	-	0	-	0
ammonium tartrate	Petiole wilted, some yellowing.	5	Moderate yellowing.	30
Modified Fries medium	Petiole wilted, some yellowing.	6	Yellow with some necrosis. Brown patches on petiole.	40
Culture filtrate of isolate				
CPB1	-	0	Slight discoloration. Some brown on petiole.	10
CPPa	Interveinal yellowing.	42	Extensive yellowing, a silvering effect apparent. Petiole brown, extending into leaf.	90
CPL2	Minor discoloration of leaf.	13	Petiole brown extending into leaf, silvering effect apparent.	57
CPC12	Minor browning of petiole, slight yellowing of leaf.	25	Petiole brown, leaf yellowing.	35
CPW1	Petiole wilting, brown minor yellowing of leaf.	6	Petiole brown extending along main vein of leaf. Silvering apparent.	45

ing of the petiole was most marked with the *C. purpureum* isolates and was not at all well developed with Modified Fries medium and was absent in the other controls. This suggests that this symptom is due to the production of phytolysin. At both 24 and 48 hours the production of phytolysin was, in decreasing order, isolates CPPa, CPL 2, CPW I, CPC 12 and CPB I. The percent discoloration with isolate CPB I at 48 hours was significantly different ($P = 0.05$) from the other isolates.

In spite of the complicating factors present in this method of assessing phytolysin production, it does appear that CPB I, the isolate from broom produced less toxin than the other isolates of *C. purpureum* tested. This may well explain the lack of silvering symptoms in cherry trees inoculated with spores from broom.

V STUDIES ON BIOLOGICAL CONTROL OF *Chondrostereum purpureum*

A. Studies on *Fusarium lateritium* Nees

Carter (1971) isolated a fungus, later identified as *Fusarium lateritium* Nees, from tissue below a 6-month-old pruning wound on an apricot tree in which *Eutypa armeniacae* Hansf. and Carter, the fungus that causes a dieback disease of apricot trees and grapevines (Carter, 1957, 1975; Bolay & Moller, 1977; Carter & Moller, 1977) had failed to become established. *F. lateritium* was subsequently investigated as a potential biological competitor of *E. armeniacae*. The application of suspensions containing 10^4 to 10^6 *F. lateritium* macroconidia per ml to pruned surfaces was found to give significant protection against *E. armeniacae*. Where *F. lateritium* was applied 24 hr before the inoculum of the pathogen (10 ascospores), the percentage of sites infected after 6 months was reduced from 49% in unprotected wounds to 12% in those treated with *F. lateritium* (Carter & Price, 1974). Work since then has confirmed the effectiveness of *F. lateritium* especially when used in association with the fungicide Benlate in giving significant control of *E. armeniacae* (Carter & Price, 1975). Carter & Mullett (1978) have now developed a 'target-orientated' system for accurate delivery of the protectants as the trees are being pruned, that can be used on a commercial scale.

Because of the success achieved with *Fusarium lateritium* as a biological control agent for *E. armeniacae,* it was decided to investigate *F. lateritium* in some detail as a possible biological control agent for *Chondrostereum purpureum*. The work with *E. armeniacae* was of particular interest because of similarities in the behaviour of *E. armeniacae* and *C. purpureum*, since both gain entry to a tree via freshly made pruning wounds, and both are vascular pathogens. Any protectant system, as was the case for *E. armeniacae*, would be designed to prevent the invasion of pruning wounds by *C. purpureum*.

Fusarium lateritium Nees as a plant pathogen

If *F. lateritium* is to be used as a biological control agent for *Chondrostereum purpureum*, it should ideally cause little or no damage to those plants on which it would be applied. A survey of the literature suggested that this is in fact the case. *F. lateritium* has been reported as causing wilt, dieback and canker on a number of trees and woody shrubs: canker of *Sophora japonica* (Massie & Peterson, 1968; Peterson & Davis, 1965), wilt of *Mimosa* sp. (Snyder, Toole & Hepting, 1949), pitch canker on several *Pinus* sp. in the south east of North America (Berry & Hepting, 1969; Hepting & Roth, 1946; Bethune & Hepting, 1963; Berry & Hepting, 1969; Hepting, 1971), canker on *Juglans major* (Kessler, 1974), and stem lesions on coffee plants (Corbett, 1959).

However, records of *F. lateritium* causing damage on fruit tree species are rare. It can be a pathogen on unthrifty citrus trees (Nattrass, 1934; Romano, 1956; Salerno, 1959), and causes bud rot and stem lesions on apple trees (Zeller, 1926; Jacks, 1953; Agarwala & Gupta, 1968). *F. lateritium* is of minor importance as a storage rot fungus on cucumber (Kessler, 1974), apple (Brien, 1937; Colhoun, 1948) and citrus (Doidge & van der Plank, 1936).

Fusarium lateritium (Nees) emend, Snyd. & Hans. f. sp. *mori* (Desm.) Matuo & Sato causes twig dieback and canker of mulberry (Wormald, 1928; Brooks, 1953; Matuo & Sato, 1962; Hepting, 1971). This *forma specialis* is probably worldwide in areas where the host is grown (Booth, 1971). *F. lateritium* f. sp. *mori* was found causing a twig dieback of mulberry *(Morus nigra)* in the Adelaide Hills. The fungus had formed abundant orange sporodochia on the dead branches.

In vitro studies of the interactions between *C. purpureum* and *F. lateritium*

The effect of *F. lateritium* on the radial growth of *C. purpureum* in culture was investigated.

Isolate CPC 12 of *C. purpureum* (Table 8) and isolate B1 of *F. lateritium,* the isolate used by Carter & Price (1974), were grown on Malt and GP/20 agars respectively and after 10 days, 8mm diameter discs were cut with a cork-borer from near the edges of the colonies. The discs were placed on the opposite sides of plates of GP/20 agar. Controls consisted of plates inoculated at the centre with discs of *C. purpureum* or *F. lateritium* alone. All treatments were replicated three times. Plates were incubated at 20°C in the dark and observed daily.

Inhibition of the growth of *C. purpureum* by *F. lateritium* was apparent after 6 or 7 days (Figure 10A). The control plates of *C. purpureum* were completely covered by day 9. These results suggest that *F. lateritium* produces a substance which is toxic to *C. purpureum*. The following experiment was designed to test the activity of this substance.

Detection of the fungitoxin by a bioassay technique

Each day for 10 consecutive days, three 15 ml plates of GP/20 agar were inoculated in the centre with a 7mm disc of *F. lateritium* taken from the edge of an actively growing colony. The plates were incubated at 20°C in the dark.

Ten days after the first inoculation, the colonies were killed by exposure to chloroform vapour for 30 minutes, the excess chloroform allowed to evaporate, and each plate was overpoured with 2.5ml of 0.2% Oxoid purified agar containing a heavy suspension of *C. purpureum* basidiospores (4.6×10^6/ml). *C. purpureum* basidiospores were found to be easily killed if placed in molten agar. Consequently, it was necessary to allow the temperature of the agar to drop to approximately 30°C before adding the spores (0.2% Oxoid purified agar gels at c. 28°C). The plates were then incubated at 20°C for a further 5 days after which time the

FIGURE 10

A. Inhibition of growth of *C. purpureum* by *F. lateritium* *in vitro.*

a = *F. lateritium*
b = *F. lateritium* and *C. purpureum*
c = *C. purpureum*

B. Bioassay of the fungitoxin produced by *F. lateritium*. c = control (*C. purpureum* only); numbers 1 - 10 refer to the age (in days) of the colony of *F. lateritium* when assayed with *C. purpureum* basidiospores.

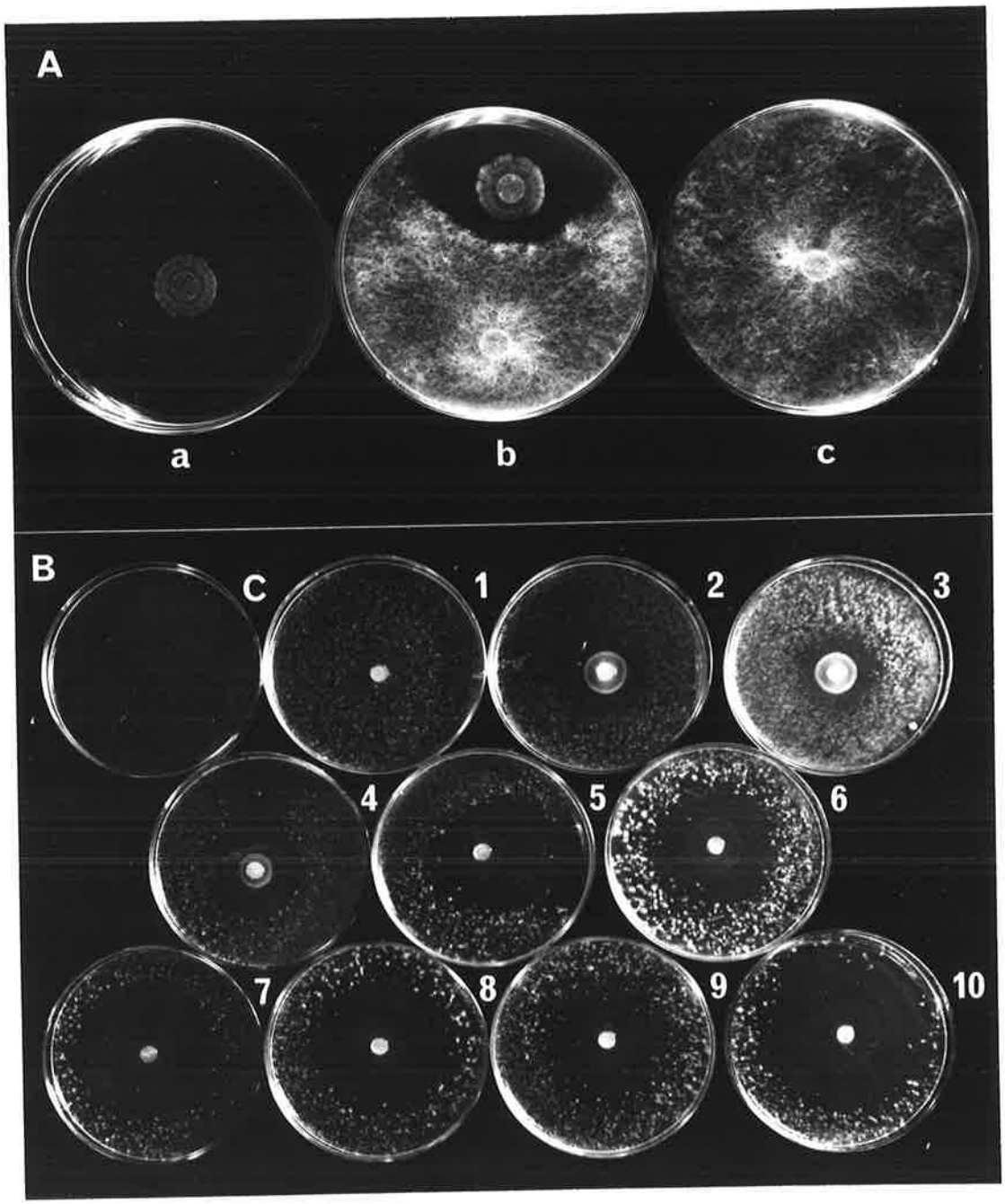

A
a
b
c
B
C
1
2
3
4
5
6
7
8
9
10

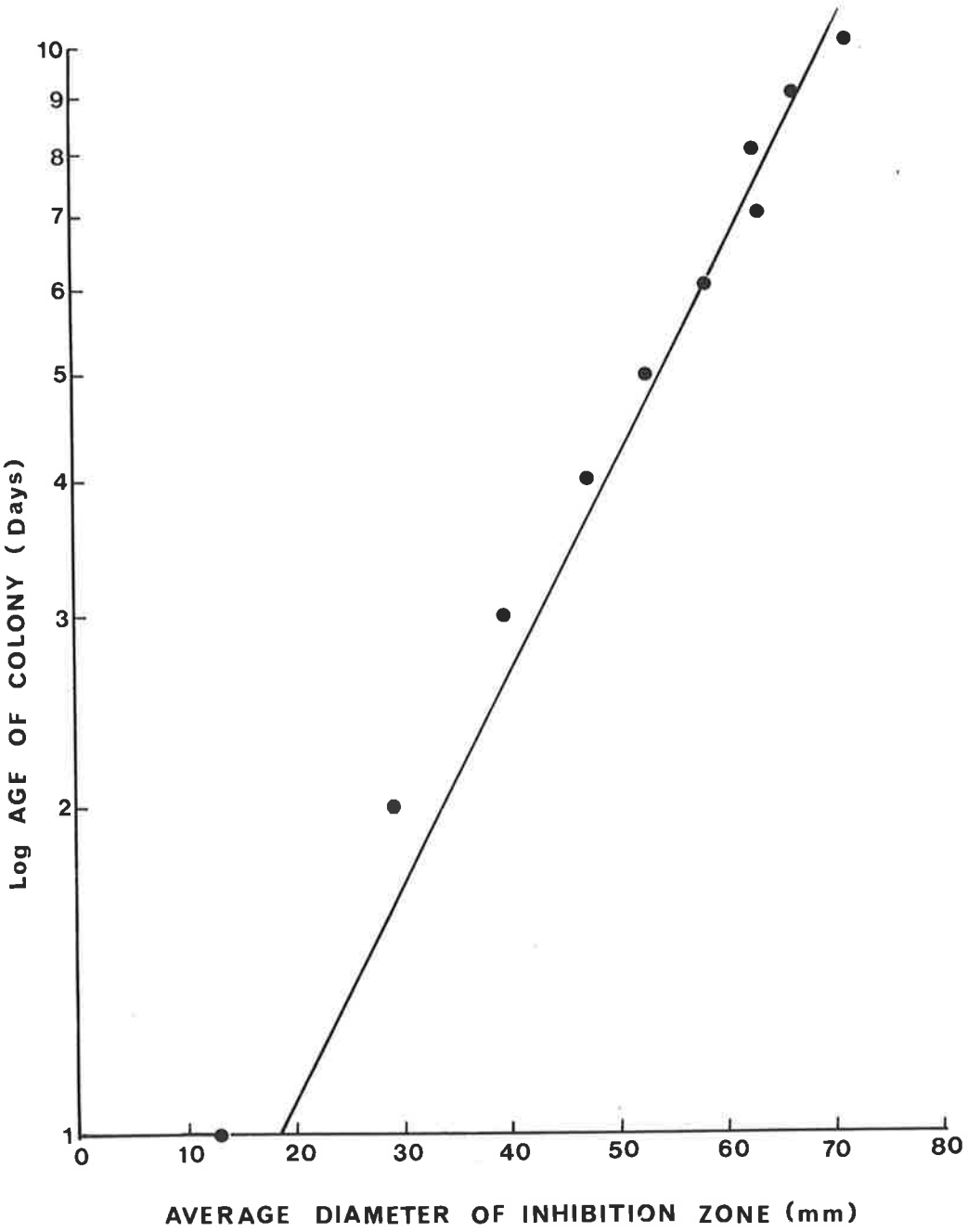
TABLE 11

Average diameter of the zone of inhibition produced on assaying *F. lateritium* colonies with *C. purpureum* basidiospores.

Age of *F. lateritium* colony (days)	**Average diameter of inhibition zone (mm) (including *F. lateritium* colony)**
1	13
2	29
3	40
4	48
5	53
6	58
7	63
8	63
9	67
10	71

FIGURE 11

The relationship between the age of *F. lateritium* colony and the size of the zone of inhibition in bioassay plates seeded with *C. purpureum* basidiospores. Y = 18.60 + 5.77X.


Log AGE OF COLONY (Days)
10
9
8
7
6
5
4
3
2
1
0
10
20
30
40
50
60
70
80
AVERAGE DIAMETER OF INHIBITION ZONE (mm)

diameters of the inhibition zones were measured, two measurements being taken at 90° to each other.

The results are given in Table 8, and the plates are shown in Figure 10B.The uneven appearance of the *C. purpureum* colonies on the plates in this photograph is due in part to the plates having been overpoured using individual lots of 2.5 ml of agar in 5 ml bottles. The quantity of basidiospores added to each of these bottles need not have been the same. The average diameter of the inhibition zone was found to be proportional to the logarithm of the age of the *F. lateritium* colony (Figure 11). These results parallel the situation that exists between *Eutypa armeniacae* and *F. lateritium* (Carter & Price, 1974). The inhibition zone tends to be slightly larger with *C. purpureum* than with *E. armeniacae.*

Inhibition of *F. lateritium* by *C. purpureum*

The reverse of the previous experiment was done to see if there is any inhibition of the growth of *F. lateritium* by *C. purpureum.*

Three 15 ml plates of GP/20 agar were inoculated in the centre with a 7 mm disc of *C. purpureum* and incubated at 20°C in the dark for 4 days. The colonies were then killed by exposure to chloroform vapour. A suspension of *F. lateritium* macroconidia from a 15-day old culture was prepared in sterile distilled water and several drops added to 2.5 ml of 0.2% purified molten agar which was poured over each of these plates and an uninoculated control. The plates were incubated at 20°C in the dark and observed daily.

After 5-6 days both the control and the plates inoculated with *C. purpureum* were evenly covered with *F. lateritium;* that is, there was no inhibition of *F. lateritium* by *C. purpureum.*

Inhibition of *C. purpureum* by enniatin

Carter & Price (1974, 1975) found that the isolate of *Fusarium lateritium* used to protect pruned apricot sapwood against invasion by *Eutypa armeniacae* produced *invitro* a nonvolatile, diffusible metabolite toxic to ascospores and mycelium of *E. armeniacae.* This inhibition of germination and growth was shown to be due to the production by *F. lateritium* of antibiotic substance(s) known by the general term 'enniatin(s)' (Carter & Ilsley, unpublished results).

Enniatins are a bioactive mixture of homologous cyclohexadepsipeptides (Audhya & Russell, 1974). A number of enniatins have been isolated from Fusaria over the years and their activity against several medically important micro-organisms tested (Gäumann, Roth, Ettlinger, Plattner & Nager, 1947; Cook, Cox & Farmer, 1949; Guérillot-Vinet, Guérillot-Vinet, Guyot, Montégut & Roux, 1950; Tirunarayanan & Sirsi, 1957).

Enniatin was extracted from the culture liquor of isolate B1 of *F. lateritium* and was tested

in vitro against *C. purpureum* to determine whether this antibiotic would inhibit the growth of *C. purpureum.*

Enniatin was dissolved in 8 ml of methanol and 2, 4, 8 and 16 µl of this solution were put on filter paper discs 6 mm in diameter. The discs were then placed at the centre of plates of GP/20 agar; 4 plates were used per dosage of enniatin tested. Each of these plates and three plates containing a 3-day-old colony of *F. lateritium* were overpoured with 2.5 ml of 0.2% Oxoid purified agar containing a heavy suspension of basidiospores of *C. purpureum.* Plates were incubated at 20°C for 5 days, after which the diameters of the zones of inhibition were measured (Table 12).

This test showed that enniatin will inhibit the growth of *C. purpureum.* The sizes of the zones of inhibition did not differ significantly between the four dosages of enniatin tested, but all were less than the zone formed with the 3-day-old *F. lateritium* colony. Subsequent investigations showed that enniatin did not have time to diffuse from the discs through the agar before the plates were seeded with basidiospores of *C. purpureum.* If the discs were left on the agar for three days before the plates were seeded, the sizes of the inhibition zones were approximately proportional to the dosage of enniatin applied to the discs.

Production of enniatin by isolates of *Fusarium lateritium*

While the population of *F. lateritium* in a commercial apple orchard was being studied, 102 fungal isolates designated as *F. lateritium* were obtained from apple trees. These isolates together with a number from a variety of other hosts, and 10 isolates received from the Commonwealth Mycological Institute (Herb. I.M.I.) and 2 (isolates F1295 and F1303) received from Dr. L. W. Burgess, University of Sydney, New South Wales as *F. lateritium* were investigated to see what proportion produced enniatin and were effective in inhibiting mycelial growth of *C. purpureum* in culture.

Materials and Methods

The isolates used and their host origin are listed in Table 13. All isolates were maintained on GP/20 agar. The production of antibiotics was detected by the plate bioassay method (see p. 52), and by the extraction of the culture filtrate and running the extract obtained on thin layer chromotography plates. For the latter, ten 8 mm diameter discs were cut from a 7-day-old culture of the particular fungal isolate growing on GP/20 agar and placed in a 1000 ml Erlenmeyer flask containing 300 ml of liquid glucose peptone medium. The isolates were grown for 4 days at 25°C in shaken culture under continuous fluorescent lighting (Audhya & Russell, 1974; Carter & Ilsley, unpublished results). Each culture was then filtered through Whatman No. 4 filter paper in a Büchner funnel and subsequently through a membrane filter

TABLE 12

Average sizes of the zones of inhibition produced when filter paper discs containing enniatin were bioassayed with basidiospores of *C. purpureum.*

	Quantity of enniatin on filter paper disc (μl)	2	4	8	16	3-day-old colony of *F. lateritium*
A.	Diameter of disc (or fungal colony) (mm)	6	6	6	6	19
B.	Average diameter of disc (or fungal colony) + inhibition zone (mm)	8	10	12	12	36
	Size of inhibition zone (mm)	2	4	6	6	17

TABLE 13

Sources of *Fusarium* isolates used in the investigation.

Isolate Number		Origin
IMI 90139a	AMARYLLIDACEAE	*Agave* sp.
B20, B21	CRUCIFERAE	*Brassica oleracea* var. *botrytis*
B19	CUPRESSACEAE	*Chamaecyparis lawsoniana*
B16		*Cupressus macrocarpa*
IMI 128749	EUPHORBIACEAE	*Manihot* sp.
B14, B15	FAGACEAE	*Castanea sativa*
IMI 134593	LEGUMINOSAE	*Laburnum* sp.
B10, B11, B12		*Robinia pseudoacacia*
B27, B28, B29	MORACEAE	*Morus nigra*
F1295, F1303, IMI 122838		*Morus* sp.
IMI 105481	OLEACEAE	*Fraxinus excelsior*
B18	PINACEAE	*Cedrus atlantica aurea*
B17	ROSACEAE	*Cydonia oblonga*
2,3,5,11,13		*Malus sylvestris*
		cv. Jonathon
10		cv. Red Delicious
14		cv. Granny Smith
21-60, 62-84, 86-117		cv. Jonared
B1		*Prunus armeniacae*
B6		*Prunus avium* cv. Early Rivers
B3, B7		cv. Lustre
B2, B5		cv. Smith's Black
B4		*Prunus cerasus*
IMI 116392		*Prunus domestica*
IMI 131457		*Rosa* sp.
B25	RUTACEAE	*Citrus limon x sinensis*
		cv. Meyer Lemon
IMI 158154, IMI 94758		*Citrus* sp.

of pore size 0.2 μm (Sartorius).

The clear filtrate (approximately 280 ml) was extracted twice using 90 ml and then 50 ml of ethyl acetate. The combined organic phase was evaporated to dryness under reduced pressure at 35°C. The dried extract was dissolved in 5 ml methanol, and 10 μl aliquots were spotted at the origin on precoated thin layer chromatography plates (Silica gel 60 F245; thickness 0.25mm; Merck). The plates were developed with ethyl acetate: hexane: methanol: water, 75:200:17:1 (Audhya & Russell, 1973). The chromatographed compounds were detected by exposing the air-dried plates to iodine vapour. The enniatin spot had an R_F value of 0.20, which was the same as that of a crystalline enniatin obtained from isolate B1 of *Fusarium lateritium* (Carter & Price, unpublished results).

Results and Discussion

As a number of the isolates tested (including several from C.M.I.) did not inhibit the growth of *C. purpureum in vitro* (Figure 12) and did not produce enniatin (Figure 13) the identity of all isolates was subsequently reviewed. It was found that of the 136 Fusaria tested, 83 were identified using morphological criteria as being *F. lateritium*, and 78 of the 83 were found to inhibit the growth of *C. purpureum* in plate bioassays and to produce enniatin (Table 14). Of the members of the group identified as not being *F. lateritium*, only 2 isolates were found to inhibit the growth of *C. purpureum* and to produce enniatin. The identity of the remaining 51 Fusaria was not determined.

The sizes of the zones of inhibition did not vary very much within the 78 isolates of *F. lateritium.* The maximum recorded was 21 mm and the minimum was 15 mm, with an overall average of 18 mm (Table 15).

The taxonomy of the genus *Fusarium* has been based mostly on morphological characteristics and host relationships. Of these, spore morphology has been the major character employed in the identification of Fusaria (Snyder & Hansen, 1940; Gordon, 1956; Tousson & Nelson, 1968; Booth, 1971). In recent years various authors have suggested that biochemical characteristics could well be used in *Fusarium* taxonomy, and in fungal taxonomy in general (Booth, 1966; Murray, 1966; Subramanian, 1967).

Biochemical criteria have been used for many years, in conjunction with morphological characteristics in the classification of yeasts (Kreger-van Rij, 1962). Characters supplied by chemical and physiological tests have come to assume considerable importance in the taxonomy of certain of the Agaricales (Watling, 1966) and of the dermatophytes (Murray, 1966). The presence of certain unidentified fungal metabolites and of specific enzymes were used by Taylor (1974) to identify mycelial cultures of four different basidiomycetes. The tests employed were sufficiently simple to be applied by a non-biochemist working in a laboratory without sophisticated equipment.

The results from the present study suggest that the production of enniatin could be a useful criterion for confirming the identity of an isolate thought to be *F. lateritium* on morphological grounds. The 5 isolates of *F. lateritium* that did not produce enniatin and did not inhibit

FIGURE 12

Example of bioassay plates. The numbers refer to the isolates listed in Table 10. Isolates 38, 60, 82, B1, 34, 59, 35b, 70, B14 are *F. lateritium*; isolates 75 and 99 were unidentified species of *Fusarium*.

C = control (*C. purpureum*).

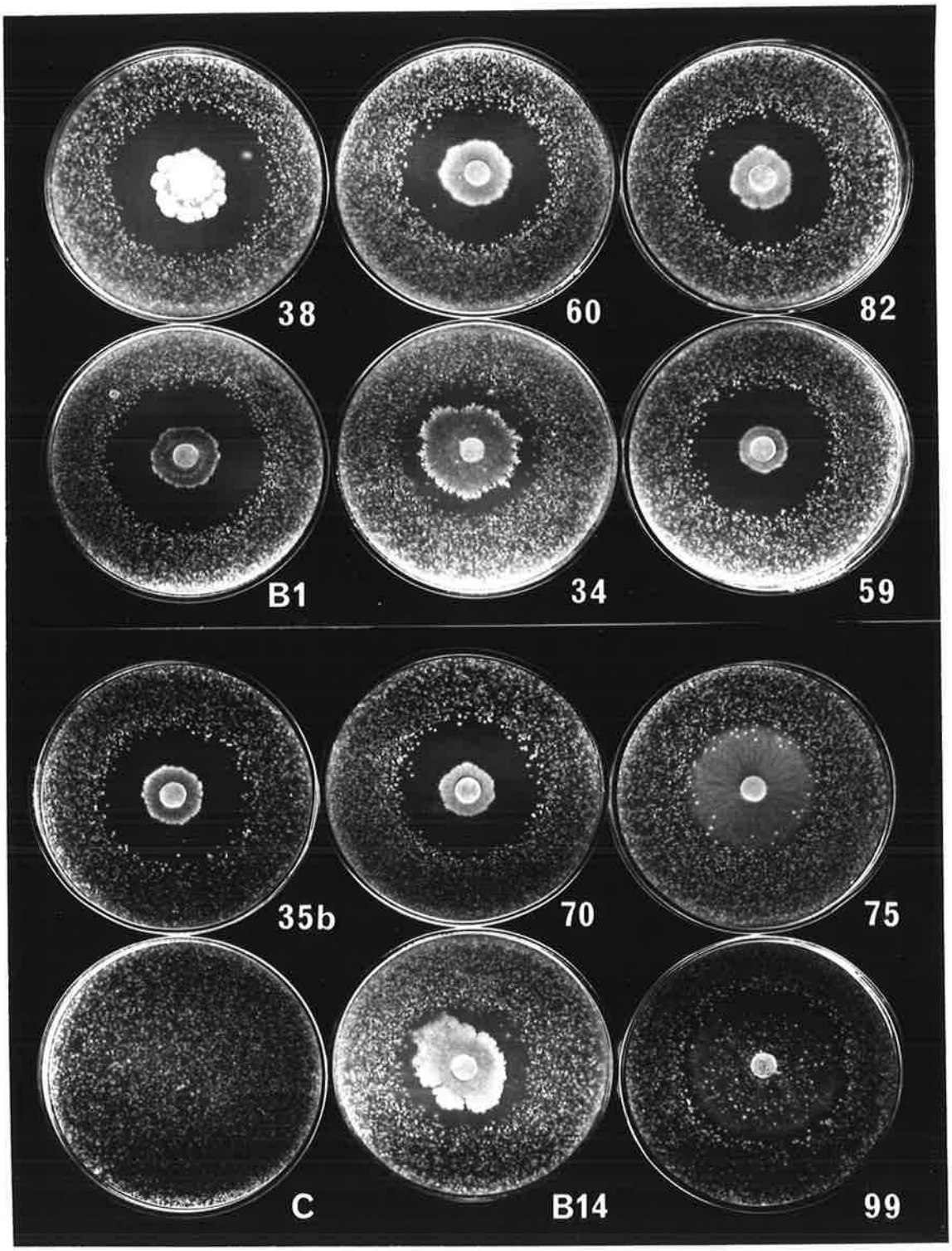

38
60
82
B1
34
59
35b
70
75
C
B14
99

FIGURE 13

Example of thin layer chromatography plates. The isolates of Fusaria on this plate (from left to right) are B1, 98, 99, 102, 103, 105, 106, 107, 108, 109, 110, 111 and 113. All isolates except 99, 103, 109, 111 and 113 are *F. lateritium.* The R_F value of the enniatin spot was 0.20.

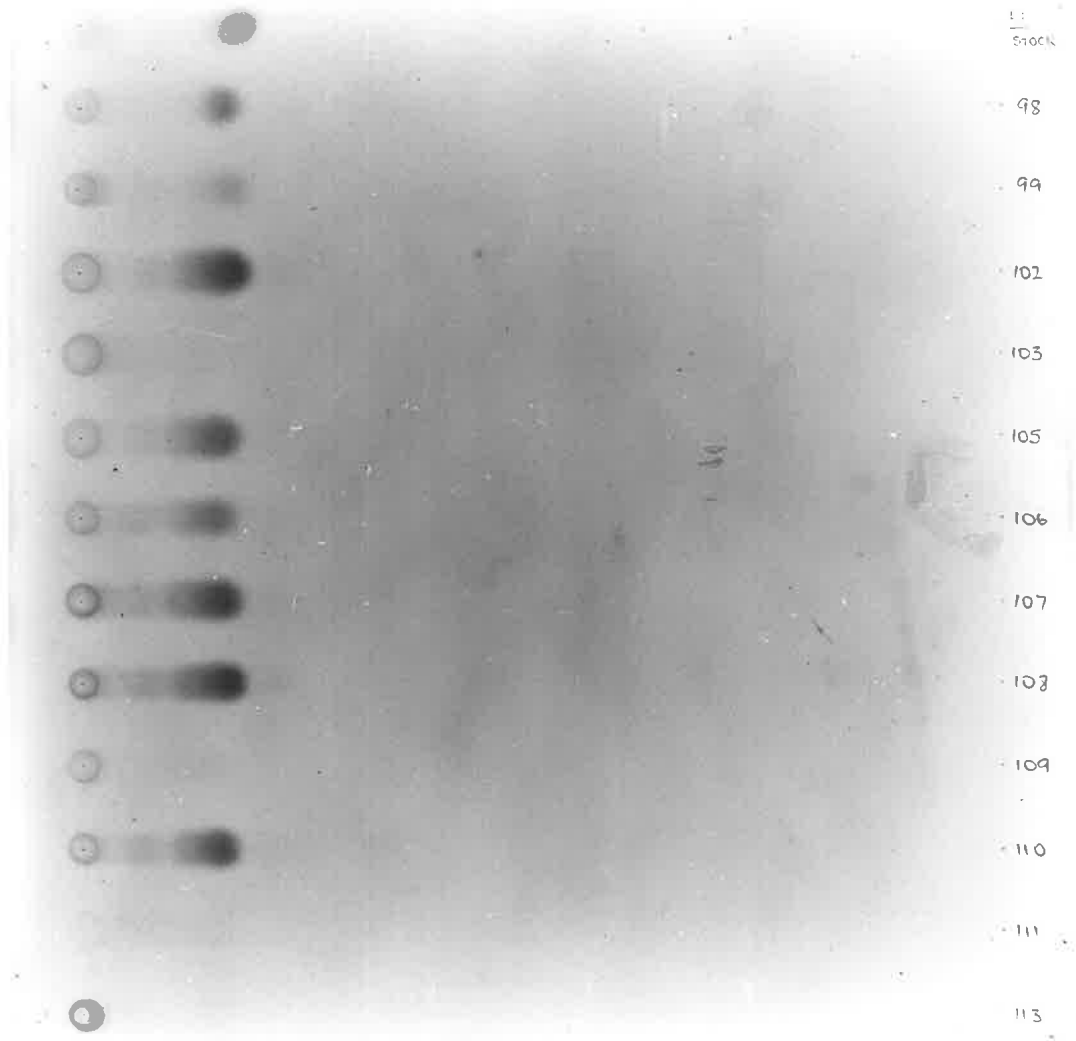

L:
STOCK
98
99
102
103
105
106
107
108
109
110
111
113

TABLE 14

Production of enniatin and inhibition of growth of *C. purpureum in vitro* by *Fusarium* isolates.

A. *Fusarium lateritium:* enniatin produced, *C. purpureum* inhibited.
2,3,10,11,13,14,21-26,32,34,35b-42,44-48,
50,52,53,55,57,59,60,65-70,74,77,82,84,
86,87,89-91,97,98,100-102,104-108,110,
112,116,117,B1,B2,B3,B4,B5,B6,B7,B10,
B11,B12,B14,B20,B21,IMI 126338,IMI 131457,
IMI 134593.

B. *Fusarium lateritium;* no enniatin produced, *C. purpureum* no inhibited.
B27,B29,IMI 122838,F1295,F1303.

C. Not *Fusarium lateritium;* enniatin produced, *C. purpureum* inhibited.
99,115

D Not *Fusarium lateritium;* no enniatin produced, *C. purpureum* not inhibited.
5,27-31,33,35a,43,49,51,54,56,58,62-64,
71-73,75,76,78-81,83,88,92-96,103,109,
111,113,114,B15,B16,B17,B18,B19,B25,B28,
IMI 90139a,IMI 94758 ,IMI 105481,IMI 116392,
IMI 128749,IMI 158154.

TABLE 15

Summary of the size of the zones of inhibition produced when *F. lateritium* isolates were bioassayed with *C. purpureum* basidiospores.

		Average*	Minimum	Maximum
A.	Diameter of *F. lateritium* colony at 4 days (mm)	19	11	32
B.	Diameter of *F. lateritium* colony + inhibition zone (mm)	37	26	53
	Size of inhibition zone	18	15	21

* Average of 78 isolates of *F. lateritium.*

the growth of *C. purpureum* were all from mulberry (*Morus* sp.). Booth (1971) recognises a physiological form of *F. lateritium* which occurs only on mulberry, called *F. lateritium* f. sp. *Mori*. The above results suggest that this *Forma specialis* should not be regarded as a subspecies of *F. lateritium.*

It must be stressed, however, that a *Fusarium* isolate producing enniatin need not necessarily be *F. lateritium.* Audhya & Russell (1973) have shown that *F. sambucinum* Fuckel produces an enniatin which they have called enniatin A. In fact, in the present study two Fusaria (isolates 99 and 115) which were not *F. lateritium*, were found to produce enniatin.

Sporulation of *Fusarium lateritium* on pruned cherry sapwood surfaces in the field.

Carter & Price (1974) noted that a biological control micro-organism will be able to compete better with a pathogen within the pathogens infection courts if it is capable of producing abundant propagules which will be transported by rainfall and deposited within the vessels exposed in pruned sapwood. A study was therefore made of the rate and magnitude of sporulation after inoculating macroconidia of *Fusarium lateritium* on freshly pruned cherry sapwood surfaces.

Selected laterals on 8-year old cherry trees (*Prunus avium* cv. Smith's Black) were pruned on June 12, 1974 just above a dormant bud to give a wound surface 1.5 - 2.0 cm in diameter. A suspension of *F. lateritium* macroconidia, adjusted to 1.0×10^3 spores per ml, was applied within a few minutes of pruning by applying to each site with a Burkard microapplicator, a measured dose of 20 macroconidia using ten 2 μl drops. A total of 35 sites were inoculated and a further 14 left untreated as controls. At each sampling 5 inoculated and 2 control sites were taken at random.

At weekly intervals for 7 weeks after inoculation, the amount of sporulation of *F. lateritium* at the pruned sapwood surfaces was estimated by removing 1 - 2 cm of stem and agitating this in a 25 ml McCartney bottle containing 10 ml of sterile distilled water and 0.1% of a non-ionic wetting agent (BASF) on a wrist-action shaker for 10 minutes. The washing was repeated three times, and the total washings filtered under vacuum through a membrane filter disc (8 μm pore size). The disc was then flooded with 1% trypan blue (Gurr) for 5 minutes and the surplus stain removed by briefly reapplying the vacuum. The disc was then thoroughly dried and mounted in lactic acid for counting. An estimate of the total number of macroconidia on a disc was made from counting those seen in 20 fields through an x25 objective.

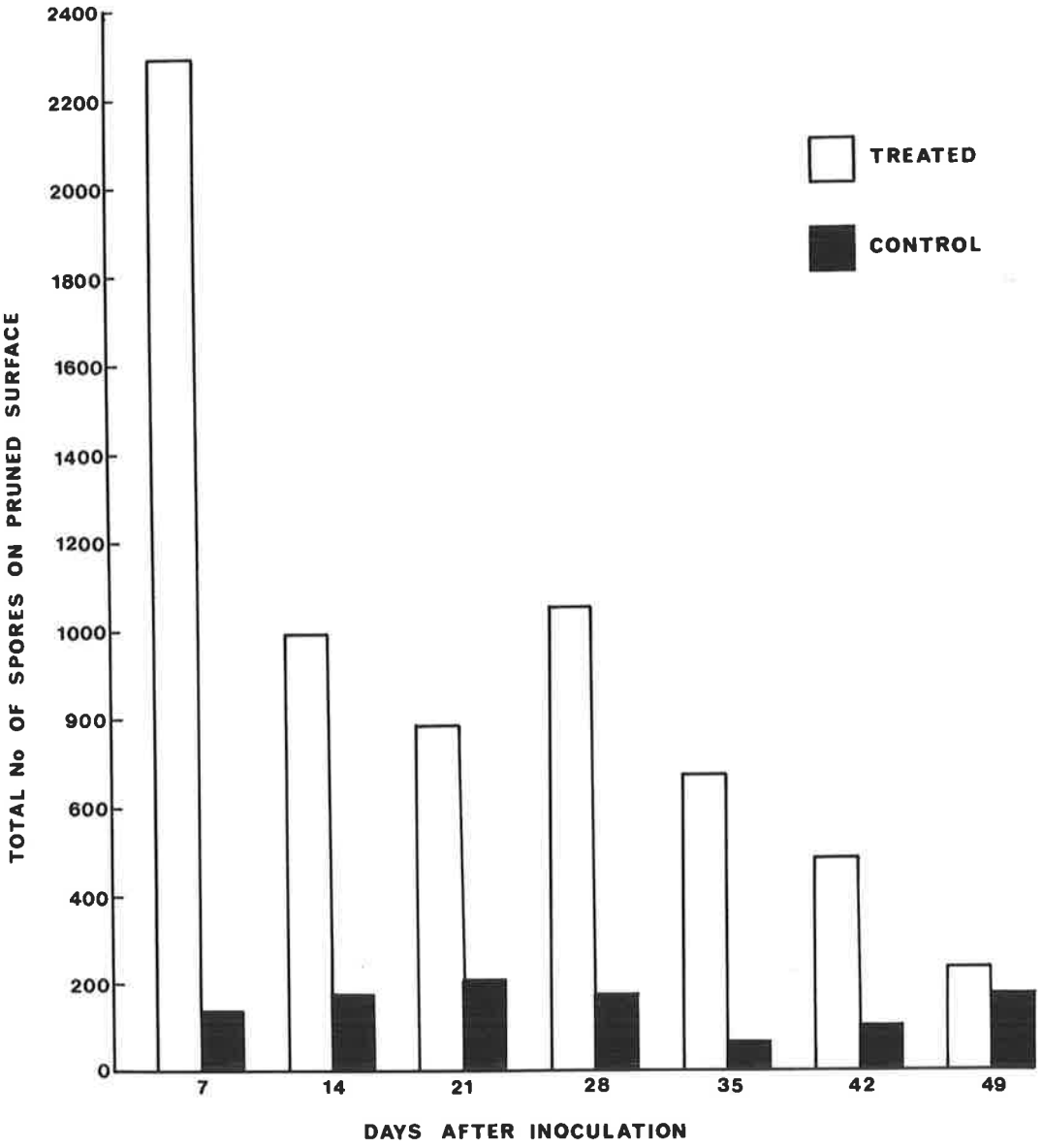
The results (Figure 14) showed that abundant sporulation was occurring at the pruned surfaces by day 7, and continued at a high level for at least 6 weeks, dropping considerably from the maximum count recorded at week 1, but at week 7 was still significantly higher than in the control ($P = 0.05$). During the experimental period, daily mean temperatures were mostly within the range 10 - 15^{o}C and rain fell on 31 of the first 50 days.

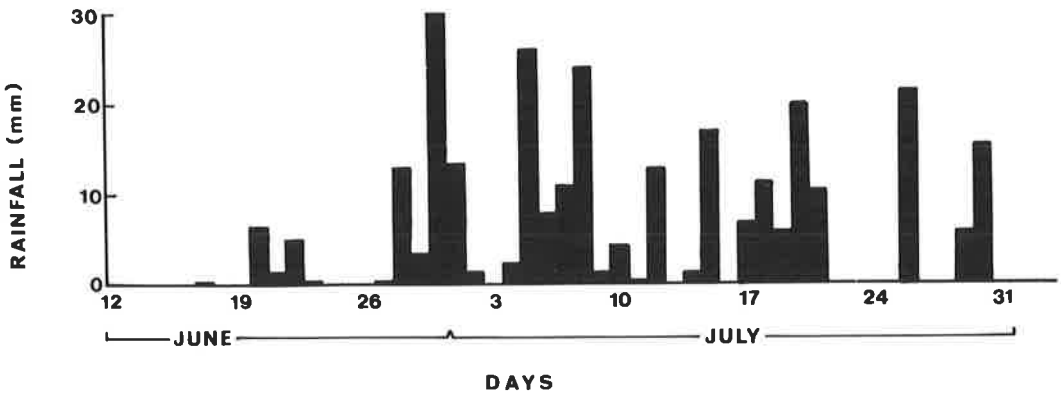
The results obtained here with cherry sapwood differed from those of Carter & Price (1974) using apricot sapwood. They found an incubation period of about 1 week for *F. lateritium* and a maximum count was obtained after about 6 weeks after which time the count declined.

FIGURE 14

Sporulation of *F. lateritium* on pruned cherry sapwood after inoculation with a suspension containing 1.0 x 10^3 macroconidia per ml.

The daily rainfall (mm) over the corresponding period is given in the bottom histogram.


TREATED
CONTROL
TOTAL No OF SPORES ON PRUNED SURFACE
2400
2200
2000
1800
1600
1400
1200
1000
900
600
400
200
0
7
14
21
28
35
42
49
DAYS AFTER INOCULATION


RAINFALL (mm)
30
20
10
0
12
19
26
3
10
17
24
31
JUNE
JULY
DAYS

Study of a natural population of *Fusarium lateritium*

At the same time as *Fusarium lateritium* was being investigated as a possible biological control agent for *Chondrostereum purpureum*, it was considered that it would be of value to know something of a natural population of *F. lateritium* existing in a commercial orchard.

An inspection of recently made pruning wounds on apple, cherry and plum trees was made in an orchard at Basket Range using cellotape impressions. Macroconidia similar to those of *F. lateritium* were found in a number of the impressions. *F. lateritium* was subsequently isolated in pure culture from impressions of the pruning wounds taken on plates of NDY/6 agar.

Few attempts have been made to monitor the population(s) of one or more micro-organisms on the external surfaces of living trees. Swinburne (1973) estimated the frequency of nineteen fungal colonists of apple leaf-scars in the period November to March of 1968-9 and 1970-1. His results were expressed as a percentage frequency of scars yielding a particular fungus at each sampling and not as an estimate of the total population of a particular species at each sampling.

An attempt has therefore been made to assess the number of viable *F. lateritium* propagules present on apple bark at different times of the year, to look at fluctuations in population levels and to assess the impact of orchard practices on the population.

Any sampling survey of the type envisaged has its inadequacies. To a certain extent these can be minimized and beyond that all that can be done is to be aware of them. For this reason a standardized procedure was devised and followed at each sampling. The survey involved sampling wood from a number of trees, washing the wood and plating the washings with an agar medium from which a count of *F. lateritium* colonies was made.

Before a procedure was decided upon, several factors had to be considered. These were, what to sample, what washing process to adopt, what agar medium to use and at what temperature to incubate the plates.

The efficiency of any washing process can be influenced by the volume of water used, the volume of the container, the total area to be washed, the intensity and duration of shaking (Preece & Dickinson, 1971). Thus, it was necessary to find a reproducable method that gave the highest count of viable spores.

In making an estimate of the population of a particular fungus, we are assuming that the number of viable propagules of that fungus in the suspension is the same as the number of colonies that develop after incubation. Preece & Dickinson (1971) recognised four requirements for a quantitative count:

(a) random dispersion of the organism throughout the washing,

(b) accurate measurement of the aliquots plated,

(c) each viable propogule to be able to produce a colony which must be counted without error, and,

(d) the development of one organism must be independent of any other.

Requirements (c) and (d) would be considerably assisted by the use of a selective medium. Swinburne (1973) found that the percentage count of *F. lateritium* from leaf-scars varied considerably with the medium used.

In this type of work the incubation temperature used is generally higher than that of the natural environment (DiMenna & Parle, 1970; Preece & Dickinson, 1971). In a preliminary test, plates were incubated at 15, 20 and 25°C. The only apparent effect of the different temperatures was that the colonies took longer to develop at 15°C than they did at 20° or 25°C.

In a situation similar to the one here, Smith & Wallace (1976) in estimating nematode population levels, found that as nematode distribution departed from random and became more patchy, more samples were required to gain an accurate estimate of mean density. This would require a large increase in the sampling effort.

For the purposes of this survey one required a part of the tree that was convenient to handle and not too destructive to the trees being used. It also had to be fairly representative of the tree as a whole. Consequently, several different pieces of apple tree (described below as 'wood') were sampled in order to select a type of wood for use in the survey that was representative of the tree as a whole. The types of apple wood sampled were as given below:

Wood Type

1.	Branch Wood (1.5 - 2.5 cm diameter, 6 cm long)		
2.	Lateral Wood (0.5 - 1.0 cm diameter, 6 cm long)		40 cm above
3.	Spur	West side	ground level
4.		East side	
5.	(6 cm long)	West side	180 cm above
6.		East side	ground level

As the trees used in the survey are close-planted and the rows run north-south, only the east and west side of the trees were suitable for obtaining any quantity of material over a long sampling period. A 'spur' is defined as a short lateral branch with the nodes close together often largely consisting of successive bourses (Hort. Education Assoc. Fruit Committee, 1955).

Five apple trees cv. Jonared were sampled at random from the twenty selected for use in this study, taking two pieces of each type of wood from each tree. The ten pieces of the different wood types were washed together in 50 ml of sterile distilled water on a wrist-action shaker for thirty-five minutes. Then, 1.0 ml portions of the washings were taken and spread with 1.0 ml lots of sterile distilled water in 9.0 cm diameter petri dishes which were then each poured with 10ml of NDY/F agar (p. 69). Three plates were prepared for each wood type and incubated at 20°C in the dark for ten days after which colony counts were made. The results are expressed as the number of viable *F. lateritium* propagules per g wet weight of material sampled.

The results are given in Table 16. As the counts for Wood Types 1 to 4 are not significantly

different and aspect does not appear to be important, it would appear that a spur is reasonably representative of the tree as a whole. In addition, of the wood types sampled, a spur is the least destructive to the trees being used. The high spurs (Wood Types 5 and 6) gave a lower count than the lower spurs. At 180 cm above the ground, conditions are likely to be subject to greater extremes and therefore less favourable as an environment for micro-organisms.

Materials and Methods

A portion of an orchard at Basket Range in the Adelaide Hills was used in this study. It contained forty-two apple trees cv. Jonared (planted 1960) of which twenty trees were selected at random for sampling.

A spur was taken from each of the twenty trees from July 1975 until January 1978 inclusive, at weekly or two-weekly intervals. When cutting the spurs from the trees the samples were handled only with forceps.

Each sample was weighed (with leaves removed if present), divided into two lots of ten spurs and placed in 100 ml Screw Cap Sample Jars (Kayline Plastics, Adelaide) with 30 ml sterile distilled water and shaken for 35 minutes on a wrist-action shaker. Washings were poured off, combined, and each lot of wood rinsed with a further 20 ml water. From the total washings (100 ml), 0.5 ml and 1.0 ml lots were taken and spread with 1.0 ml sterile distilled water in 9.0 cm diameter petri dishes which were then poured with 10 ml of agar. When colony counts were tending to exceed 50 per plate, the dilution of the washings was adjusted accordingly. Ten plates were prepared from each sampling. Plates were incubated at 20°C in the dark for seven to fourteen days after which time colony counts were made.

The selective agar medium used was one modified from Kerr (1963). To a basal NDY medium was added PCNB (pentachloronitrobenzene) 360 p.p.m. (Nash & Snyder, 1962; Papavizas, 1967), rose bengal 250 p.p.m., streptomycin sulphate 200 p.p.m. and kanamycin sulphate 100 p.p.m. Rose bengal and PCNB were used to restrict the growth of moulds. Rose bengal is also inhibitory to bacteria, and this effect is reinforced by the addition of streptomycin and kanamycin (Beech & Davenport, 1970). The medium is hereafter designated as NDY/F (Figure 14).

For thirty-four samplings (October 1975 to May 1976), the leaves were removed from the spurs, washed and plated with NDY/F. Only on one occasion was *F. lateritium* found on plates prepared from leaf washings. Subsequent to this, the leaves were removed and discarded.

Results

Figure 15 gives the monthly mean number of viable *F. lateritium* propagules per g wet weight of material sampled over the period of sampling, July 1975 to January 1978. This figure was obtained by dividing the estimated number of *F. lateritium* propagules in total

TABLE 16

Number of viable propagules of *F. lateritium* per g wet weight of material sampled on different types of apple wood.

Wood Type		No. per g wet weight
1.	branch	42
2.	lateral	79
3.	spur low W	47
4.	spur low E	54
5.	spur high W	20
6.	spur high E	24

FIGURE 14

Plate of the selective agar medium (NDY/F) used in isolating *F. lateritium* from the surface of spurs from apple trees.

The white colonies are *F. lateritium.*

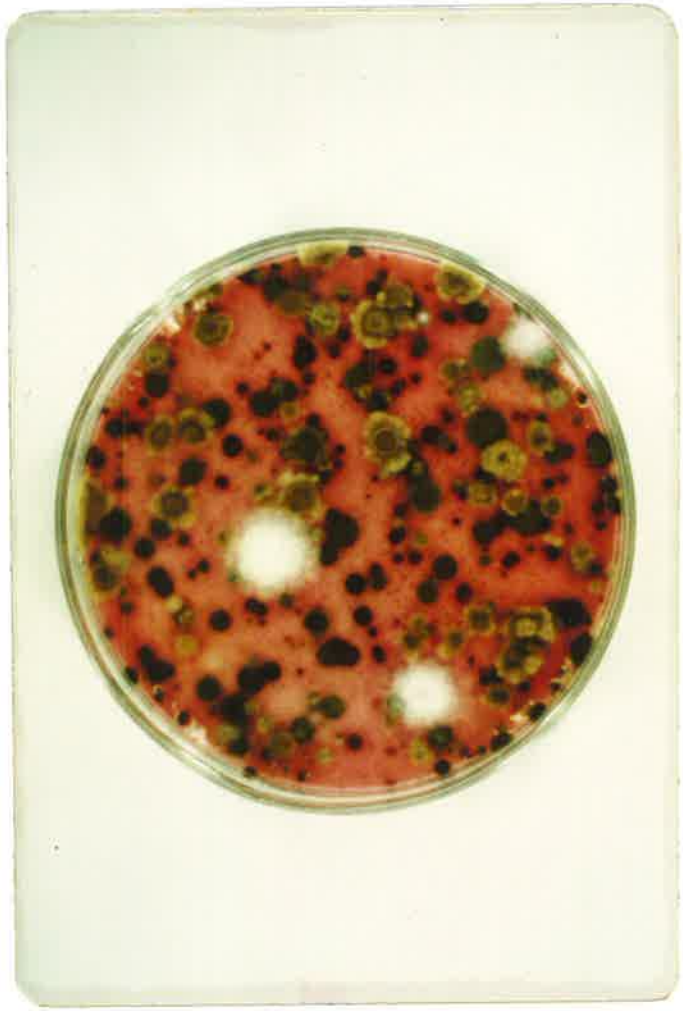


FIGURE 15

Monthly mean number of viable *Fusarium lateritium* propagules per g wet weight of material sampled, and total monthly rainfall (mm) over the period July 1975 to January 1978.

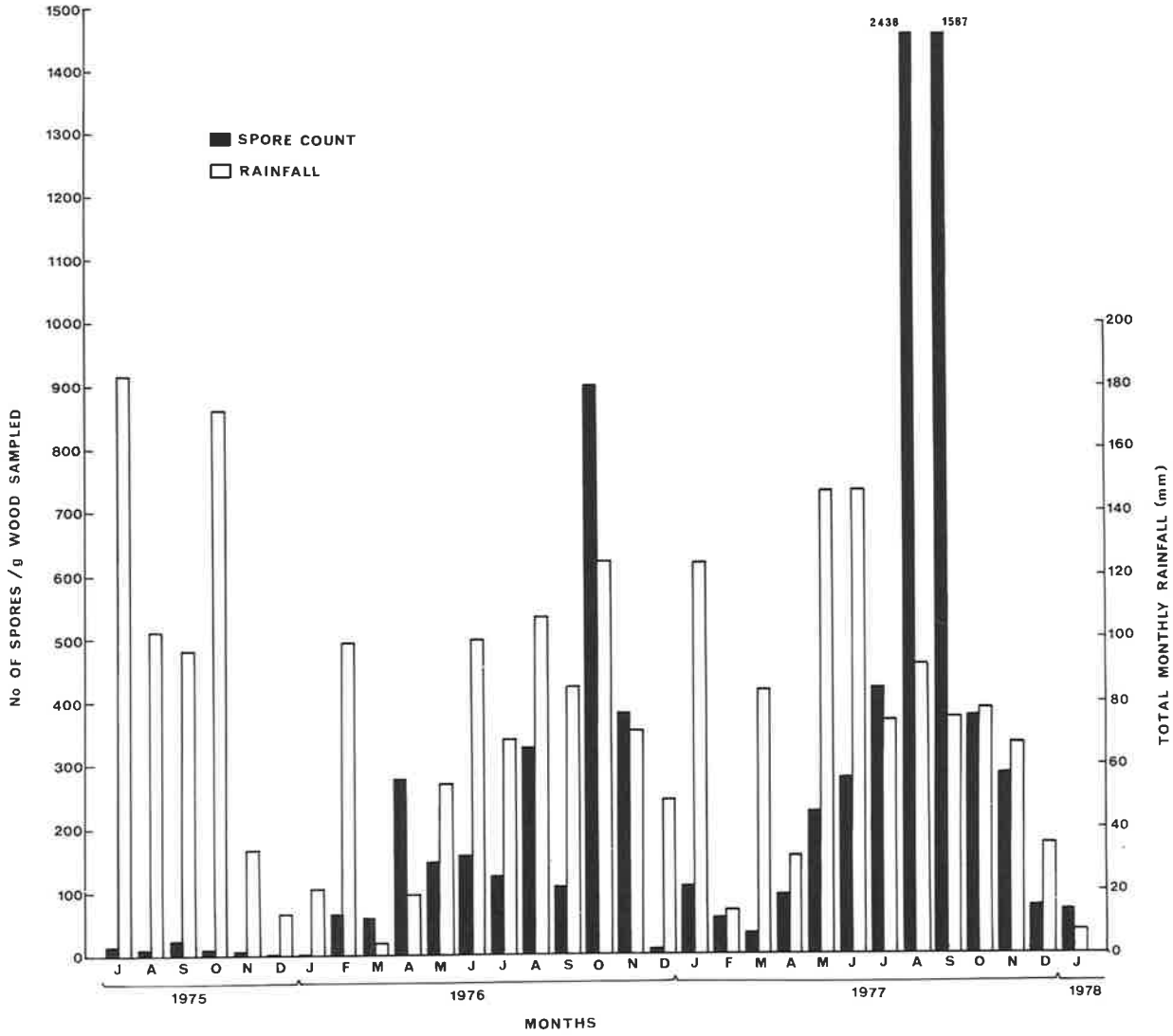
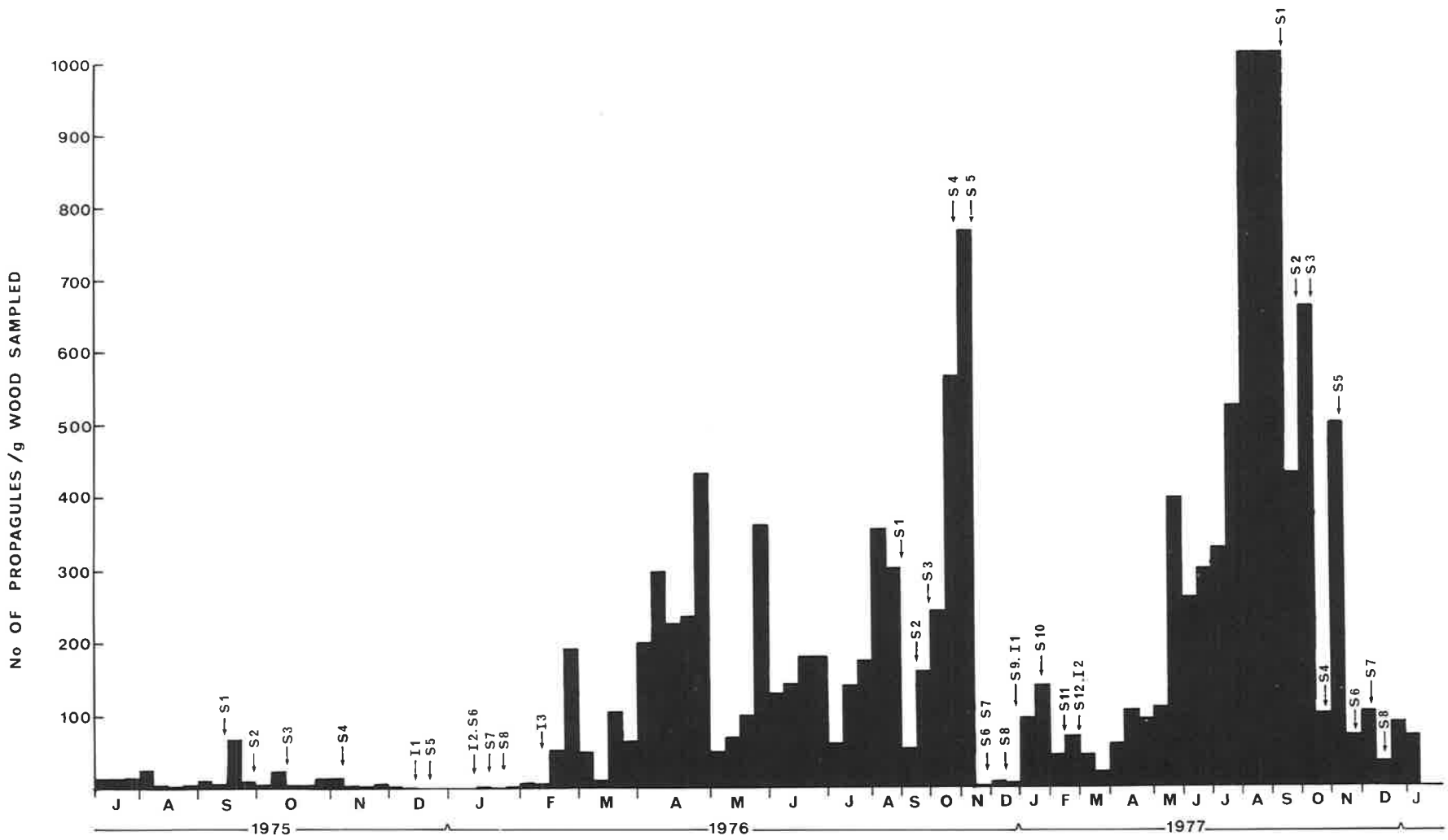

SPORE COUNT
RAINFALL
2438
1587
No OF SPORES /g WOOD SAMPLED
TOTAL MONTHLY RAINFALL (mm)
MONTHS
1975
1976
1977
1978

FIGURE 16

Mean number of viable *F. lateritium* propagules per g wet weight of material sampled at each sampling over the period July 1975 to January 1978.

The legend to the spraying programme (indicated by the letter S in the figure) is given overleaf in Table 17. Note that the sprays are positioned on the graph in respect to the time of sampling and not strictly on a date-of-spraying basis.

The letter I indicates where an irrigation was applied.


No OF PROPAGULES /g WOOD SAMPLED
1000
900
800
700
600
500
400
300
200
100
J A S O N D J F M A M J J A S O N D J F M A M J J A S O N D J
1975
1976
1977
S1
S2
S3
S4
I1
S5
I2. S6
S7
S8
I3
S1
S2
S3
S4
S5
S6 S7
S8
S9. I1
S10
S11
S12. I2
S1
S2
S3
S4
S5
S6
S7
S8

washings (100 ml) by the wet weight (g) of the sample taken.

Fewer propagules of *F. lateritium* were isolated in 1976 than on comparable dates in 1977. The overall pattern was much the same in both years; a low count over summer with an increase in autumn and winter reaching a maximum level in late winter-spring, October in 1976 and August in 1977. The counts then dropped off to a low level in December.

Trends in this histogram were influenced to some extent by the orchard spraying programme. Ideally, one would like to have data on the population of *F. lateritium* in an unsprayed orchard in the same district with which to compare these results.

Figure 16 gives the results in detail, that is, the mean number of viable *F. lateritium* propagules per g wet weight of material sampled at each sampling over the period July 1975 to January 1978. In addition to the spore count, this figure also indicates the time of application of fungicidal and other sprays and of irrigations in respect to the date of sampling.

Rainfall (see Appendix III) alone does not account for the variation observed in weekly counts. No significant correlations were found between spore count and rain that fell in twenty-four hour intervals up to seven days prior to sampling (see Appendix V). It would appear from this that more than one factor is probably influencing spore count at any one sampling.

On several occasions during the growing season fungicidal sprays applied to the trees near the sampling date appear to have had a direct influence on the spore count. As can be seen from the list given in Table 17 the spray programme varied from year to year depending on seasonal conditions. November 1975 to January 1976 was relatively dry (see Appendix III) and fewer sprays were applied than in the same period of 1976-7. Details of the sprays referred to in Table 17 are given in Appendix IV.

In the 1975-6 growing season, few sprays were applied within one to three days of a sample being taken. Of those that were, Melprex (S4 and S6) tended to keep the *F. lateritium* population low. The effect of Melprex seems to vary in this and in other years. Melprex and Topfoliar (S1) was followed by an increase in the *F. lateritium* count. This could reflect the presence of the nutrient spray, Topfoliar. Co-thion and Captafol (S5) on December 24 1975 reduced an already low count to zero. It is worth noting that very little rain was recorded in the ensuing two to three weeks (see Appendix III) during which time *F. lateritium* was not seen on any isolation plates.

A large decrease in the *F. lateritium* count followed the application of Dithane (S1) and Captafol (S5) within two to five days of sampling in the 1976-7 growing season. Dithane (S6, S7) was probably responsible for maintaining the population of *F. lateritium* at a low level following the effect of S5. The effect of Melprex was again variable, for example, a decrease followed S10 and S12, but an increase followed S8.

In the 1977-8 growing season, Nimrod (S3, S5) applied within three days of sampling

resulted in a considerable decrease in the *F. lateritium* count. As in previous seasons, the application of Dithane (S1) was followed by a decrease in the count. Melprex was not applied within five days of a sampling in either of S4 or S7, so its actual effect on *F. lateritium* may not have been recorded here. A slight increase was noted following the application of Melprex with the nutrient spray, Topfoliar (S4).

On two occasions an irrigation applied to the trees near a sampling date appears to have had a direct influence on the spore count. An irrigation consisted of 85 mm of water applied to trees by means of overhead sprinklers during an overnight irrigation period of twelve hours. In the 1975-6 season, a large increase followed I3. Spore count increased for the two weeks following this irrigation. In this same period, 10.2.1976 to 26.2.1976, 66 mm of rain were recorded (see Appendix III), and this also may have affected the spore count.

In 1976-7, an increase in spore count had commenced prior to I1, and continued to rise until Melprex (S10) was applied. The count declined after Melprex (S12) and only started to rise again after I2.

TABLE 17

Legend to Figure 16: Sampling dates, and the date and details of the spray(s), foliar fertilizers and irrigations applied in the seasons 1975-6, 1976-7 and 1977-8.

A. 1975-6 Growing Season

Sampling dates (at weekly intervals) 11/9, 18/9, 25/9, 2/10, 9/10, 16/10, 23/10, 30/10, 6/11, 13/11, 20/11, 27/11, 4/12, 11/12, 18/12, 26/12, 1/1, 8/1, 15/1, 22/1, 29/1, 5/2, 12/2 and 19/2.

S1	16/9	Melprex, Topfoliar
S2	27/9	Melprex
S3	11/10	Captan, Thiodan
S4	11/11	Melprex
I1	12/12	Irrigation
S5	24/12	Co-thion, Captafol
I2	11/1	Irrigation
S6	14/1	Melprex, $CaNO_3$
S7	19/1	Alar
S8	24/1	$CaNO_3$
I3	10/2	Irrigation

B. 1976-7 Growing Season

Sampling dates (at two-week intervals) 9/9, 23/9, 7/10, 21/10, 4/11, 18/11, 2/12, 16/12, 6/1, 20/1, 3/2, 17/2, 3/3 and 17/3.

S1	6/9	Dithane
S2	27/9	Benlate, Melprex
S3	12/10	Melprex
S4	28/10	Ethrel
S5	12/11	Captafol
S6	23/11	Benlate, Dithane, Phosvel, Topfoliar
S7	27/11	Co-thion, Dithane, Topfoliar
S8	14/12	Melprex
S9	27/12	Melprex, Phosvel, $CaNO_3$
I1	4/1	Irrigation
S10	17/1	Melprex, $CaNO_3$, Alar
S11	18/2	Plictran, Dithane, $CaNO_3$
S12	28/2	Melprex, $CaNO_3$
I2	11/3	Irrigation

C. 1977-8 Growing Season

Sampling dates (at two-week intervals) 15/9, 30/9, 21/10, 3/11, 17/11, 2/12, 16/12 and 29/12.

S1	12/9	Dithane
S2	30/9	Ethrel
S3	18/10	Nimrod
S4	28/10	Melprex, Topfoliar
S5	15/11	Nimrod
S6	23/11	Benlate, Phosvel, Topfoliar
S7	10/12	Melprex
S8	18/12	Saprol

(Details of the common and trade names, and of the active constituents of the sprays referred to above and in Figure 19 are given in Appendix IV).

Toxicity of orchard fungicides to *F. lateritium*

Four of the fungicidal sprays used in the orchard spray programme, Benlate, Dithane M45, Melprex and Difolitan were tested in the laboratory for their relative toxicities to *F. lateritium*. The chemical names and formulations of the fungicides tested are given in Appendix IV.

The sensitivity and resistance of *F. lateritium* isolates to various fungicides used in the orchard were determined by measuring radial growth on agar in the following way. Chemicals were added as a suspension in water to 10 ml lots of molten purified agar (Oxoid). It is important to shake the suspension thoroughly before adding it to the agar and then to stir it evenly through the agar. Plates were allowed to stand for twenty-four hours and each was then inoculated in the centre with a 6 mm diameter disc cut from near the margin of a two-week-old culture of isolate B1 of *F. lateritium*. All tests were at least in duplicate. Radial growth was measured after two to twelve days incubation at 25°C. Radial growth on different concentrations of the fungicide was expressed as an average rate of growth (mm/day) for comparison with the rate on the control without fungicide (Van Tuyl, 1977). Relative toxicity was expressed as ED_{50}, that is the concentration causing 50% reduction in radial growth (American Phytopath. Soc., 1943).

The germination of macroconidia was also examined over the same range of fungicide concentrations. Drops of a spore suspension were placed on the fungicide plates and on purified agar without fungicide as a control. The presence or absence of germination was scored after twenty-four hours at 25°C. The comparative ED_{50} values for the fungicides against mycelium of *F. lateritium* are given in Table 18. The concentration of fungicide (in p.p.m. A.i.) that completely inhibits germination of macroconidia was found to be < 5 for Difolitan and Dithane and 15 - 20 for Melprex. Spores will germinate in concentrations of at least 25 p.p.m. A.i. of Benlate, but germtubes fail to elongate. As seen in Table 19, 3 p.p.m. Benlate is sufficient to inhibit hyphal growth. Some hyphal growth will still occur with Dithane and Melprex at concentrations of 60 p.p.m., whereas with Difolitan hyphal growth ceases at approximately 4 p.p.m. A.i.

In order to see if the response of isolate B1 was typical of the species *F. lateritium*, twenty other isolates were subsequently tested at the concentration of the different fungicides that inhibited the growth of isolate B1. It was found that isolate B1 was typical in respect to sensitivity to Difolitan and Dithane, but was less tolerant to Melprex and Benlate than the other isolates of *F. lateritium* tested. The results showed that 0/20 grew with 4 p.p.m. Difolitan, 3/20 with 60 p.p.m. Dithane and 9/20 with 60 p.p.m. Melprex (average rate of growth was calculated to be 0.03 mm/day). With Benlate at 1.5 p.p.m. all isolates grew. This was reduced to 15/20 at 3 p.p.m. and 6/20 at 5 p.p.m.

TABLE 18

Comparative ED_{50} values for fungicides against *Fusarium lateritium* hyphae.

Fungicide	ED_{50} (p.p.m. active ingredient)
Benlate	0.075
Difolitan	1.5
Dithane M45	5.0
Melprex	10.0

TABLE 19

Rate of growth (mm/day) of *F. lateritium* on purified agar containing different concentrations of fungicides.

p.p.m.	Dithane M45	Melprex	p.p.m.	Benlate	p.p.m.	Difolitan
0	3.4	3.4	0	3.4	0	3.4
2.5	3.2	3.2	0.05	3.2	2	1.4
5	1.5	2.4	0.1	1.4	3	0.4
10	1.35	1.7	0.5	1.4	5	0
15	1.25	1.4	1.0	0.3	7.5	0
20	1.25	1.4	1.5	0.25	10	0
25	1.15	1.2	2.0	0.1		
30	0.7	1.0	3.0	0		
40	0.5	0.8	4.0	0		
50	0.4	0.7	5.0	0		
60	0.2	0.45				
70	0	0				

Discussion

The seasonal pattern of the population of *F. lateritium* found in this study under South Australian conditions is not unlike that found by Swinburne (1973) in England. Although he only looked at the period November to March in two different years, he found that *F. lateritium* propagules isolated from leaf scars increased from leaf fall onwards in both seasons. This period of increase corresponds to the increase observed in this study from autumn through to late spring.

When doing their work on *Nectria galligena,* Swinburne, Flack & Brown (1975) found that no information was available on what effect, if any, fungicides used in commercial orchard practice might have on apple leaf scar microflora. They looked at the effect in the field of fungicides on eight fungi commonly isolated from leaf scars on apple trees. They found that neither Melprex nor Benlate had much effect on the population of *F. lateritium.* Their study differed from the present one in that there was a considerable time lapse between the application of the fungicides and the taking of samples. Hislop & Cox (1969) reported a considerable reduction in the microflora of apple leaves following the application of Captan shortly before sampling.

The laboratory tests of the toxicity to *F. lateritium* tend to elucidate the effects of the fungicides on the population of this fungus in the orchard. The high toxicity of Difolitan to both spores and hyphal growth may well explain the decreases in the count of *F. lateritium* propagules following the application of Difolitan in December 1975 and November 1976. The decrease in the count following the applications of Dithane in 1976-7 and 1977-8 seasons is probably due to the low concentration of this fungicide needed to inhibit germination of *F. lateritium* spores. Similarly, the higher concentration of Melprex required to inhibit the germination of *F. lateritium* spores may explain the variable effects of this fungicide in the orchard. If on any occasion the amount delivered to any one site is not sufficient to prevent germination, growth would proceed.

Protection of cherry sapwood by *Fusarium lateritium* against invasion by *Chondrostereum purpureum* in the field.

The aim of this field experiment was to evaluate the protection afforded by immediate post-pruning application of *F. lateritium,* which has been found in the present study to inhibit the growth of *C. purpureum in vitro* by the production of an antibiotic substance, against inocula of *C. purpureum* introduced on to pruned cherry sapwood soon after pruning.*

Materials and Methods

Branches on cherry trees cv. Waterloo**, of diameter 1.0 - 1.5 cm, were labelled and pruned

* This experiment was previously unsuccessfully attempted using apple trees (see Appendix VI).

** Waterloo is a light cherry, the correct cultivar name of which is Florence (Grubb, 1949, p.109).

FIGURE 17

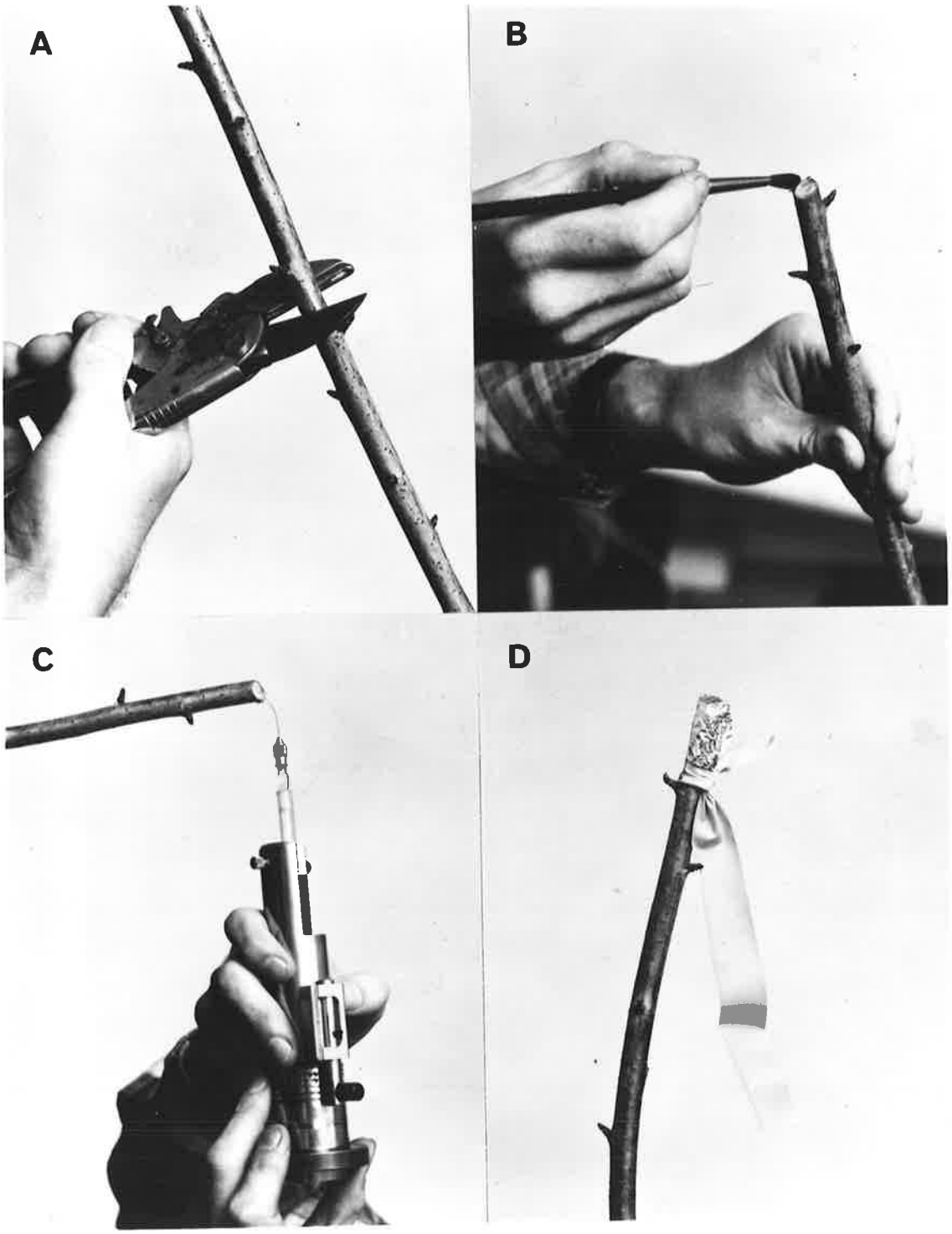
Inoculation of pruned cherry sapwood

A. Limb cut just above a dormant leaf bud.

B. Suspension of *F. lateritium* macroconidia applied with a brush.

C. Measured dose of *C. purpureum* basidiospores applied with a Burkard micro-applicator.

D. Site covered with sterile aluminium foil and labelled with coloured tape.


A
B
C
D

above a dormant bud on September 3 1976. A total of 25 sites were inoculated per treatment and the treatments were assigned at random to the sites. Details of the treatments are given in Table 20. In treatments 4 to 9 inclusive, the pruned surface was inoculated immediately by brushing on sufficient of a suspension of *F. lateritium* macroconidia containing 10^4 spores per ml to saturate the cut surface. The controls were left uninoculated. Control 3 was inoculated on Day 0, treatments 4 and 7 on Day 1, treatments 5 and 8 on Day 3 and treatments 6 and 9 on Day 5 with five 2 μl drops of a suspension of *C. purpureum* basidiospores containing 1 x 10^4 spores per ml, that is, 100 basidiospores per pruned site, using a Burkard microapplicator. *C. purpureum* was not applied to Controls 1 and 2. Inoculated sites in Controls 2 and 3 and treatments 4, 5 and 6 were covered with sterile aluminium foil for 14 days following inoculation. The inoculation procedures are illustrated in Figure 17.

The branches were harvested 16 weeks later (January 1977) and the presence of the pathogen was assessed visually using foliar silvering symptoms and by culturing tissue from the discoloured portion of the wood. All wood chips were cultured on two different agars: GP/20 agar to which had been added 100 p.p.m. streptomycin and 250 p.p.m. guaiacol and Malt agar to which had been added 100 p.p.m. streptomycin. *F. lateritium* was found to be more readily identified on Malt agar than on GP/20. Plates were incubated at 20°C and the presence of the two fungi assessed after about 5 days (Figure 18). If any uncertainty existed about the identity of the fungi reisolated, transfers were made to fresh agar plates, and a reappraisal made.

Results and Discussion

The details of the reisolation of *F. lateritium* and *C. purpureum* from each of the treatments are given in Table 20. In the covered sites, *F. lateritium* was consistently reisolated from 90 - 100% of sites. *C. purpureum* was reisolated from 64, 60 and 40% of sites (treatments 4, 5 and 6 respectively). Where only *C. purpureum* was applied (Control 3), 100% infection resulted. In the uncovered sites, *F. lateritium* successfully colonized between 12 and 36% of the sites inoculated, and *C. purpureum* was reisolated from 88, 80 and 68% of sites (treatments 7, 8 and 9 respectively).

The results of this field experiment indicate that *F. lateritium* would not be particularly effective as a biological control agent for *C. purpureum*. In those sites not covered with aluminium foil, the reduction in the number of sites invaded by *C. purpureum* was not great. A maximum reduction in the number of sites invaded by *C. purpureum* of 32% was recorded where *F. lateritium* was applied 5 days before the arrival of basidiospores of the pathogen. Carter & Price (1974) found that a higher level of protection was obtained with larger dosages of macroconidia when the pathogen, *Eutypa armeniacae,* was introduced by inoculation one day later. This suggests that the success of this form of protection may depend very much upon the proportion of wood vessels occupied by *F. lateritium* at the time the spores of the pathogen arrive. Consequently, a larger dosage of macroconidia may be more effective in preventing the establishment of *C. purpureum* than that tested in this experiment. However,

FIGURE 18

Reisolation of *C. purpureum* and *F. lateritium* from cherry wood.

a. *F. lateritium* on Malt agar (photographed on a black background).

b. *C. purpureum* on GP/20 agar plus 250 p.p.m. guaiacol.

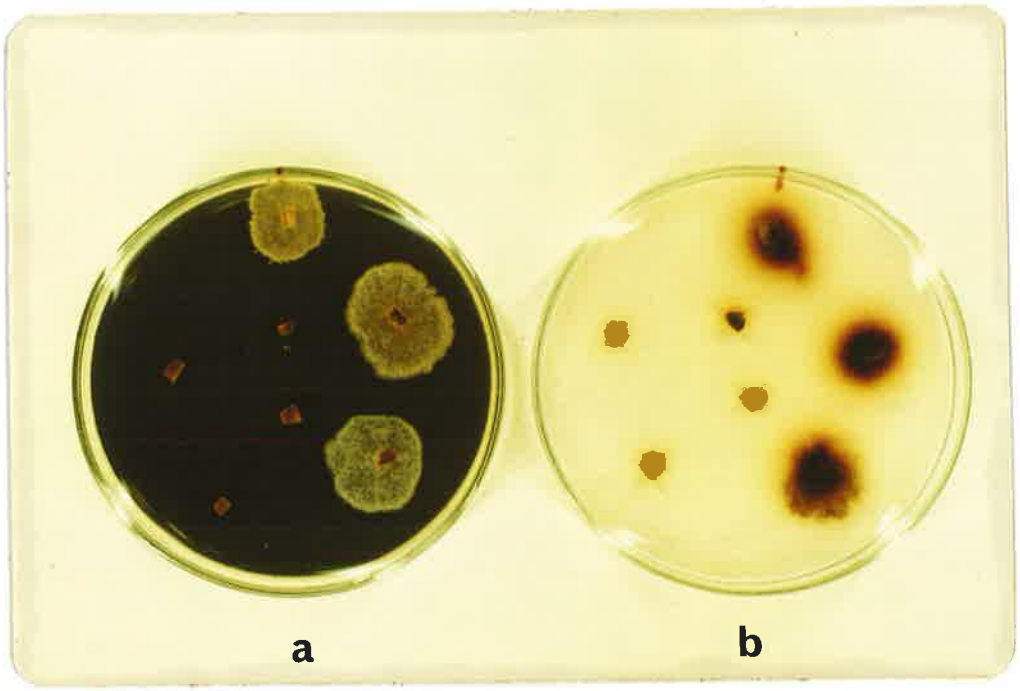

a
b

TABLE 20

Treatments applied and number of sites on pruned cherry sapwood yielding *F. lateritium* and/or *C. purpureum* 4 months after inoculation

Treatment No.	Treatment: *F. lateritium* applied on day	Treatment: *C. purpureum* applied on day	Treatment: Covered with foil	Number of sites yielding *F. lateritium* or *C. purpureum* at harvest (total 25): *F. lateritium*	Number of sites yielding *F. lateritium* or *C. purpureum* at harvest (total 25): *C. purpureum*
Control 1	-*	-	-	2	5
Control 2	-	-	+	0	1
Control 3	-	0	+	0	25
4	0	1	+	23	16
5	0	3	+	25	15
6	0	5	+	25	10
7	0	1	-	9	22
8	0	3	-	3	20
9	0	5	-	3	17

*not applied

Carter & Price (1974) suggested that if every vessel exposed at pruning is a potential infection court for the pathogen, very large numbers of macroconidia would be required at each pruned surface to make the entry of a basidiospore into a vessel not occupied by *F. lateritium* highly unlikely.

The results also show that *in vitro* tests, as were conducted prior to field evaluation of *F. lateritium*, need not indicate the type of effect that occurs in the field. Just because *F. lateritium* produces an antibiotic substance that inhibits the growth of *C. purpureum in vitro*, it does not necessarily mean that *F. lateritium* will produce this substance when it grows in cherry sapwood, or if it is produced, that it will have the same effect as it does in culture. Carter & Ilsley (unpublished results) have recently demonstrated that although *F. lateritium* is effective in preventing the establishment of *Eutypa armeniacae* in pruned apricot sapwood, the purified antibiotic (enniatin) obtained from *F. lateritium* is not effective in preventing the invasion of wounds on apricot trees by *E. armeniacae.*

B. Selection of a biological control agent for *Chondrostereum purpureum*

Introduction

D'Aeth (1939) found that the type of interaction occurring between two micro-organisms on artificial media may be quite different from that occurring between them in nature. Such a situation has been found in the present study. *Fusarium lateritium* effectively inhibited the growth of *C. purpureum in vitro* but failed in this respect in the field. This leads one to the problem of how best to screen micro-organisms as potential biological control agents for a particular pathogen. Broadbent, Baker & Waterworth (1971) screened 3,500 isolates as potential antagonists to fungal root pathogens and found that 40% inhibited 1 or more of 9 pathogens being tested on agar, but only 4% of these were effective in the field. They also suggested that an even lower percentage might be effective under commercial conditions.

The control of *C. purpureum* would involve what Cook (1977) termed the protection of an infection court. It would require an organism to take early possession of the infection court (that is, the pruning wound) and adjacent tissues, cause little or no disease, and prevent a more potent pathogen from getting into the tissues. This is the principle involved in the control of *Eutypa armeniacae* by *F. lateritium* (Carter & Price, 1974), the control of *Fomes annosus* by *Peniophora gigantea* (Rishbeth, 1975) and the control of *Poria carbonica* by *Scytalidium* sp. (Ricard & Bollen, 1968).

Carter (1971) listed four properties that a micro-organism would have to possess to compete with *E. armeniacae*. These would be equally applicable to the case of *C. purpureum*. These properties are as follows:

(a) ability to produce abundant propagules capable of being deposited within the vessels exposed at pruning wounds,

(b) ability to germinate and grow rapidly at temperatures $\leqslant 10^{o}C$,

(c) ability to rapidly colonize the zone of dying cells below a wound, but be non-pathogenic to the host, and

(d) ability to prevent the establishment of mycelia of *C. purpureum* from basidiospores that arrive simultaneously, or later, at the same site. This would require that the control agent should produce metabolites toxic to the pathogen or it should exhaust the available nutrients faster and thereby eliminate the mycelium of the fungus.

An added requirement in the case of *C. purpureum* is that the antagonistic micro-organism should be equally efficient at growing on pruned sapwood of a number of hosts belonging to different genera in the family Rosaceae.

As a preliminary test for selecting fungi for possible use against *C. purpureum*, 20 different fungi isolated in the main from pruning wounds and on the bark of cherry and apple trees were grown *in vitro* with *C. purpureum* and after four weeks an attempt was made to reisolate *C. purpureum* from these plates. From these tests, the fungi listed below were selected for further study.

The fungi that were looked at in further detail were:

(a) two isolates of a red *Fusarium*, one isolated from the surface of apple tree bark (isolate F 92) and the other from a week-old pruned surface on a cherry tree (isolate F 73. 1B). These fungi have tentatively been identified as *Fusarium graminearum* Schwabe. This species has not been recorded as a pathogen on any member of the Rosaceae (Booth, 1971).

(b) two different species of *Trichoderma*, one received from Dr. J.H. Warcup, Department of Plant Pathology, Waite Agricultural Research Institute (isolate T311) and another isolated from a pruned surface on a cherry tree (isolate T9).

Trichodermas have been under investigation as potential biological control agents for several pathogens for a number of years. Only recently has the genus *Trichoderma* been subjected to a monographic treatment which has made possible a reliable determination of species groups (Domsch & Gams, 1970). However, this is not to say that any one species that has been used in several different biological control studies is necessarily exactly the same fungus in all cases. A case in point here could well be *Trichoderma viride* Pers. ex Fr. According to Dingley (1957) the species *T. viride* represents the conidial form of at least 9 different *Hypocrea* species. *T. viride* has been investigated as a potential antagonist for *C. purpureum* (Grosclaude, 1973a; Dubos & Ricard, 1974; Corke, 1974). Grosclaude, Dubos & Ricard (1974) found that in large quantities ungerminated spores of *T. viride* would inhibit the growth of hyphae of *C. purpureum*. *T. harzianum (= T. viride)* successfully protects wounds on *Acer rubrum* from invasion by wound-invading Hymenomycetes, even though it is not a natural pioneer of fresh wounds on *A. rubrum* (Pettle, Shigo & Blanchard, 1977). The reverse of this situation was found to occur with *Trichoderma pseudokoningii* which was isolated from apples with dry eye rot disease caused by *Botrytis cinerea*. *T. pseudokoningii* will completely suppress the pathogen in apples under laboratory conditions but is not particularly effective in the orchard (Tronsmo & Raa, 1977). Thus, there is no assurance that a micro-organism which was selected from the microflora of the infection court and inhibited the growth of a particular pathogen in culture, will necessarily be as effective against that pathogen in the field.

Sporulation of *Fusarium* isolates

In order to be able to test the effectiveness of isolates F92 and F73.1B in controlling *C. purpureum* it was necessary to induce them to sporulate abundantly *in vitro*. Various artificial media and a variety of light conditions were tested as suggested in Booth (1977) and Tousson & Nelson (1968).

The media tested included Coon's (Rawlins, 1933; Tschanz, Horst & Nelson, 1975), Nash (Nash & Snyder, 1962), Sporulation Medium for *Fusarium sambucinum* (Audhya & Russell, 1974), Potato Sucrose (Booth, 1971), brown rice grains (Tousson & Nelson, 1968) and various dilutions of NDY (1/20, 1/6) and Glucose Peptone (full strength, 1/20). Booth (1971) suggested that the use of a medium containing neutral phosphate and starch (instead of glucose) can increase sporulation in Fusaria. Consequently, Brown's medium (Mayo, 1925) and a modified form of Brown's containing a larger amount of neutral phosphate and no glucose (see Appendix II) were tested along with those listed above under continuous white light (Philips TLF 40W/33RS) at 25°C.

Limited sporulation of both isolates occurred with plates of NDY/6 and Audhya & Russell's sporulation medium at fourteen days after inoculation.

Near-ultraviolet Black Light (320-420mm) has been found to stimulate sporulation in a number of fungi including *Fusarium nivale* (Leach, 1962, 1967). Black Light was tried with isolate F92 on the media listed above. Sporulation was not observed on any of the media tested over a period of fourteen days.

Sterols have been shown to increase the production of conidia in a number of fungi, (Hendrix, 1970). Baniecki & Bloss (1969) found that ergosterol and other steroids, with light from cool white or blue fluorescent lamps, enhances the production and viability of conidia of *Phymatotrichum omnivorum*.

Isolates F92 and F73.1B were grown on agar plates to which had been added 0.1 g of a sterol per litre of agar. The media used were those that had given some sporulation previously, namely NDY/6 and sporulation medium. The steroids tested were ergosterol, β - sitosterol and β - stigmasterol. Sterols were added to the culture media by dissolving 0.0lg of the crystalline steroid on 0.5 ml of hot Tween 20 and this was carefully stirred into 100 ml of the molten agar. The plates were inoculated at the centre with isolates F92 or F73.1B and placed 40 cm beneath four fluorescent lamps (Philips TLF 40W/33RS) at 25°C. Plates were also prepared containing only the basal medium and the basal medium plus Tween 20. A duplicate set of plates was incubated at 25°C in the dark.

Abundant sporulation occurred with isolate F92 on NDY/6 plus ergosterol under white light within four days. Sparse sporulation occurred with NDY/6 plus sitosterol. With both of these steroids, sporulation was greater with isolate F92 than with F.73.1B. Virtually no sporu-

lation occurred in any of the plates stored in the dark. Tween 20 by itself in NDY/6 did not induce sporulation.

Once F92 had been induced to sporulate *in vitro*, it produced macroconidia readily on just NDY/6 agar when grown under white light. Spores from cultures on NDY/6 plus ergosterol and from the transfer from this to NDY/6 were found to have a germination rate of 90 - 100%.

Crossplating tests with the selected fungi

The four fungi selected for further study as potential control agents for *Chondrostereum purpureum* were subjected to further crossplating tests using a larger number of replicates.

Plates of GP/20, Cherry Wood and Malt agars were each inoculated with a disc of *C. purpureum* (isolate CPA 8 from apple cv. Jonathon) and one of the test fungi, *Fusarium* isolates F92, F73.1B and *Trichoderma* isolates T311, T9, placed on the opposite side of the plate. Plates were incubated for six weeks at 15°C, a figure representative of air temperatures in the field over the winter-spring period, by which time the fungi had met and grown over each other. The media selected were; one that was high (Malt) and one low (GP/20) in nutrients, and an agar prepared from cherry wood sawdust (see Appendix II). Control plates were inoculated with each fungus alone.

Reisolation of the fungi on each plate was attempted after six weeks. Material from different positions on each plate was plated on Malt agar and GP/20 plus 200 p.p.m. guaiacol agar and incubated at 25°C. On Malt agar the *Trichoderma* isolates grow and sporulate readily, and the *Fusarium* isolates form distinctively coloured colonies. The GP/20 agar plus guaiacol was used to detect the presence of *C. purpureum.*

No inhibition zones were formed between *C. purpureum* and the test fungi on any of the agars used. *C. purpureum* was not reisolated from any of the plates which had been inoculated with either species of *Trichoderma. C. purpureum* was reisolated from 2/5 plates with F92 grown on Cherry Wood agar and 1/5 on GP/20 agar. With F73.1B, *C. purpureum* was reisolated on 2/5 plates of both Cherry Wood and GP/20 agars and 1/5 on Malt agar.

Growth of *C. purpureum* isolates and test fungi in cherry wood blocks

The rate of growth of the test fungi in cherry wood blocks was investigated. Ideally a control agent should be able to germinate and grow on and in the wood beneath a freshly pruned sapwood surface more rapidly than *C. purpureum.* These aspects were investigated in the following experiment.

The average rate of growth of five isolates of *C. purpureum*, CPA8 (from apple cv. Jonathon), CPN1 (from nectarine), CPC3 (from cherry cv. Waterloo), CPW1 (Table 9) and CPB1 (Table 9), and that of *Fusarium* isolates F92 and F73.1B in cherry wood blocks were tested using the method of Rishbeth (1976). For this the inoculum applied to the pruned surface was in the form of wood discs inoculated with the fungus. This was later repeated using a spore suspension as the inoculum as this more closely parallels the situation in the field.

Cherry branches 15 mm in diameter were cut into 5 cm lengths. The bark was scrubbed with a brush and the blocks rinsed in distilled water. They were then surface-sterilized by soaking in 'Milton' solution for 30 minutes. Excess hypochlorite was removed by rinsing the blocks in sterile distilled water. They were then placed in sterile wide-necked McCartney bottles, labelled and spore suspension applied. If wood discs overgrown with fungus were being used, the wood blocks were buried in moist sterile sand in 300 ml beakers (Rishbeth, 1976). Wood blocks were incubated at 15°C and reisolations attempted 4, 9, 12 and 16 days after inoculation using wood discs and after 7, 14, 28 and 35 days where a spore suspension was used.

When using spores as inoculum, macroconidia of Fusaria were washed off a culture on an NDY/6 agar slope giving a suspension of concentration 0.35 x 10^6 per ml. Spores were applied using a High Peak No. 2 brush which delivered approximately 1200 - 1400 spores to the pruned surface. Basidiospores of *C. purpureum* (isolate CPC15 from cherry cv. Waterloo) were applied with a High Peak No.0 brush which delivered approximately 250 - 300 viable spores to the pruned surface. The viability of the suspension of basidiospores was 50% on GP/20 agar after 48 hr at 20°C.

The media used for the reisolations were as in the Crossplating tests above; Malt agar for the Fusaria and GP/20 agar plus 200 p.p.m. guaiacol for *C. purpureum*. The blocks were stripped of their bark and cut into 5 mm wide cross-sections with a pair of secateurs and the sections placed on the agar media. Plates were incubated at 25°C for four to seven days after which the presence of the various fungi was assessed. Attempts at estimating the rate of growth of the *Trichoderma* isolates were abandoned because it was not possible to reisolate the fungus from wood at different depths beneath the inoculated surface without interference from contamination. *Trichoderma* sporulates very readily on the pruned surface and on bark.

The results are given in Table 21. The *Fusarium* isolates grew faster than *C. purpureum* (isolate CPC15) when wood blocks were inoculated with spores, 1.10 and 0.95 mm/day compared with 0.73 mm/day for *C. purpureum*. The rate of growth from basidiospores was similar to that found by Rishbeth (1976) for growth of *C. purpureum* in poplar wood inoculated with basidiospores. It was found that the Fusaria caused no visible internal discolouration of the wood with either type of inoculum, which is in direct contrast to *C. purpureum*.

TABLE 21

Growth of *C. purpureum* isolates and test fungi in lengths of cherry wood after inoculation with spores or wood discs inoculated with fungus.

Isolate		Method of Inoculation	Growth Rate * (mm/day)
Fusarium	F92	Wood discs	1.7
		spores	1.1
Fusarium	F73.1B	Wood discs	1.8
		spores	0.95
C. purpureum isolate	CPC3	Wood discs	3.0
	CPN1	"	3.3
	CPA8	"	2.6
	CPW1	"	2.3
	CPB1	"	4.3
	CPC15	spores	0.73

* average rate of growth over a period of 16 days where inoculated with wood discs and 35 days where inoculated with spores.

The rates of growth of the five isolates of *C. purpureum* from wood disc inocula ranged from 2.3 to 4.2 mm/day. In poplar wood the rates of growth of the isolates from cherry and apple (the only ones tested), 10.0 and 6.0 mm/day respectively, were considerably greater than in cherry wood, but were similar to those found by Rishbeth (1976) in beech and birch wood. The rate of growth in cherry wood was similar to that found by Bennett (1968) for *C. purpureum* in plum wood.

The time taken for spores of the Fusaria to germinate on the surface of the wood blocks was not investigated, but on plates of distilled water agar, they germinated within twelve hours and made good growth by 48 hours by which time *C. purpureum* had progressed very little. A similar situation was found to exist with the two isolates of *Trichoderma*.

Protection of charry sapwood *in vitro*

This experiment was to evaluate *in vitro* the protection afforded by the application of spores of a test fungus, applied in suspension, against inoculum of *C. purpureum* introduced on cherry sapwood soon after the application of the other fungus. The conditions of this experiment are probably an exaggeration of the situation in the orchard. Provided fruit trees are pruned when weather conditions are not favourable for the release of basidiospores, it is unlikely that spores of the pathogen would be deposited on a pruned surface so soon after the application of a control agent.

The fungi tested against *C. purpureum* on cherry wood blocks were *Fusarium* isolate F92 and the *Trichoderma* isolates T311 and T9. Isolate F92 was used in preference to isolate F73.1B because although they grew in wood blocks at much the same rate, F92 sporulated more readily on agar media, a consideration which would be important if it were to be used in a control programme.

The wood blocks were prepared as previously (p. 90) and placed in sterile wide-necked McCartney bottles. The treatments were as follows:

1.	Control-uninoculated	6 blocks
2 - 4.	F92, T311 or T9 only	6 blocks each
5.	*C. purpureum* only	10 blocks
6.	*C. purpureum* and F92	10 blocks
7.	*C. purpureum* and T311	10 blocks
8.	*C. purpureum* and T9	10 blocks

Suspensions of spores of F92, T311 and T9 were made from week old cultures growing on NDY/6 agar slopes and the concentrations of all three adjusted to 1.0×10^6/ml. The spores

TABLE 22

Treatments applied and number of wood blocks from which *C. purpureum* and test fungi were reisolated.

	Treatment	Fungi Reisolated				Total number of blocks
		C. purpureum	F92	T9	T311	
1.	Uninoculated	- *	-	-	-	6
2.	*C. purpureum*	10 **	-	-	-	10
3.	*Fusarium* F92	-	6	-	-	6
4.	*C. purpureum* + F92	1	10	-	-	10
5.	*Trichoderma* T9	-	-	6	-	6
6.	*C. purpureum* + T9	0	-	10	-	10
7.	*Trichoderma* T311	-	-	-	6	6
8.	*C. purpureum* + T311	0	-	-	10	10

* not applied

** number of blocks from which fungus reisolated

were applied using a High Peak No. 2 brush, which would deliver approximately 30,000 to 40,000 spores per block. Within five minutes of applying the test fungus, 100 viable basidiospores of *C. purpureum* (isolate CPC15) were applied with a burkard microapplicator. The spores used were from a sporophore from cherry and had been stored at 2°C for ten weeks. The viability of the spores was approximately 65% after 48 hours at 20°C on GP/20 agar.

The wood blocks were incubated at 15°C, a figure representative of temperatures over the winter-spring period in the field. Half of the blocks were sampled after two weeks and the remainder after three weeks. The blocks were stripped of their bark, cut into cross-sections with a pair of secateurs and the sections placed on agar media; Malt agar and GP/20 agar to which had been added 200 p.p.m. guaiacol. Plates were incubated at 25°C for 5 - 10 days after which time the presence of the various fungi was assessed.

The results are given in Table 22. *C. purpureum* was reisolated from all of those wood blocks where it was applied by itself. Where applied along with one of the test fungi, *C. purpureum* was not reisolated from any of the blocks inoculated with either of the two *Trichoderma* spp. and from only one block inoculated with *Fusarium* isolate F92.

The conditions under which this test was conducted reasonably represented those in the field. That is to say, spores of the test fungi were placed on freshly pruned surfaces immediately after pruning and before the arrival of the pathogen. The dosage of *C. purpureum* spores applied was probably in excess of what would be encountered naturally and their arrival so soon after pruning would probably be a rare occurrence in the field. Despite these constraints, the three test fungi appeared to stop *C. purpureum* from becoming established in cherry wood blocks. They were probably effective because all three germinate and start growing well in advance of *C. purpureum,* and therefore put the pathogen at a disadvantage with regard to nutrients that were available on the pruned surface. Both of the *Trichoderma* isolates sporulated readily on the pruned surface and on the bark within 7 - 10 days of being applied.

VI GENERAL DISCUSSION

In this study *Chondrostereum purpureum* has been found to occur on a number of plants that were not formerly recognised as hosts in South Australia. It was also found that basidiospores of *C. purpureum* from sporophores on four different host plants, cherry, apple, willow and a broom, were equally effective in invading pruned cherry sapwood. Although only one of the newly identified hosts (the broom *Teline monspessulana)* was included in the experiment, the results suggest that most, if not all, hosts of *C. purpureum* should be regarded as potential sources of inoculum for infection of fruit trees.

The results from this investigation differed from those of work done in England by Bennett (1962a). She found variation in pathogenicity of different isolates of *C. purpureum* to Victoria plum trees. Isolates from poplar and sycamore were pathogenic to Victoria plum but, in terms of the volume of wood colonized and intensity of the foliar symptoms induced, they were less virulent than an isolate from plum. She considered that foliar symptoms increased in intensity with the volume of wood colonized by the pathogen. The present results do not support this hypothesis.

The isolates from willow, apple and cherry did not differ significantly in their ability to cause silvering of trees, but the willow isolate had on the average colonized less wood than the other two isolates. In addition, the isolates from broom, cherry and apple did not differ significantly in the amount of wood colonized, whereas the isolate from broom gave no silvering, silvering was apparent with the other two isolates. It was subsequently found that the isolate from broom produced *in vitro* relatively little phytolysin, the toxin which is responsible for the silvering of the foliage (Naef-Roth, Kern & Toth, 1963). Thus, the amount of wood invaded by an isolate of *C. purpureum* need not be an accurate indication of the amount and intensity of the silvering of foliage that can be expected. The amount of silvering depends upon the quantity of phytolysin produced by an isolate, and from the results of the experiment on phytolysin production this varies considerably between isolates.

It was not ascertained in the experiment with the different isolates of *C. purpureum* whether or not invasion by the different isolates would ultimately lead to the death of the tree. An experiment of that kind would be of many years' duration and was therefore outside the scope of the present study. In fact, most experimentation involving diseases of perennial crops such as stone and pome fruit trees are of long duration because many aspects of diseases can only be studied in the field over one period every 12 months. For example, the constraints on an

investigation of the invasion of pruning wounds by *C. purpureum* in the field are those of pruning time and how soon after inoculation can *C. purpureum* be successfully recovered from the wood. Since fruit trees are pruned in winter when they are dormant, and it is approximately 6 months before *C. purpureum* has grown sufficiently to be detected either by visual appraisal or by reisolation from the wood, such an experiment can be attempted only once every 12 months.

Biological control of *C. purpureum* would require a nonpathogenic micro-organism to take early possession of the pruning wound and adjacent tissues and thus prevent the pathogen from invading the branch. In this study, *Fusarium lateritium*, which has been successfully used to control another vascular pathogen, *Eutypa armeniacae* (Carter & Price, 1974, 1975), was investigated as a potential biological control agent for *C. purpureum.*

Carter (1971) suggested four properties that a micro-organism should posses to be able to compete with a pathogen that invades pruning wounds. *F. lateritium* was found to fulfil three of these requirements. It sporulated readily in culture thus providing an ample supply of propagules that could be delivered on to the pruned surface. It germinated and grew rapidly *in vitro* at temperatures $< 10^{o}C$, and thirdly, it was able to colonize the zone of dying cells beneath a wound and to sporulate on the surface of the wound. *F. lateritium* was found however, not to be particularly effective in the field in preventing the establishment of mycelia of *C. purpureum* from basidiospores that arrived soon after macroconidia of *F. lateritium* had been applied to a pruning wound. The fact that *F. lateritium* was also found to occur in an apple orchard where natural infection by *C. purpureum* is relatively high is perhaps further indirect evidence that *F. lateritium* is not a particula ly effective organism for the biological control of *C. purpureum.*

The basis on which *F. lateritium* was first selected for investigation as a potential control agent for *Eutypa armeniacae* was that it inhibited the growth of *E. armeniacae in vitro* (Carter & Price, 1974). The proposition that *F. lateritium* might be expected to act similarly against other pathogens that invade pruning wounds was subsequently confirmed when *F. lateritium* was grown in culture with *C. purpureum.* The antibiotic that was responsible for inhibiting the growth of *E. armeniacae* and *C. purpureum* in agar culture was subsequently identified, but when tested in the field it failed to prevent the establishment of *E. armeniacae* (Carter & Ilsley, unpublished results) whereas the fungus itself did prevent *E. armeniacae* from becoming established in fresh pruning wounds (Carter & Price, 1974, 1975). As to why *F. lateritium* is effective against *E. armeniacae* but not against *C. purpureum* in the field is not known. Whatever the factors are that operate in this biological control of *E. armeniacae,* they do not appear to operate anywhere near as effectively with *C. purpureum.* One possible reason for this difference was proven unlikely when it was found that *F. lateritium* grows and sporulates just as readily on cherry sapwood as on apricot.

The use of *in vitro* inhibition of growth as the criterion for selecting control agents was questioned by Huber & Watson (1966). Data thus obtained are frequently extrapolated to conditions in the field, yet few positive results have been obtained by using such antagonists in the field (Huber & Watson, 1966; Broadbent, Baker & Waterworth, 1971). One notable exception to this has been the control of crown gall (caused by *Agrobacterium radiobacter* var. *tumefaciens*) by a closely related non-pathogenic bacterium, *A. radiobacter* var. *radiobacter*. The control achieved both *in vitro* and in the field is due to the production of a bacteriocin by the controlling organism (Kerr & Htay, 1974).

After the experience with *F. lateritium*, other methods of screening potential biological control agents in the laboratory were investigated. If a micro-organism showed promise in these tests, it would then be desirable to extend the study to the field.

Several fungi were investigated by observing the effect that they had on *C. purpureum* when grown together on agar media. Three fungi , a *Fusarium* sp. and two *Trichoderma* spp. selected from this test for further study were from those plates where *C. purpureum* could no longer be reisolated. When these fungi were tested for their ability to protect pruned cherry sapwood from invasion by *C. purpureum in vitro*, all three were found to afford protection. All three fungi germinated and started to grow well in advance of *C. purpureum*. This feature would tend to put the pathogen at a disadvantage with regard to the nutrients that would be available at the pruned surface and therefore be important in preventing the establishment of mycelia of the pathogen from basidiospores that arrive simultaneously or later at the same site.

The laboratory tests to which these potential biological control agents were subjected were conducted under conditions which reasonably represented the situation in the field and would therefore seem to provide a better basis for testing fungi in the field against *C. purpureum*, than those tests that select possible control agents purely on the basis of inhibition of mycelial growth in culture.

Biological control of Silver Leaf Disease by the protection of pruning wounds is not beyond reason once an efficient control agent has been found. In this study, several fungi were shown to have considerable potential as control agents in laboratory tests which were designed to represent the field situation as far as possible. Further study would involve testing these fungi in an orchard since that is where their worth must finally be decided.

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VIII APPENDICES

APPENDIX I : Publication

Bishop, G.C. & Ilsley, A.H. (1978). Production of enniatin as a criterion for confirming the identity of *Fusarium lateritium* isolates. *Aust. J. Biol. Sci. 31:* 93 - 96.

Bishop, G. C. & Ilsley, A. H. (1978). Production of enniatin as a criterion for confirming the identity of Fusarium lateritium isolates. *Australian Journal of Biological Sciences, 31*(1), 93-96.

APPENDIX II : Media

Brown's agar (Mayo, 1925)

Glucose	2g
Asparagine	2g
Starch (water soluble)	10g
K_3PO_4	1.25g
$MgSO_4$	0.75g
Agar (Davis)	15g
Distilled Water	1 litre

Starch dissolved separately, then added to other ingredients.
Modified Brown's agar contained 2g of K_3PO_4, and no glucose.

Cherry wood agar

To each 5g of freshly prepared cherry wood sawdust add 100 ml distilled water. Steam in autoclave for 20 minutes. Filter. Make volume up to 1 litre. Add 15g Bacto-Agar (Difco), and autoclave.

Coon's agar (Rawlins, 1933, p. 82)

Sucrose	7.2g
Glucose	3.6g
$MgSO_4$	1.23g
K_2HPO_4	2.72g
KNO_3	2.02g
Bacto-Agar (Difco)	15g
Distilled Water	1 litre

Glucose Peptone agar (GP and GP/20)

	GP	GP/20
Bacto-Peptone (Difco)	10g	0.5g
Glucose	10g	0.5g
NaCl	5g	0.25g
Bacto-Agar (Difco)	15g	15g
Distilled Water	1 litre	1 litre

Dissolve ingredients with agar. Check pH = 7.0.

Malt agar

Malt extract (Difco)	20g
Bacto-Peptone (Difco)	1g
Glucose	10g
Agar (Davis)	20g
Distilled Water	1 litre

Modified Brown's agar. See under Brown's agar.

Modified Fries' agar (Naef-Roth, Kern & Toth, 1963)

Glucose	20g
Ammonium tartrate	5g
KH_2PO_4	0.5 g
$MgSO_4 \cdot 7H_2O$	0.5 g
$FeCl_3 \cdot 7H_2O$	0.01g
Agar (Davis)	15g
Distilled Water	1 litre

Dissolve glucose separately, then add to other ingredients.

Nash Agar (after Nash & Snyder, 1962)

Bacto-Peptone (Difco)	15g
KH_2PO_4	1g
$MgSO_4 \cdot 7H_2O$	0.5 g
Agar (Davis)	20g
Distilled water	1 litre

Neutral Dox Yeast agar (NDY and NDY/6)

	NDY	NDY/6
$NaNO_3$	2.0g	0.33g
KH_2PO_4	1.0g	0.16g
KCl	0.5g	0.08g
$MgSO_4 \cdot 7H_2O$	0.5g	0.08g
$FeSO_4$	0.01g	0.001g
Sucrose	30g	5g
Yeast Extract (Difco)	0.5 g	0.08g
Agar (Davis)	15g	15g
Distilled Water	1 litre	1 litre

Dissolve ingredients. Then add to molten agar. Check pH = 5.6 - 5.8

Potato sucrose agar

'Proto' dehydrated potato	22g (Rosella Foods Pty. Ltd., Melbourne)
Sucrose	10g
Bacto-Agar (Difco)	15g
Distilled water	1 litre

Add potato to boiling water, boil until soft. Filter through muslin, make up to 1 litre. Add sucrose and agar, heating slowly until agar has dissolved. Autoclave.

Sporulation agar (Audhya & Russell, 1974)

Sodium acetate (trihydrate)	2.0g
Bacto-tryptone (Difco)	2.0g
Potassium chloride	0.25g
$MgSO_4 \cdot 7H_2O$	0.25g
KH_2PO_4	0.5g
$FeSO_4 \cdot 7H_2O$	0.005g
$Ca(NO_3)_2 \cdot 4H_2O$	0.03g
Trace Metal Solution	5ml
Agar (Davis)	15g
Distilled Water	1 litre

Trace Metal Solution (in 1 litre) –

$FeCl_3 \cdot 6H_2O$	0.29g
$CuSO_4 \cdot 5H_2O$	0.039g
$MnCl_2 \cdot 4H_2O$	0.018g
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$	0.0075g

Note: All media and distilled water were sterilized by autoclaving for 15 minutes at 120°C and 102kPa.

APPENDIX III : Rainfall (mm) data for "Ferndale", Basket Range from July 1975 to January 1978

Date/Month	1975 July	Aug.	Sep.	Oct.	Nov.	Dec.	1976 Jan.	Feb.	Mar.	Apr.	May	June
1	-	1.0	0.7	6.2	5.2	-	-	-	0.5	-	-	-
2	-	-	-	2.5	13.2	-	-	-	-	1.0	-	-
3	-	8.7	18.7	4.2	-	6.5	-	-	-	-	-	24.0
4	0.7	12.2	1.7	0.5	2.0	-	0,5	14.5	-	-	-	1.5
5	2.5	6.5	1.2	-	0.2	-	-	-	-	1.5	-	13.2
6	15.2	4.2	-	-	0,5	-	-	-	-	-	-	-
7	1.2	0,5	-	8.0	-	-	-	-	-	-	-	6.7
8	1.2	-	-	13.2	-	-	-	-	-	-	1.7	3.5
9	-	-	-	1.0	2.5	-	7.0	5.7	-	-	0.7	4.7
10	-	-	0.7	3.7	-	-	-	0.5	-	-	-	1.5
11	6.7	4.7	-	2.0	-	-	-	-	-	-	-	0.5
12	25.7	7.8	-	4.0	-	0.7	9.5	-	-	12.5	-	-
13	1.2	6.0	5.5	1.7	-	4.0	0.5	-	-	-	-	-
14	32.2	1.2	-	2,2	-	1.5	-	-	-	1.2	-	5.0
15	7.7	-	-	-	-	-	-	-	-	-	-	-
16	5.2	0.5	8.5	-	-	-	-	-	1.7	-	11.0	7.7
17	0.4	-	0.5	-	-	-	-	-	-	7.7	7.2	4.5
18	5.2	-	2.2	0.5	-	-	-	1.2	-	2.2	2.2	0.7
19	8.2	-	11.5	5.5	-	-	-	9.7	-	-	-	-
20	7.5	18.7	-	5.2	4.7	-	-	-	1.2	-	-	-
21	8.2	6.2	5.5	1.5	1.5	-	-	-	0.7	-	-	-
22	0.5	7.5	2.7	-	-	-	-	-	-	-	5.0	-
23	-	1.2	7.7	-	3.7	-	-	-	-	-	5.2	-
24	4.5	1.2	-	31.7	-	-	-	-	-	-	8.0	3.7
25	0.7	2.2	-	34.7	-	-	0.7	32.0	-	-	4.2	0.7
26	-	1.0	-	1.2	-	-	1.0	23.0	-	5.7	8.5	-
27	-	4.7	6.7	-	-	-	1.7	-	-	9.2	-	-
28	0.2	1.2	8.7	-	-	1.0	-	-	-	-	-	11.5
29	-	3.2	4.7	-	-	-	-	9.7	-	6.7	-	7.0
30	-	0.7	9.5	3.2	-	-	-		-	-	-	2.0
31	44.5	-		3.6		-	-		-		-	
Monthly Total	182.7	102.0	96.2	172.0	33.3	13.5	20.9	97.3	4.1	48.7	53.7	98.4

Annual Total 1975 - 1171mm

Date/Month	1976 July	Aug.	Sep.	Oct.	Nov.	Dec.	1977 Jan.	Feb.	Mar.	Apr.	May	June
1	-	15.2	8.5	-	-	-	2.0	-	-	-	-	4.5
2	-	6.2	0.7	6.2	20.5	-	-	-	-	-	-	3.0
3	20.2	9.2	2.7	10.7	6.5	6.5	-	-	-	-	-	5.7
4	15.0	-	-	0.7	3.2	-	-	-	-	-	-	-
5	5.7	-	-	-	2.2	-	-	-	-	-	-	-
6	-	10.0	0.5	21.0	0.5	-	-	-	-	1.2	44.2	-
7	-	1.7	-	19.0	-	0.2	-	-	-	21.7	-	-
8	-	-	2.2	2.2	-	-	-	-	-	4.2	1.7	-
9	-	-	20.2	6.5	-	-	-	-	-	1.2	4.7	0.7
10	-	6.7	9.2	1.0	-	-	-	-	-	-	10.7	1.7
11	-	5.7	7.0	-	-	-	-	-	-	-	1.5	3.5
12	-	1.2	-	-	1.0	7.0	-	-	-	-	1.0	-
13	-	6.0	-	2.2	-	-	-	6.7	-	-	1.0	-
14	-	-	-	8.2	-	-	23.5	-	-	-	0.5	1.0
15	2.2	-	-	1.5	2.2	1.0	57.5	-	9.0	-	-	9.5
16	0.2	-	-	7.2	2.5	13.0	30.7	-	1.0	0.7	-	12.0
17	-	8.5	6.0	22.2	-	0,5	0.5	-	-	1.5	-	26.5
18	-	1.0	-	2.5	-	2.5	-	-	-	-	-	4.7
19	-	-	-	1.2	-	-	-	-	-	-	-	-
20	-	-	1.4	-	-	-	-	-	-	-	-	1.2
21	-	-	11.0	-	-	-	-	-	-	-	20.0	0.5
22	-	2.7	7,7	4,2	-	-	2.7	-	-	-	3.0	-
23	-	17.5	2.2	-	-	18,7	3.5	-	-	-	10.5	-
24	-	11.2	1.0	-	-	-	3.0	-	9.2	-	24.7	6.2
25	-	3.7	-	6.7	13.5	-	-	0.4	2.0	-	8.7	0.5
26	0.7	-	-	-	4.2	-	-	7.0	53.0	-	-	-
27	-	-	-	-	-	-	-	-	3.4	-	-	5.0
28	-	-	-	-	-	-	-	-	1.7	-	15.0	18.2
29	5.7	-	3.0	-	4.0	-	-	-	0.5	1.0	1.2	35.0
30	10.0	-	2.0	-	10.0	-	-	-	2.5	-	4.2	7.2
31	8.0	-		-		-	-		1.5		15.7	
Monthly Total	67.7	106.5	85.3	124.3	70.3	49.4	124.4	14.1	83.8	31.5	146.5	140.6

Annual Total 1976 - 827mm

Date/Month	1977 July	Aug.	Sep.	Oct.	Nov.	Dec.	1978 Jan.
1	0.7	7.7	7.2	-	8.2	-	-
2	-	0.7	4.0	33.7	-	27.0	-
3	-	16.0	2.2	3.7	-	0.5	1.5
4	-	10.0	2.0	-	2.7	1.2	-
5	0.2	2.5	-	1.0	-	-	-
6	-	3.0	-	-	-	-	-
7	-	6.0	7.0	-	-	-	-
8	-	-	-	-	-	-	-
9	-	-	18.2	-	-	-	-
10	0.2	-	1.5	-	44.2	-	-
11	-	-	0.2	-	3.5	-	-
12	-	2.7	17.0	-	-	-	-
13	-	9.5	3.7	0.5	-	-	-
14	1.5	-	4.2	15.0	-	-	-
15	2.0	-	3.0	2.2	0.7	0.7	-
16	4.0	-	1.2	-	-	-	-
17	1.0	-	-	-	-	-	-
18	-	-	-	-	-	-	-
19	-	-	-	14.7	-	1.2	-
20	-	-	-	2.5	0.5	-	-
21	-	7.7	-	2.2	-	-	-
22	2.2	11.0	-	0.2	-	-	-
23	25.0	-	-	-	-	-	3.7
24	6.5	-	-	-	-	-	1.5
25	18.2	-	-	-	-	3.5	-
26	0.7	-	-	-	-	-	-
27	7.7	-	-	-	0.2	3.5	-
28	3.5	-	-	-	3.7	-	-
29	-	-	3.0	-	25.7	-	-
30	-	1.7	-	-	-	-	-
31	-	10.5		2.5		-	-
Monthly Total	74.2	89.1	75.4	78.3	67.4	37.6	6.7

Annual Total 1977 - 996mm

APPENDIX IV :

Orchard Sprays

Details of the orchard sprays (fungicides, insecticides and foliar fertilizers) referred to in Figure 16 (p. 73).

Common Name	Trade Name	Active Constituent
dimazide	Alar 85(1)	85% w/w Daminozide
benomyl	Benlate(4)	50% w/w methyl 1-(butylcarbamoyl) benzimidazol-2-ylcarbamate
	Calcium nitrate(1)	
captan	Captan A(1)	83% w/w N-trichloromethylthiotetra-hydrophthalimide
azinphos methyl	Co-thion(1)	50% w/w azinphos methyl
captafol	Difolitan 80(1)	80% N-(1,1,2,2-tetrachloroethylthio) -4-cyclohexene-1,2-dicarboximide
mancozeb	Dithane M45(1)	80% w/w zinc and manganese ethylene dibithiocarbamate
	Ethrel	48% w/w 2-chloroethyl phosphonic acid
dodine	Melprex A(1)	65% w/w N-Dodecyl guamidine acetate
	Nimrod(1)	25% w/v bupirimate
leptophos	Phosvel(1)	45% w/w O-(4-bromo-2,5 dichlorophenyl) O-methylphenylphosphonothioate
	Plictran 50W(2)	50% w/w tricyclohexyltin hydroxide
	Saprol(1)	10% w/v triforine
	Topfoliar(3)	Nutrient Spray; w/v 9.11% N, 3.95% P, 5.90% K
endosulfan	Thiodan(5)	35% w/v Endosulfan

Manufacturers (1) ICI Aust. Ltd., Melbourne (2) Dow Chemical (Aust.) Ltd., Sydney (3) Adelaide & Wallaroo Fertilizers Ltd., Adelaide (4) DuPont (Aust.) Ltd., Sydney (5) Hoechst Aust. Ltd., Melbourne.

APPENDIX V :

Correlations of spore number per g and rainfall prior to sampling.

Sample period July 1975 - January 1978

Number of samples - 86

Correlation Coefficients (ρ)

No. of *F. lateritium* spores/g material sampled and

(a) rain that fell on the nth day prior to sampling

n	ρ
1	0.15268
2	0.094645
3	0.066240
4	0.12495
5	0.050776
6	0.039828
7	0.039828

(b) rain that fell in the period (hrs) prior to sampling

hrs	ρ
48	0.12560
72	0.15842
96	0.15009
120	0.16315
144	0.18918
168	0.23647*

* significant at P = 0.05

APPENDIX VI :

Failure of an attempt to artificially infect apple trees with *Chondrostereum purpureum*

An experiment to evaluate the protection afforded by *F. lateritium* against invasion of sapwood by *C. purpureum* was started in July 1975 using apple trees. The method used was very similar to that employed when this experiment was repeated using cherry trees (see p. 80).

Branches 1.5 - 2.0 cm in diameter on 10 year old apple trees cv. Granny Smith growing in Claremont Orchard at the Waite Institute, Glen Osmond, were labelled and pruned above a dormant bud on July 18 1975. A total of 40 sites were inoculated per treatment and were assigned at random to the sites which were on 8 trees. The treatments were, (a) *F. lateritium* only, applied at pruning, (b) *F. lateritium* applied at pruning and *C. purpureum* after 24 hr and (c) *C. purpureum* only, applied 24 hr after pruning. Treatments (a) and (b) were inoculated by brushing on sufficient of a suspension of *F. lateritium* macroconidia containing 10^6 spores per ml to saturate the cut surface. After 24 hr, 100 basidiospores of *C. purpureum* were applied to treatments (b) and (c), via five 2 μl drops of a suspension of basidiospores containing 1×10^4 per ml, using a Burkard microapplicator. Inoculated sites were covered with sterile aluminium foil for 14 days following inoculation.

Results

Branches were sampled at random from the 8 trees after 6 months, and wood chips from the centre of the stems were plated on Malt agar or GP/20 plus 250 p.p.m. guaiacol agar (see p. 39 for full details).

C. purpureum was not reisolated from any of the sites where it was applied by itself or along with *F. lateritium*. *F. lateritium* was reisolated from all of the 27 sites investigated from treatment (a), 8/27 in treatment (c) and 22/27 in treatment (b).

Discussion

The reasons why *C. purpureum* failed to colonize pruning wounds on the apple trees are not obvious. The basidiospores used were from a sporophore on an apple tree, the spores having been collected in June 1975 and stored at $2^{\circ}C$ until required. Before being used their rate of germination was tested and was found to be 95 - 100% after 48 hr on GP/20 agar at $25^{\circ}C$. Consequently, it would seem unlikely that the spores were responsible for failure of the pathogen to grow.

From observations made in the field, the cultivar Granny Smith is not resistant to invasion

by *C. purpureum*. The trees in Claremont Orchard were on rootstocks 778 and 111, which are not used in commercial plantings of Granny Smith in South Australia. Rootstocks have been found that confer a degree of resistance to Silver Leaf Disease in plum scions (Brooks & Storey, 1923; Grosjean, 1943) and this may well be the situation with Granny Smith on rootstocks 778 and 111. From what occurred in this experiment, it would seem to be advisable to sample a number of the sites where only *C. purpureum* was applied, say 4 to 8 weeks after inoculation, to determine whether *C. purpureum* has grown or not. This procedure would help to avoid the situation encountered here where an experiment was allowed to run for 6 months as planned before being sampled, only then to find that the pathogen had not grown. Field experiments with fruit trees are of necessity of long duration and therefore problems of this nature need to be identified, where possible, earlier than they were in this experiment.