STUDIES OF CAMELLIA FLOWER BLIGHT

(*Ciborinia camelliae*)

IN NEW ZEALAND

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ABSTRACT

Camellias are popular ornamental plants grown for their verdant evergreen foliage and spectacular flowers, which come in many colours, sizes and forms. The fungal pathogen (*Ciborinia camelliae*), which causes camellia flower blight, is considered the most serious disease of the camellia genus as it attacks the flowers, causing them to turn brown and fall early. The pathogen was described in Japan in 1919, spread to the USA in 1938 and was discovered in New Zealand in 1993. Relatively little research has been done on the pathogen or the disease that it causes. Chemical and cultural control methods have not proved particularly effective.

The fungus was isolated from sclerotial medulla and isolates grew significantly better on Camellia Petal PDA, Oxoid PDA, Difco PDA and Oxoid MEA than on Homemade PDA, expired Oxoid PDA and Merck PDA. Cultures were maintained on Difco PDA and optimum temperatures for growth and sclerotial formation were between 15°C and 20°C.

Surveys of the North Island in 1997 and both North and South Island in 1998 found the pathogen was more widely distributed than previously thought. It is widespread in the central, western and lower North Island and present in the north of the South Island and Christchurch. Although the outbreaks in Auckland and in Christchurch were probably the result of the transfer of infected material, dispersal by windborne ascospores appears to be the main method of spread.

Conditions that stimulate sclerotial germination out of season were investigated using both protocols established for other fungi and novel methods that involved incubation at various temperatures, combinations of temperatures, and light. Artificial stimulation of germination was not achieved.

Infection of petals was investigated using agar plug inoculation and, during the disease season, ascospores. Wounding was required for infection from plug inoculum but not from ascospores. Younger buds appeared to have more resistance to both types of inoculum. Ascospore inoculations of species and varieties showed that there were levels of resistance within the genus but this was not quantified.

Several potential biocontrol agents, effective against the ascospore stage, were isolated but could not be evaluated due to the limited ascospore production season.

Further research is required to study the pathogen/host interaction, the infection process, levels of resistance and mechanisms of resistance. Breeding for resistance appears to be possible and offers the best long term prospects for the control of this disease.

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Finally, I would like to dedicate this thesis to Nana, Aunty Janet and Cousin Bell.

Joyce Taylor, 5.5.1916 – 22.7.1998

Isabel McCallum 22.12.1915 – 14.9.1998

Janet Hamlin 23.1.1910 – 2.11.1998

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CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION TO THE GENUS *CAMELLIA*

The Family THEACEAE contains about 30 genera (Kobuski 1978), of which the most important is the genus *Camellia*. Named by Linnaeus in 1735 (*Systema Naturae*) it has become one of the most popular flowering tree groups, along with roses and rhododendrons (Hagiya 1997). Members of the genus are slow growing, evergreen shrubs or trees (Sealy 1958; Feathers 1978), occurring naturally in the tropical and subtropical forests of the Far East (Clere 1991). They are found over a large part of south-east Asia, inland and coastal China, Korea, Japan, Taiwan and islands (Durrant 1982; Clere 1991; Macoboy 1992). Only one species originated outside Asia; *C. lanceolata* (Bl.) Seem. is a native of the mountainous areas of the Philippines and Indonesia (Durrant 1982; Macoboy 1992). There is confusion over the exact number of *Camellia* species (Clere 1991), but more than 200 are known (Chang & Bartholomew 1984; Hagiya 1997) and the International Camellia Register lists 267 species. Some of these will probably be natural hybrids (Macoboy 1992), but as many camellia regions remain botanically unexplored, more species remain to be discovered.

Camellia cultivation has a long history. They were grown both as garden ornamentals, and for their commercial products. The crushed seeds of some species produce high quality oils, which are used in cosmetics (Nuccio, 1975; Durrant, 1982) or as cooking oils (Sealy 1958; Durrant 1982; Guomei 1986). The pharmaceutical and manufacturing industries also use camellia products (Chang & Bartholomew, 1984). The most important economic product - tea - is made from the leaves of *C. sinensis* L. var. *sinensis* Kuntze and *C. sinensis* var. *assamica* (Mast.) Kitamura. The Chinese probably drank tea since before the birth of Christ (Sealy 1958; Brown 1978; Durrant 1982; Macoboy 1992; Macoboy 1998) and they introduced it to Japan during the Tang dynasty (A.D. 618-905) (Tanaka 1973; Brown 1978; Chang & Bartholomew 1984; Macoboy

1998). The Japanese Tea Ceremony (*cha-no-yu*) became an integral part of Japanese culture and tradition. Tea was brought to Europe in the early 17th Century and quickly became a popular, if expensive commodity. At this time, all tea was produced in China and it was to break this monopoly, that European traders (notably the East India Company) began transporting camellia plants and seeds back to Europe (Durrant 1982; Macoboy 1992). Whilst the commercial production of tea was not viable in Europe (Durrant 1982), it did flourish in countries where labour was cheap. Today, more than one million tonnes of tea is produced annually (Durrant 1982).

In addition to *C. sinensis*, a number of other camellias were introduced from China and Japan and as their ornamental qualities became apparent, more varieties and species were sought by enthusiasts (Macoboy 1992). Several *C. japonica* Linn. varieties were already well established in Europe by the 1800s, and these were joined by many more, notably *C. reticulata* Lindl., *C. maliflora* Lindl. *C. oleifera* Abel and *C. sasanqua* Thunb. (Booth 1829; Macoboy 1992). New species were imported as they were discovered, for example *C. hongkongensis* Seem. in 1874, *C. saluenensis* Stapf. ex Bean in 1924 (Macoboy 1992) and *C. granthamiana* Sealy in 1955 (Durrant 1982). Camellias are now widely cultivated in most countries around the world (Clere 1991) and there are a number of societies dedicated to their appreciation, breeding and propagation.

The camellia offers a wide range of flower forms (eg, single, anemone, formal), colours (white, cream, pink, rose pink, red, black red, purple), and sizes (~1cm-23cm diameter). This range is the result of many centuries of selective breeding for desirable characteristics, first in China and Japan (Durrant 1982; Chang & Bartholomew 1984; Guomei 1986), and later in Europe. Breeding continues in backyards and nurseries around the world, with many new varieties registered each year. In New Zealand alone, eight new varieties were registered in 1996 (New Zealand Camellia Register) and ten in 1998 (New Zealand Camellia Register). Although many species are available, the three most popular are *C. sasanqua*, *C. japonica* and *C. reticulata*. *C. sasanqua* are lightly fragrant, autumn flowering plants, native to southern Japan and the Ryukyu Islands (Macoboy 1992). *C. japonica* flower from early winter through to early summer, in a plethora of forms, colours and sizes, and they account for around three-quarters of the

camellia varieties grown worldwide (Macoboy 1992). Most *C. reticulata* have comparatively large blooms, some up to 23cm in diameter (Nuccio 1975) and like *C. japonica*, they flower in spring. They are trees, rather than shrubs, and have fewer and larger leaves (Nuccio 1975). Interspecific hybridisation is possible, with about 5600 hybrids registered (Anonymous 1996).

1.2 INTRODUCTION TO *CIBORINIA CAMELLIAE*

Camellia flower blight is caused by the fungus *Ciborinia camelliae* Kohn. It is a specialist pathogen, attacking only the floral parts of species belonging to the genus *Camellia* (Raabe et al 1978; Kohn & Nagasawa 1984). There is no evidence that any other part of the plant is affected (Hansen & Thomas 1940; Young & Milbrath 1950), and the health of the plant does not appear to be affected. Infected petals turn brown and the flowers fall early (Kohn & Nagasawa 1984). Since camellias are specifically cultivated for their beautiful flowers, the depredations of *C. camelliae* detract from their aesthetic value. All varieties and species are thought susceptible to the disease, but no programme of resistance screening has been carried out. Camellias are relatively untroubled by pests and diseases (Bieleski 1991; Bond 1994) and this blight is considered the most serious disease of the genus *Camellia* (Raabe et al 1978).

1.2.1 Classification

C. camelliae is an inoperculate Discomycete in the Order Helotiales (Alexopoulos & Mims, 1979). It belongs to the Family SCLEROTINIACEAE; members of this Family produce apothecia from stroma or sclerotia (Alexopoulos & Mims 1979; Agrios 1988) and include a number of economically important plant pathogens such as *Sclerotinia* spp. and *Botrytis* spp. (Alexopoulos & Mims 1979). In 1945, Whetzel subdivided the Family into 14 genera. Formerly classified as *Sclerotinia camelliae* Fuckel., Whetzel erected the genus *Ciborinia* to contain species in which either wholly or partly digested host tissue is incorporated into the sclerotial medulla. Sclerotia of the genus *Sclerotinia* do not contain host tissue (Whetzel 1945).

Twenty *Ciborinia* species are known, and all are host specific pathogens. Most are limited to one host species or to several species within a genus. In general, they attack the leaves or floral parts of their host, although some also infect twigs, bark, bulbs, stolons and sepals (Groves & Bowerman 1955; Batra & Korf 1959; Batra 1960). The life cycle is annual, with sclerotia forming in infected host tissues over summer and autumn, then producing apothecia the following spring. Microconidia have been recorded for most species (Groves & Bowerman 1955; Batra & Korf 1959; Batra 1960). Macroconidia have not been recorded in any *Ciborinia* species (Kohn 1979). Table 1.1 summarises the basic features of each species; its host(s), plant part(s) infected, and whether an annual cycle or microconidia have been observed. Little research has been carried out on members of the genus, as they are generally not economically important pathogens. The details of life cycles and host/parasite interactions are sparse.

1.2.2 Description of *C. camelliae*

Camellia flower blight was first described in Japan by Hara in 1919 and he named it *Sclerotinia camelliae* Hara. Hansen & Thomas (1940) identified it from camellias in California in 1938. Unaware of Hara's work they believed it to be a new species, which they described as *Sclerotinia camelliae* Hansen & Thomas. In 1946, now conscious/cognizant of Hara's description, Thomas & Hansen recognised (1946) *S. camelliae* Hara, but noted that the dimensions of asci and ascospores were smaller in their samples than those recorded by Hara. When Kohn (1979) compared Hara's holotype with fresh samples collected in California, she concluded that the two species were not the same and described the Californian specimens as *Ciborinia camelliae* Kohn. After studying the holotypes of *S. camelliae* Hara. and *C. camelliae* Kohn., Kohn & Nagasawa (1984) adjudged the two to be synonymous. Although Hara's epithet has priority in *Sclerotinia*, because it is best accommodated in the genus *Ciborinia,* the correct name for this species is *C. camelliae* Kohn.

Table 1.1 Summary of Features Found in *Ciborinia* Species

Species	**Host(s)**	**Plant Part(s) Infected**	**Season /Month Apothecia Observed: (Northern Hemisphere)**	**Apothecia Produced in Culture:**	**Microconidia Observed:**	**Reference**
C. allii (Saw.) Kohn	*Allium fistulosum*	leaves	late autumn-spring	yes	no	Leu & Wu 1985
C. candolleana (Lév) Whet.	*Querus rubra*	leaves	-	-	yes	Batra 1960
	Querus rubra *Q. pedunculata* *Q. robur* *Q. sessiliflora* *Castanea sativa*	leaves, bud sclaes and bark of small twigs	May and June	-	-	Wilson & Waldie 1927
C. bresadolae (Rick) Palmer †	*Querus* *Castanea*	leaves, twigs	March-June	-	-	Palmer 1992
C. camelliae Kohn	*Camellia japonica* *C. rusticana*	petals	spring	-	yes	Kohn & Nagasawa 1984 Iriyama 1980
C. ciborium (Vahl:Fr) Schumacher & Kohn	*Carex* spp. *Eriophorum* spp.	base of leaf	(Finnish) summer	-	yes	Schumacher & Kohn 1985
C. davidsoniana Whet. ex Groves and Bowerman	*Populus tremuloidis*	leaves	-	-	-	Groves & Bowerman 1955
C. erythronii (Whet.) Whet.	*Erythronium albidum* *E. americanum*	leaves, bulbs, stolons and petioles	April and May	yes	yes	Batra & Korf 1959
C. foliicola (Cash and Davidson) Whet.	*Salix*	leaves	June and July	-	yes	Batra 1960

- information not available

† may be synonym of *C. hirtella*

Table 1.1 (cont.) Summary of Features Found in *Ciborinia* Species

C. graacilipes (Cooke) Seaver	*Magnolia glauca*	petals	-	-	yes	Batra 1960
C. gracilis (Clements) Whet.	*Erythronium albidum*	bulbs, stolons and petioles	May	-	no	Batra & Korf 1959
C. hemisphaerica Zhuang & Wang	unidentified	-	-	-	no	Zhuang & Wang 1997
C. hirtella (Boud.) Batra and Korf	*Castanea*	leaves and bark of small twigs	-	-	-	Batra 1960
C. hirsuta Kohn & Korf	*Vaccinium* Liliaceae	-	-	yes	yes	Kohn 1982
C. jinggangensis Zhuang & Wang	unidentified	-	-	-	no	Zhuang & Wang 1997
C. pseudobifrons Whet. ex Groves and Bowerman	*Populus*	leaves and twigs	April and May	yes	-	Groves & Bowerman 1955
C. seaveri Groves and Bowerman	*Populus*	leaves	"when leaves budding"	-	yes	Groves & Bowerman 1955
C. trillii Batra and Korf	*Trillium decumbens* *T. erectum* *T. grandiflorum* *T. maculatum*	leaves, petioles, floral peduncles, sepals and petals	May	-	no	Batra & Korf 1959
C. violae Batra and Korf	*Viola papilionacea* *V. pubescens* *V. rotundifolia*	leaves, petioles, floral peduncles, main stems	May	-	no	Batra & Korf 1959
C. whetzelii (Seaver) Seaver	*Populus*	leaves	May	-	-	Groves & Bowerman 1955
				-	yes	Whetzel 1945
C. wisconsinensis Batra	*Salix petiolaris*	leaves	late June	yes	yes	Batra 1960

- information not available

1.2.3 Life Cycle of *Ciborinia camelliae*

The life cycle of *C. camelliae* is shown in Figure 1.1. Infection occurs in spring, when an ascospore germinates on a petal and successfully penetrates the host tissue. Each infection causes an irregular, small brown spot on the petal and these infections enlarge and coalesce until the entire petal is brown. When the infection reaches the base of the petal, it spreads to, and up, other petals of the flower until the entire flower shows symptoms of the blight. At the base of the flower, a white or grey ring of mycelium can be seen when the calyx is removed. Fungal pseudoparenychema in the base of the petals eventually form sclerotia. The symptoms and signs of disease are shown in Figures 1.2 and 1.3. Sclerotia in fallen flowers lie dormant on the ground or in plant debris over summer, autumn and winter. Towards the end of winter, the sclerotia begin to germinate, producing apothecia from which ascospores are released. Sclerotial germination continues for two to three months. Not all sclerotia germinate the season following formation; they may remain viable in the soil for at least four years (Baxter & Thomas 1995).

1.2.4 Origin and Distribution

Although discovered in Japan in 1919 (Hara 1919), the disease does not appear to have become widespread in Japan until mid 1970s (Matsumoto 1995). In the United States, it was found on camellias in a nursery near Hayward, in 1938 (Hansen & Thomas 1940) and later, at a second Californian nursery (Thomas & Hansen, 1946). After its identification, nurserymen reported that, prior to 1938, infected flowers had been in seen in "scattered areas in northern California" (Raabe et al 1958). Both nurseries imported camellia plants from Japan and it is likely that the disease was introduced on these imported plants (Hansen & Thomas 1940; Thomas & Hansen 1946). The disease progressively spread to other states, first Georgia (Gill 1948), then Oregon (Richmond 1949), Louisiana (Plakidas 1950), North Carolina (Watson 1950), Texas (Plakidas 1957), South Carolina (Brown 1958), Virginia

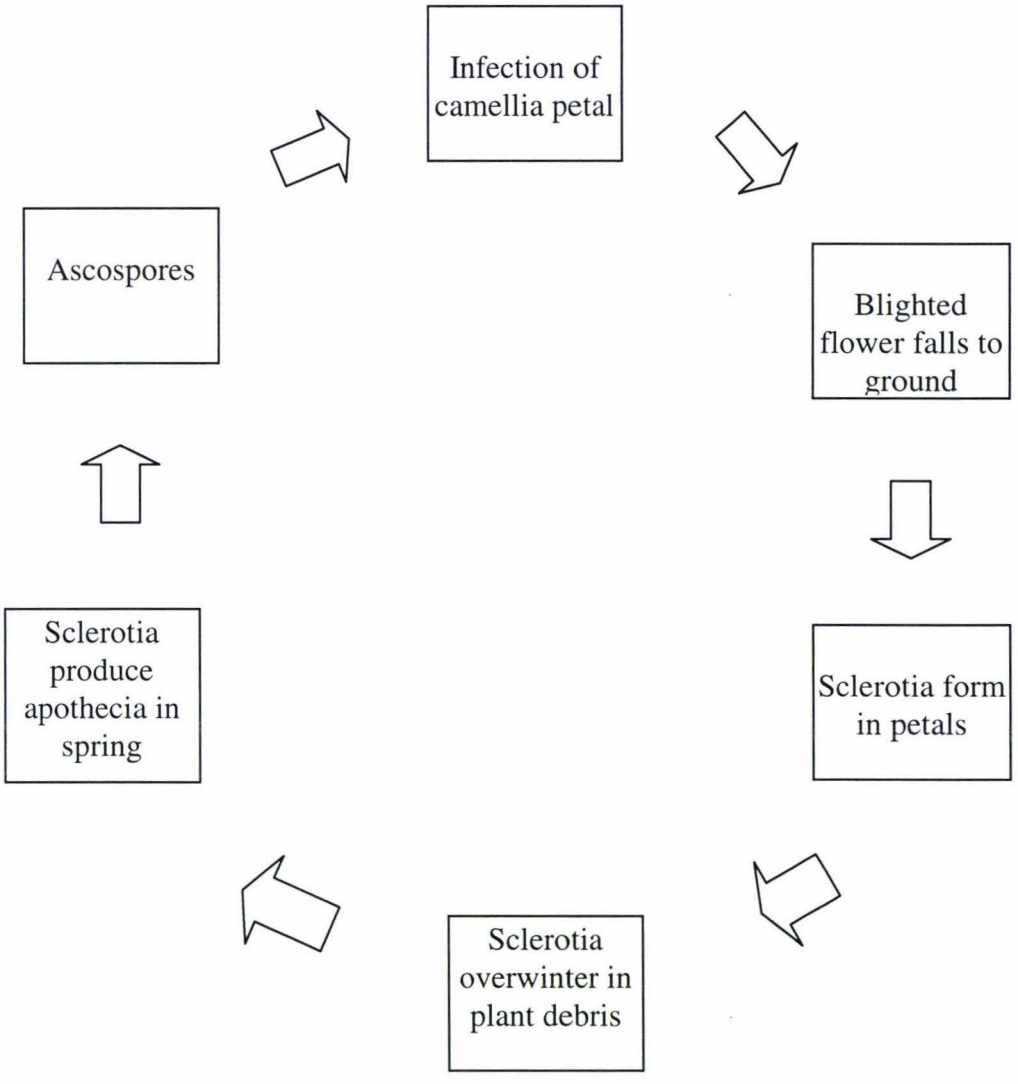

Figure 1.1 Life cycle of *Ciborinia camelliae*.

Figure 1.2
Signs and symptoms of *C. camelliae* infection. There is a white ring of mycelium where the calyx has been removed and a brown rot is spreading from the base of the flower.

Figure 1.3
Black sclerotia at the base of an infected flower.

(Brown 1958) and Mississippi (Cochran 1962). It has been reported in Florida and is probably also in other southern states (Raabe et al 1978). Thus, within 20 years of its arrival in the United States, *C. camelliae* has spread to all regions where camellias are grown (the 'camellia belt' of America).

In the Georgia, Louisiana and Texas infections, the owners of the gardens or nurseries where the disease was initially found had all imported camellias from California (Gill 1948; Plakidas 1950; Plakidas 1957). The origin of the infections in other states is not known.

In 1993, the disease was found in a garden in Wellington, New Zealand and identified by Stewart & Neilson (1993) who believed it had been there at least two seasons. A survey conducted by the Ministry of Agriculture and Fisheries (MAF) (Hill 1993) found the disease to be widespread in Wellington and no eradication or containment measures were undertaken (Frampton, Pers. Comm.). In 1996, the disease was reported in Waikanae, Wanganui and from a farm at Kauangaroa, 22 km east of Wanganui (Neall et al 1998). Human activity was suspected of introducing it to these locations and it was thought to be limited to these sites.

1.3 CONTROL

When Hansen & Thomas (1940) described this 'new' disease of camellias, they concluded that there was "little danger of the disease becoming established in parks or private gardens" and that it would be "a relatively simple matter to control the disease and eradicate the pathogen by gathering and destroying all fallen flowers". During their observations no apothecia were found under plants grown in the open without mulching. However, by 1943, the disease was known to occur in parks and gardens (Stout, letter to Alford et al 1961) but since infection was by ascospores, and only the flowers were infected, it was thought that it could be eradicated through the destruction of infected flowers combined with ground fungicide sprays to kill or inhibit the sclerotia (Hansen & Thomas 1940; Thomas & Hansen 1946). However, the disease continued to spread

within the United States and the current distribution demonstrates that once the disease is established in an area, eradication is unlikely and even control can be problematic.

Nevertheless, while no control method has been completely effective (Baxter & Thomas 1996) there are a number of practices which will slow the spread of the disease and/or minimise its effects in disease areas. These can be classified as either prevention or as management practices.

1.3.1 Prevention

Prevention involves some form of quarantine, an isolation procedure that prevents the entry/importation and spread of unwanted organisms.

- Quarantine

  A quarantine against flower blight would for example, prohibit the transport of camellia plants, flowers, or camellia material from regions that have the disease. The infections in Louisiana, USA, occurred on plants imported from California in violation of Louisiana State quarantine (Plakidas 1950).

- Sanitation

  Camellia plants can be sold to the public with all flowers and buds removed (infected or not) and the upper 5cm of soil removed and replaced with a sterile medium (Raabe et al 1958). Alternatively, the roots may be laid bare of soil. These measures aim to prevent old sclerotia in the soil, or new sclerotia forming in flowers, from being transported along with the plant. Many nurseries in the United States ship plants bare rooted (Brown 1983). These precautions are equally important in limiting the spread of the disease within a diseased area, as well as in preventing its introduction to areas where it is not present.

1.3.2 Management

Once the disease is established in a region, then a range of chemical and cultural methods have been suggested for management of the disease (Raabe et al 1958; Zummo & Plakidas 1959; Raabe et al 1978):

- Chemical

  Fungicides can be used as sprays or drenches on sclerotia in the soil, thus preventing the formation of apothecia. When sprayed on flowers, they may kill or prevent infections in the petals. Frequent fungicide applications are required, as both apothecial formation and flowering are continuous throughout the season. The fungicides that have been tested against *C. camelliae* are detailed in Tables 1.2 and 1.3.

- Cultural

  The disease can be avoided completely by growing autumn flowering species or varieties that flower early in the season, before flower blight is present. The majority of camellias, however, flower during the blight season and will become infected if ascospores are present.

  By keeping the ground under the camellia plant bare of vegetation, the ground will dry out more rapidly to the detriment of the sclerotia (Baxter et al 1987). In addition, apothecia are more readily observed and removed as they appear.

  All infected flowers should be collected and burned. Flowers should not be composted as the sclerotia can survive this process and be respread around the garden. A plastic ground sheet laid underneath a plant makes collection of fallen flowers easier, and prevents apothecial development. Where the terrain is steep or many camellias are planted, these measures are frequently impractical, especially when the grower is unable, or is disinclined, to carry them out.

Some of the methods used to control *C. camelliae* have been adopted from methods used in the control of *Sclerotinia* diseases. *Sclerotinia* diseases are major economic pathogens, and because they are closely related to *Ciborinia* and have similar infection cycles, improvements in the control of *Sclerotinia* are likely to lead to similar improvements in the control of *Ciborinia* (Holcomb 1983).

1.3.3 Chemical Control

The most common method of controlling plant diseases is through the use of chemical compounds (Agrios 1988). These compounds may be toxic, killing the pathogen, or inhibit its germination, growth or reproduction. Protectant fungicides cannot stop or cure an infection already begun. They are applied to the plant surface in anticipation of the pathogen's arrival. They require frequent application to maintain their efficacy as a) they may be washed off in rain, b) they are readily degraded and c), new growth (eg. young leaves, fruits or flowers) must be protected. Most are multi-site inhibitors which act against several different enzyme systems involved in energy production and pathogen resistance to these materials is rare (Agrios, 1988). Many newer chemicals have both a protective and curative ability and can be effective even after the pathogen has established within the host. Some are absorbed and translocated systemically by the plant. Systemic fungicides are effective at low dose rates. However, systemic fungicides are site-specific inhibitors and are vulnerable to mutations in the pathogen which enable it to become resistant to that fungicide or group of related fungicides. Such resistance has been documented in a growing number of cases. For example, resistance to benzimidazoles and dicarboximide in strains of *B. cinerea* (grey mould on grapes) has been documented (Beever & Brien 1983; Löcher 1987; Beever et al 1989) and resistance to phenylamide has been reported in *Phytophthora infestans* (Cook 1981; Hartill et al 1983; Deahl et al 1993).

In the past 60 years, various fungicides have been evaluated for control of *C. camelliae* in trials ranging from small laboratory tests, to large field trials, conducted over several years. Key features of these trials are given in Tables 1.2 and 1.3. No fungicide has been found to give total control of the disease. Several have produced satisfactory results (eg. Terrachlor and Lynx) but Holcomb (1994) found that the level of disease

Table 1.2 Summary of Fungicide Trials Against Sclerotia of *C. camelliae*

Trade Name	Chemical	Rate of Application	Method of Application	Results*	Reference
Alto	cyproconazole	200ml/5L .4ml/100ml	not specified soil spray	46/336 (86%) "highly effective"	Cooper et al 1997 Fullerton et al 1998
Chloro IPC	isopropyl-N-(3chlorophenyl) carbamate	20lb/acre	dry to soil in white sand	22/46 (53%)	Haasis & Nelson 1953
Cyanamid	calcium cyanamid	200lb/ acre 400lb/acre 800lb/acre	dry to soil in white sand dry to soil in white sand dry to soil in white sand	11/62 (82%) 1/40 (97.5%) 1†/46 (98%)	Haasis & Nelson 1953 Haasis & Nelson 1953 Haasis & Nelson 1953
Ferbam/Fermate	ferbam	4lb/100gal 300lb/acre 6lb/1000ft^2 + wetting agents	not specified soil drench soil drench	"seemed to inhibit formation of … apothecia" 15/306 (95%) "sclerotia … have been suppressed"	Thomas & Hansen 1940 Anzalone & Plakidas 1958 Harvey & Hansen 1950
Folicur 250EC	tebuconazole	12.5ml/5L	not specified	42/268 (84%)	Cooper et al 1997
Folicur 430SC	tebuconazole	43%ai	soil spray		Fullerton et al 1998
Krenite-26	19% sodium dinitrocresylate, 5% sodium butylnapthalene sulfonate, 2% sodium chromate	200gal/acre 800gal/acre	1% soluble drench 1% soluble drench	7/69 (90%) 4‡/72 (94.5%)	Haasis & Nelson 1953 Haasis & Nelson 1953
Orthocide	captan	300lb/acre 2lb/100gal	soil drench soil spray	41/306 (87%) 90% reduction	Anzalone & Plakidas 1958 Hanson 1955
Shirlan	fluazinam	50%ai	soil spray	"highly effective"	Fullerton et al 1998
Sulphur	sulphur	.05lb/yard^2 200lb/acre	not specified soil drench	"effective in suppressing apothecia" 223/306 (27%)	Harvey & Hansen 1950 Anzalone & Plakidas 1958

* where data are given, they are presented as: no. of apothecia produced in treated area/no. in control area (= % survival)

† stipe only

‡ average of two duplicate plots

Table 1.2 (cont.) Summary of Fungicide Trials Against Sclerotia of *C. camelliae*

Sumisclex 25	procymidone	12.5ml/5L	not specified	97/203 (84%)	Cooper et al 1997
TCA	sodium trichloroacetate	10lb/acre	dry to soil in white sand	6/38 (84%)	Haasis & Nelson 1953
		20lb/acre	dry to soil in white sand	5/102 (95%)	Haasis & Nelson 1953
		40lb/acre	dry to soil in white sand	5/61 (92%)	Haasis & Nelson 1953
Terrachlor	pentachloronitrobenzene	300lb/acre	soil drench	2/306 (35%)	Anzalone & Plakidas 1958
		15lb/100gal	soil drench	75%	Tourje 1958
			dry to soil in white sand	25/71 (65%)	Haasis & Nelson 1955
		50lb/acre	dry to soil in white sand	7/25 (72%)	Haasis & Nelson 1955
		100lb/acre	dry to soil in white sand	1/53 (98%)	Haasis & Nelson 1955
		200lb/acre	ground spray or soil drench	"has given excellent control"	Raabe et al 1978
		3lb/1000ft^2 + 1-4oz detergent			

* where data are given, they are presented as: no. of apothecia produced in treated area/no. in control area (= % survival)

† stipe only

‡ average of two duplicate plots

Table 1.3 Summary of Fungicide Trials Against Infection of Camellia Flowers or Prevention of Sclerotial Formation by *C. camelliae*.

Trade Name	Chemical	Rate of Application	Method of Application	Results*	Reference
Acti-dione RZ	cycloheximide	1tsp/gal	sprayed	"excellent control"	Mathews 1975
			sprayed	40%/25%	Alford 1961§
Acti-spray	cycloheximide	-	sprayed	45%/25%	Alford 1961§
Agrimycin	streptomycin	-	sprayed	40%/25%	Alford 1961§
Arasan 428	thiram	-	sprayed	30%/25%	Alford 1961§
Baycor	bitertanol	1lb/100gal	sprayed	35%/57%	Holcomb 1994
Bayleton	triadimefon	1lb/100gal	sprayed	28%/54%	Holcomb 1981
		1tsp/gal	soaked	0-4%/58-90%	Holcomb 1994
		1lb/100gal	sprayed	10%/43%†	Holcomb 1994
		1lb/100gal	sprayed	27%/68%†	Holcomb 1994
		1lb/100gal	sprayed	45%/69%†	Holcomb 1994
		1lb/100gal	sprayed	21%/72%†	Holcomb 1994
		1lb/100gal	sprayed	29%/54%†	Holcomb 1994
		1lb/100gal	sprayed	47%/68%†	Holcomb 1994
		1tsp/gal + Ivory detergent	soaked	50%	Hotchkiss & Baxter 1994
Benlate	benomyl	2.27g/gal	sprayed	~50%	Baxter et al 1987
		1tsp/gal	soaked	0-4%/58-90%	Holcomb 1994
		1tsp/gal + Ivory detergent	soaked	all showed symptoms‡	Hotchkiss & Baxter 1994
Cleary 3336	-	1tsp/gal = Ivory detergent	soaked	all showed symptoms‡	Hotchkiss & Baxter 1994
Cyprex	dodine	-	sprayed	35%/25%	Alford 1961§
Draconil 2787 75W	chlorothalonil	.75 + 1.5 pints/100gal	sprayed	43%/68%	Holcomb 1994
Draconil 2787 54EC	chlorothalonil	.75 + 1.5 pints/100gal	sprayed	47%/68%	Holcomb 1994
Endomycin	-	-	sprayed	20%/25%	Alford 1961§

- information not available

* where given: treatment infection rate (%)/ untreated control infection rate (%)

† trials conducted over six successive years

‡ when subsequently dipped in Lynx stopped advance

§ % blighted flowers 16 days after spraying

Table 1.3 (cont.) Summary of Fungicide Trials Against Infection of Camellia Flowers or Prevention of Sclerotial Formation by *C. camelliae*.

Fermate	ferbam	-	sprayed	35%/25%	Alford 1961[§]
Griseofulvin	-	-	sprayed	45%/25%	Alford 1961[§]
Lynx (Cereous)	triadimenol	2g/gal 2tsp/gal + ¼ tsp liquid detergent 2tsp/gal + Ivory detergent	sprayed soaked soaked	0% 0% 0%	Baxter et al 1987 Baxter & Thomas 1994 Hotchkiss & Baxter 1994
Manzate	mancozeb	-	sprayed	45%/25%	Alford 1961[§]
Ornalin	-	1lb/100gal	sprayed	32%/84%	Holcomb 1994
Orthocide	captan	2tsp/100gal + Ivory detergent	soaked	all showed symptoms[‡]	Hotchkiss & Baxter 1994
Parzate	nabam	-	sprayed	40%/25%	Alford 1961[§]
Ronilan	vinclozolin	9.08g/gal	sprayed	~50%	Baxter et al 1987
Tilt	propiconazole	3.785g/gal	sprayed	~50%	Baxter et al 1987
2635	-	-	sprayed	40%/25%	Alford 1961[§]
1456	-	-	sprayed	0%/25%	Alford 1961[§]
1564	-	-	sprayed	30%/25%	Alford 1961[§]
2235	-	-	sprayed	35%25%	Alford 1961[§]
4283	-	-	sprayed	40%/25%	Alford 1961[§]
2235	-	-	sprayed	45%/25%	Alford 1961[§]

[-] information not available

[*] where given: treatment infection rate (%)/ untreated control infection rate (%)

[†] trials conducted over six successive years

[‡] when subsequently dipped in Lynx stopped advance

[§] % blighted flowers 16 days after spraying

fluctuates though out the season and between years, thus good results one year are not necessarily indicative of consistant control. The systemic fungicides have not proved any more successful than protectants, and Baxter & Thomas (1994) have shown that they are not translocated into the flower. The camellia enthusiast, therefore, must decide whether the reduction in disease is worth the cost in materials and effort.

1.3.4 Biological Control

The National Academy of Sciences (1987) defines biological control as the "use of natural or modified organisms, genes, or gene products to reduce the effects of undesirable organisms, and to favour desirable organisms such as crops, trees, animals and beneficial insects and microorganisms". There is increasing interest in replacing synthetic chemical pesticides with biological control agents (BCAs), driven by a) the increasing cost of pesticides, b) pathogen resistance to pesticides and c), the perceived negative effects of pesticides on the environment and human health.

There has been no published work on biological control of *C. camelliae* but there would appear to be two useful approaches. The first would be to test fungi shown to attack sclerotia of other fungal plant pathogens, and this approach is being tested by one group in New Zealand (Jones & Stewart, 1995). The second approach would be to evaluate BCAs for use on flowers to prevent infection. This approach will be used in this work using the procedures of Cook et al (1997a,b).

1.4 SUMMARY

C. camelliae is the most destructive pest, insect or disease that affects the camellia plant. Camellias are popular ornamental plants world wide, and are grown for their beautiful blooms. The disease attacks only the floral parts of the plant, causing the petals to turn brown and fall early, thereby negating the aesthetic charm of the plant.

The pathogen is related to the common and widespread plant pathogens *Sclerotinia* spp. and *Botrytis* spp. It does not produce macroconidia, infection is by ascospores only, and these are produced in spring. Sclerotia form in the petals and these remain dormant

amongst plant debris until the next season. Early in spring, apothecia are produced from the sclerotia and these release ascospores to be transported by wind. All *Ciborinia* species described have an annual cycle of infection.

Hara (1919) first identified and described the disease from infected flowers in Japan. It is thought to have spread to the United States on imported camellia plants around 1938 (Hansen & Thomas 1940; Raabe et al 1958) and it is believed to have spread to New Zealand by the same method. Since the disease was discovered in Wellington, New Zealand in 1993 (Stewart & Neilson 1993) it has spread to Waikanae and Wanganui. Officially, the disease is limited to Japan, the United States of America and New Zealand, but there are reports that it may be present in Europe and the United Kingdom (R. J. Cook, 1998. Pers. Comm.).

Control of this disease has proved difficult, this is despite the fact that a) only the flowers are infected b) there is no secondary infection, and c) spores are present for only 2-3 months a year. To date, various fungicides have been tested for control of the disease, but the results have also been less than satisfactory. Interest in potential biocontrol agents is growing, but remains an unexplored alternative.

Very little is known about the genus *Ciborinia*. *C. camelliae* is the most studied species, but such studies have been largely limited to fungicide trials, observations of the infection cycle and growth in culture, as shown by the limited data in Table 1.1. Four post-graduate theses have been completed in the USA (Anzalone 1959; Zummo 1960; Alford 1961; Johnson 1971) but only the thesis of Alford (1961) has been seen; copies of the other three have been ordered. Little is known of the pathogen basic biology, e.g. whether it is homothallic or heterothallic or of host/parasite relationships. Further work is urgently required to provide a scientific basis for formulating more effective disease management strategies.

1.5 RESEARCH AIMS

This work has five aims. To:

1. Determine the current distribution, method and rate of spread of *C. camelliae* in New Zealand.

2. Elucidate the basic biology of *C. camelliae*, in particular, to ascertain the
   (a) optimum conditions for culturing the fungus
   (b) critical conditions required for carpogenic germination of sclerotia.

3. Extend knowledge of the infection biology and in particular to determine
   (a) the factors affecting the process of infection
   (b) whether flowers change in susceptibility during development.

4. Isolate potential yeast and bacterial biocontrol agents.

5. Determine whether resistance exists within the genus *Camellia* and whether the development of resistant varieties is feasible.

CHAPTER TWO

ISOLATION AND CULTURE OF *C. CAMELLIAE*

2.1 INTRODUCTION

The successful isolation and culture of *C. camelliae* is important in all aspects of work with this pathogen. While symptoms and signs of the pathogen are distinctive, its isolation in to culture from diseased petals is essential for positive identification of the pathogen. The fungus must also be maintained reliably and consistently to produce pure cultures for laboratory work.

While some workers have reported that *C. camelliae* is readily recovered from diseased petals (Plakidas 1950; Anzalone 1959; Alford 1961; Zummo 1961; Alford & Sinclair 1962; Fullerton et al 1998) others have found it difficult to isolate in this way (Watson 1950; Winstead et al 1954). In comparison with other fungi, *C. camelliae* is relatively slow growing, taking 3-4 days before hyphae are seen on the growth medium (Fullerton 1998). Failure to recover it from diseased petals may be due to its suppression by other fungi and bacteria present on the petal (Winstead et al 1954). Winstead et al (1954) reported that *Pestalotia, Alternaria, Penicillium,* yeasts and bacteria were commonly recovered and Watson (1950) lists 32 fungi which were regularly recovered from petals when attempting to isolate *C. camelliae*.

The pathogen can be isolated from sclerotia, but contamination by other microorganisms is also a problem (Alford 1961). Alford & Sinclair (1962) found that the pathogen could be isolated when small pieces of tissue were taken from within a surface-sterilised sclerotium.

A number of workers have grown the pathogen in culture. The majority have used potato dextrose agar (PDA) (Hansen & Thomas 1940; Watson 1950; Haasis 1953; McCain 1963; Kohn & Nagasawa 1984; Holcomb 1994; Fullerton et al 1998), but

potato sucrose agar (PSA) (Alford 1961; Alford & Sinclair 1962), water agar (WA) (Watson 1950), malt extract agar (MEA) (Kohn & Nagasawa 1984), bean pod agar (Alford 1961), oatmeal agar (Alford 1961), Leonian's Medium (LM) (Kohn & Nagasawa 1984) and sterilised wheat grains (Hansen & Thomas 1940; Haasis 1953) have all been used to successfully culture the pathogen. None of the above reports have considered whether the age of the agar medium could affect growth of the pathogen.

2.2 OBJECTIVES

Preliminary attempts to isolate and grow the fungus in culture met with an erratic response, consequently a number of isolation procedures and culture conditions were investigated to find consistent and successful techniques for isolation and culture of the fungus.

The two main objectives of this section of the work were to:

1. Find a procedure for isolation of *C. camelliae* into pure culture.
2. Determine the optimum conditions for growth and maintenance of *C. camelliae*.

The first objective was investigated using a range of surface sterilisation procedures on petals and sclerotia. The second by a series of experiments to investigate the effect of temperature and agar media on vegetative growth (Experiments 1-3), whether plant tissue or iron supplements improved growth on agar (Experiments 4 and 5) and whether repeated sub-culturing led to a reduction in virulence (Experiment 6).

2.3 MATERIALS AND METHODS

2.3.1 Isolation of *C. camelliae* from Petals and Sclerotia

Diseased camellia petals, with infections ranging from 3 d old to ~20 d old, were surface sterilised in either 1:1 sodium hypochlorite (42 g/Litre) (Janola) and SROW (Sterile Reverse Osmosis Water) for 1 or 5 min or in 1:5 sodium hypochlorite (Janola) and SROW 1 min, rinsed in SROW water and air-dried. Using aseptic technique, petals were cut in half and placed cut edge foremost on Merck PDA or Difco PDA. Nonsterilised petals were similarly cut and placed on agar. Plates were incubated at 20°C in 12 h

light/12 h dark for 3-7 d until fungal growth or contaminants were observed. The tips of hyphal growth were subcultured onto fresh plates of Merck or Difco PDA and further incubated at 20°C in 12 h light/12 h dark. Cultures were assessed as *C. camelliae* or 'other' on the basis of colony form as described by Kohn & Nagasawa (1984).

Sclerotia collected from Wellington Botanic Gardens in November 1997 were surface sterilised as described above. Using aseptic technique, pieces cut from sclerotia (including rind) were placed on either Merck PDA or Difco PDA. Small pieces of tissue cut from the centre of sclerotia (medulla only) were placed on Difco PDA. Plates were incubated at 20°C in 12 h light/12 h dark for 3-7 d until fungal growth or contaminants were observed. Plugs of 5 mm diameter were cut from mycelial growth and placed inverted on Merck PDA or Difco PDA and incubated at 20°C in 12 h light/12 h dark. Cultures were assessed as above.

2.3.2 Effect of Temperature and Growth Medium on Growth of *C. camelliae*

2.3.2.1 Experiment 1 - Growth of four isolates, on six media at six temperatures.

Isolates: Cc1, Cc2, Cc3 and Cc5

Media: Difco PDA (label expiry 5/2002)
Merck PDA (label expiry 8/1997)
Oxoid PDA (label expiry 3/1990)
Oxoid PDA (label expiry 7/2001)
Oxoid MEA (Malt Extract Agar) (label expiry 2/2001)
Homemade PDA (Appendix I) (cv. 'Nadine')

Media were prepared as per the manufacturers' recommendation. At the time this experiment was conducted (22.2.1998-5.3.1998), only new potato tubers were available for preparing Homemade PDA.

Incubation/Light Conditions:

5°C 24 h dark
10°C 12 h light/12h dark
15°C 24 h dark
20°C 12 h light/12h dark

25°C 24 h dark

30°C 24 h dark

Insufficient incubators capable of operating a 12 h light/12 h dark regime were available and while all the treatments could have been subjected to 24 h dark, two treatments of 12 h light/12 h dark were chosen to assess whether light significantly altered growth.

Four mm diameter plugs cut from the actively growing margin of cultures grown on Difco PDA were inverted, one per dish, in the centre of 90 mm petri plates containing the appropriate agar medium. One plate of each isolate on each medium was incubated at each temperature. Growth of colonies was measured on Day 3, 6, 8, 10, 12 and 14. Diameter across the plug was measured on Day 3 (when all colonies had commenced growth) and subtracted from the growth attained at Day 8 (just prior to colonies reaching the edge of the dish) to calculate daily radial growth rate. Two measurements were taken across the colony diameter. Data were analysed using SAS (Statistical Analysis System) for Windows Version 6.12. A two factorial ANOVA (ANalysis Of VAriance) was performed on the raw data, which met the assumptions of normality of distribution and constant variance of residuals. An example of the SAS code is given in Appendix II.

2.3.2.2 Experiment 2 - Growth of four isolates, on six media at five temperatures

This experiment was carried out as for Experiment 1 except that;

1. Old potato tubers were available for the Homemade PDA as recommended by the Commonwealth Mycological Institute (CMI) Plant Pathology Pocketbook.
2. 30°C was not used since there was no growth at this temperature in Experiment 1.
3. All treatments were incubated in the dark.
4. There were 3 replicate plates for each isolate/medium/temperature combination.
5. Diameter across the plug was measured on Day 2 and subtracted from the growth attained at Day 8 to calculate daily radial growth rate.
6. A three factorial ANOVA was performed.
7. Prior to analysis, the data were log transformed to ensure assumptions of normality of distribution and constant variance of residuals were met.

2.3.3 Effect of Age of Agar on Growth of *C. camelliae*

2.3.3.1 Experiment 3 - Growth of two isolates on six agars of Difco PDA of different age

Isolates: Cc1 and Cc3

Media: Difco PDA (label expiry 5/2002) 0 d old
Difco PDA (label expiry 5/2002) 4 d old
Difco PDA (label expiry 5/2002) 7 d old
Difco PDA (label expiry 5/2002) 14 d old
Difco PDA (label expiry 5/2002) 21 d old
Difco PDA (label expiry 5/2002) 50 d old

Media were prepared as per the manufacturers' recommendation.

Incubation/Light Conditions:

20°C 24 h dark

This experiment was carried out as for Experiment 1 except;

1. There were 10 plates of each isolate on each agar medium.
2. Diameter across the plug was measured on Day 2 and subtracted from the growth attained at Day 6 to calculate daily radial growth rate.
3. A three factorial ANOVA was performed.
4. Prior to analysis, the data were log transformed to ensure assumptions of normality of distribution and constant variance of residuals were met.

2.3.4 Effect of Camellia Petal or Iron Supplements on Growth of *C. camelliae*

2.3.4.1 Experiment 4 - Growth of four isolates on ten petal agars at one temperature

Isolates: Cc1, Cc2, Cc3 and Cc5.

Media: Difco PDA (label expiry 5/2002) and supplement of:
Propylene oxide sterilised *C. japonica* petals
Propylene oxide sterilised *C. sasanqua* petals
Autoclaved *C. japonica* petals
Autoclaved *C. sasanqua* petals
Boiled *C. japonica* petals
Boiled *C. sasanqua* petals
Boiled infusion of *C. japonica* petals

Boiled infusion of *C. sasanqua* petals

Homogenised *C. japonica* petals

Homogenised *C. sasanqua* petals

No supplement

The preparation methods for these agars are given in Appendix I.

Incubation/Light Conditions:

20°C 24 h dark

This experiment was carried out as for Experiment 1 except;

1. There were 3 replicate plates for each isolate and medium combination.
2. Diameter across the plug was measured on Day 3 and subtracted from the growth attained at Day 5 to calculate daily radial growth rate.
5. A three factorial ANOVA was performed on the raw data (which met the assumptions of normality of distribution and constant variance of residuals).

2.3.4.2 Experiment 5 – Growth of four isolates, on four agars supplemented with two concentrations of iron

The method for detection of siderophore production was adapted from the bacterial method of Kloepper et al (1980). Four PDA agars where supplemented with ferric iron and inoculated once.

Isolates: Cc1, Cc2, Cc3 and Cc5

Media: Difco PDA (label expiry 5/2002)

Difco PDA (label expiry 5/2002) + 0.1 μm ferric iron

Difco PDA (label expiry 5/2002) + 1.0 μm ferric iron

Merck PDA (label expiry 8/1997)

Merck PDA (label expiry 8/1997) + 0.1 μm ferric iron

Merck PDA (label expiry 8/1997) + 1.0 μm ferric iron

Oxoid PDA (label expiry 7/2001)

Oxoid PDA (label expiry 7/2001) + 0.1 μm ferric iron

Oxoid PDA (label expiry 7/2001) + 1.0 μm ferric iron

Homemade PDA

Homemade PDA + 0.1 μm ferric iron

Media were prepared as per the manufacturers' recommendation.

Incubation/Light Conditions:

20°C 12 h light/12 h dark

This experiment was carried out as for Experiment 1 except that;

1. One temperature was used.
2. Colonies were not measured, but fluorescence was assessed after 14 d by holding the plates under near-UV (Ultraviolet) light and scored as 'not fluorescent', 'slightly fluorescent' and 'fluorescent'.

2.3.5 Effect of Repeated Sub-Culturing on Pathogenicity

2.3.5.1 Experiment 6 - Growth of a repeatedly sub-cultured isolate, a sub-cultured isolate recovered after infecting a petal, and three freshly isolated cultures on seven media at five temperatures

Isolates: Cc3, Cc3R (re-isolated from petal), Cc6, Cc9 and Cc10

Media: Difco PDA (label expiry 5/2002)
Merck PDA (label expiry 8/1997)
Oxoid PDA (label expiry 3/1990)
Oxoid PDA (label expiry 7/2001)
Oxoid MEA (label expiry 2/2001)
Homemade PDA (cv. 'Nadine')

Media were prepared as per the manufacterurs' recommendation.

Incubation/Light Conditions:

5°C 24 h dark
10°C 24 h dark
15°C 24 h dark
20°C 24 h dark
25°C 24 h dark

This experiment was carried out as for Experiment 1 except that;

1. Old potato tubers were available for the homemade PDA as recommended by CMI Plant Pathology Pocketbook.
2. 30°C was not used since there was no growth at this temperature at Experiment 1.
3. There were 3 replicate plates for each isolate/medium combination.

3. Diameter across the plug was measured on Day 2 and subtracted from the growth attained at Day 6 to calculate daily radial growth rate.
6. A three factorial ANOVA was performed on data from Cc3, Cc3Reisolated and Cc6. Growth data from Cc9 and Cc10 were removed from the analysis because the assumptions of normality of distribution and constant variance of residuals could not be met with any transformation because more than 50% of the inoculation plugs failed to establish for no apparent reason.

2.4 RESULTS

2.4.1 Isolation of *C. camelliae* from Petals and Sclerotia

No effort was made to identify 'other' fungi recovered from diseased petals, although *Botrytis cinerea* was frequently found.

C. camelliae was not isolated from diseased petals. There was no difference between non-sterilised and surface sterilised treatments, or age of petal infection.

C. camelliae was not isolated from pieces of surface-sterilised sclerotia. *Penicillium* spp. contamination was common.

C. camelliae was consistently isolated from small pieces of tissue taken from the sclerotial medulla. None was contaminated and >80% of sclerotial tissue pieces plated out established colonies. Isolations from individual sclerotia were labeled as Cc1 (*C. camelliae* sclerotium no. 1), Cc2, Cc3, Cc5, Cc6, Cc9 and Cc10. Isolates Cc1, Cc2, Cc3 and Cc5 were routinely used in other experiments.

2.4.2 Effect of Temperature and Growth Medium on Growth of *C. camelliae*

2.4.2.1 Experiment 1 - Growth of four isolates, on six media at six temperatures

There was strong evidence for differences between isolates ($F_{3, 75}=5.08$, $P=0.0003$), for differences between media ($F_{5, 75}=29.21$, $P=0.0001$), for differences between temperature ($F_{5, 75}=107.6$, $P=0.0001$) and that differences between media are

dependent on temperature (F25, 75=6.28, P=0.0001). There was some evidence that the differences between isolates depends on media (F15, 75=1.89, P=0.0373), but no evidence that the difference between isolates depended on temperature (F15, 75=1.58, P=0.101).

The only significant difference between growth of isolates occurred on Oxoid PDA (old), where the growth rate of Cc1 was significantly less than that of Cc2, Cc3 and Cc5 (Figure 2.1) on the same medium. On all other media, there was no significant difference between the growth rate of the isolates.

Growth of all isolates was significantly greater on Oxoid PDA (new) and Difco PDA than on Merck PDA (Figure 2.1). Growth of isolates Cc2, Cc3 and Cc5 was significantly greater on Oxoid MEA than on Merck PDA.

Growth of all isolates showed a similar response to temperature (Figure 2.2). There was little or no growth at 5°C, some growth at 10°C and a peak growth rate at 15°C. Above 15°C there was a steady decline in growth rate to none at 30°C.

The overall pattern of growth on agar media was not affected by temperature although media could be separated into two groups: those where the peak growth was at 20°C (Oxoid MEA and Difco PDA) and the remainder where peak growth was at 15°C (Figure 2.3).

Differences in growth on the various media were dependent on temperature (Figure 2.3). Growth on Homemade PDA was greater than Oxoid PDA (old) at all temperatures except 20°C.

The difference between isolates did not depend on temperature (Figure 2.2).

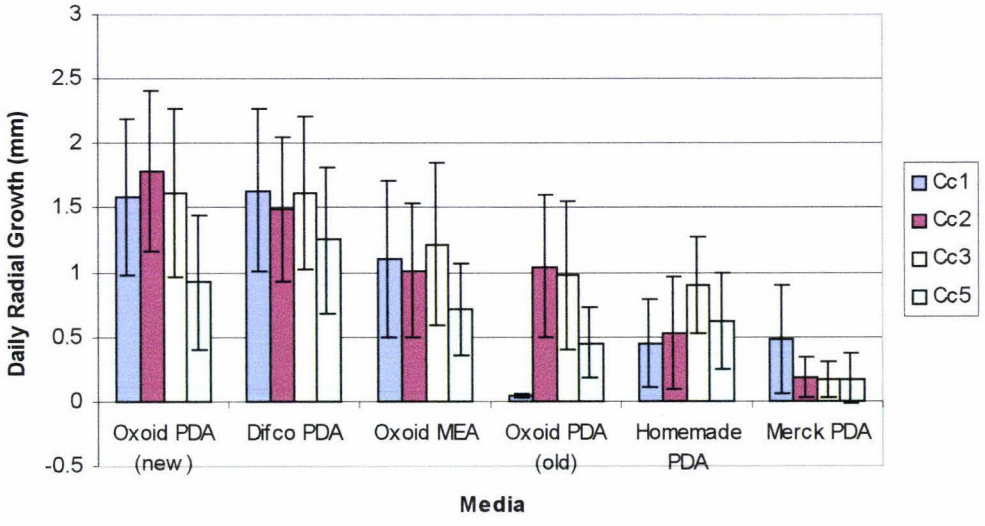

Figure 2.1
Mean isolate growth over all temperatures on each medium

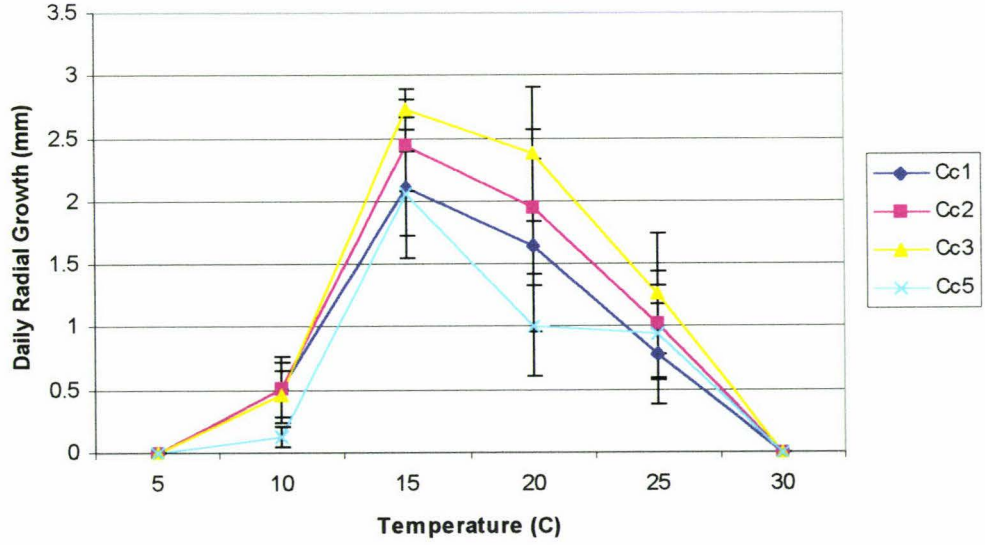

Figure 2.2
Mean isolate growth over all media at each temperature.

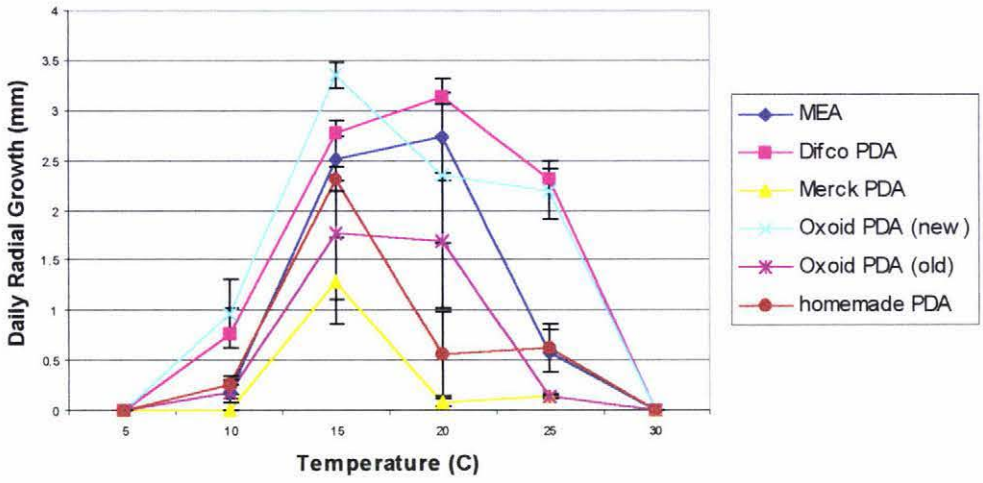

Figure 2.3
Mean isolate growth on each medium at each temperature.

2.4.2.2 Experiment 2 - Growth of four isolates, on six media at five temperatures

There was strong evidence for significant differences between isolates (F3, 240=946.92, P=0.0001), between media (F5, 240=318.31, P=0.0001), for differences between temperature (F4, 240=110.99, P=0.0001), that differences between isolates were dependent on medium (F15, 240=41.53, P=0.0001), that differences between isolates were dependent on temperature (F12, 240=18.39, P=0.0001), that differences between media were dependent on temperature (F20, 240=23.4, P=0.0001) and that the differences between isolates were dependent on media and temperature (F60, 240=11.42, P=0.0001).

Differences between the growth of isolates are shown in Figures 2.4 and 2.5. The growth of Cc1 and Cc3 were not significantly different from each other over all temperatures (Figure 2.4) and on all media except Merck PDA on which growth of all isolates was poor (Figure 2.5). Growth was significantly greater than that of either Cc2 or Cc5 over all temperatures and on all media, except Cc5 on Oxoid PDA (old) (Figure 2.5). Isolates Cc2 and Cc5 were significantly different from each other over all temperatures (Figure 2.4) with Cc2 having the lowest growth rate of all four isolates.

The interaction between temperature and isolate was because isolate Cc2 did not show a peak growth at 15°C or 20°C.

For Cc1 and Cc3, the highest growth rate occurred on Oxoid PDA (new) followed by Difco PDA, Homemade PDA, Oxoid MEA, Merck PDA and Oxoid PDA (old). The difference in growth of these two isolates on Oxoid PDA (new) and Difco PDA was not significant, nor was that between Difco PDA and Homemade PDA, but the difference between Oxoid PDA (new) and Homemade PDA was significantly different. Isolates Cc2 and Cc5 were more variable (Figure 2.5).

Averaged over all isolates, the differences between media were more complex (Figure 2.6). Peak growth was at 20°C on all media except Merck PDA where it was at 10°C. Growth on Oxoid PDA (old) was consistently poor and that on Merck PDA was little better except at 10°C and at 15°C.

The media/isolate interaction was because isolate Cc3 grew significantly better than isolate Cc1 on Merck PDA but Cc1 grew significantly better than Cc3 on Oxoid PDA (old).

On most agars the fungus grew best at 20°C but on Merck PDA it grew best at 10°C, on Oxoid PDA (old) there was no peak, and growth on Homemade PDA was greatly reduced at 25°C compared with the other media.

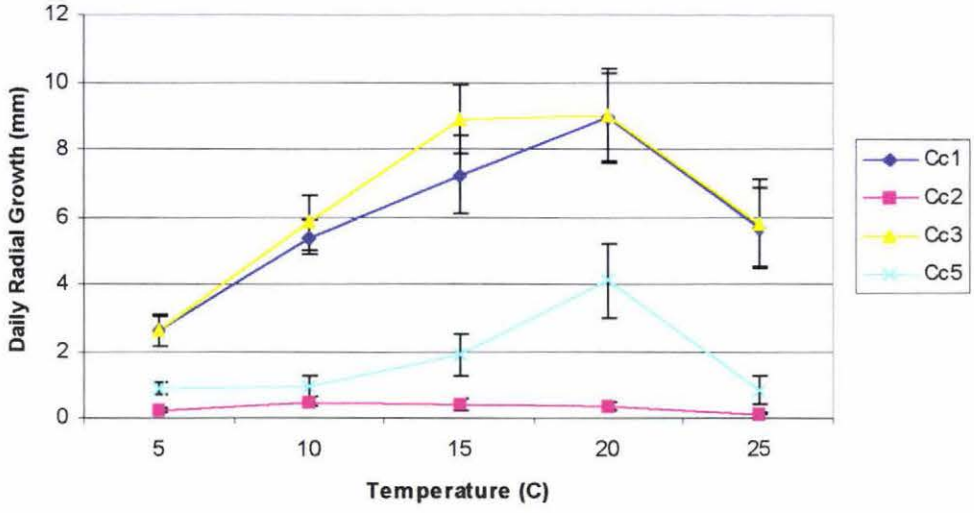

Figure 2.4
Mean isolate growth over all media at each temperature.

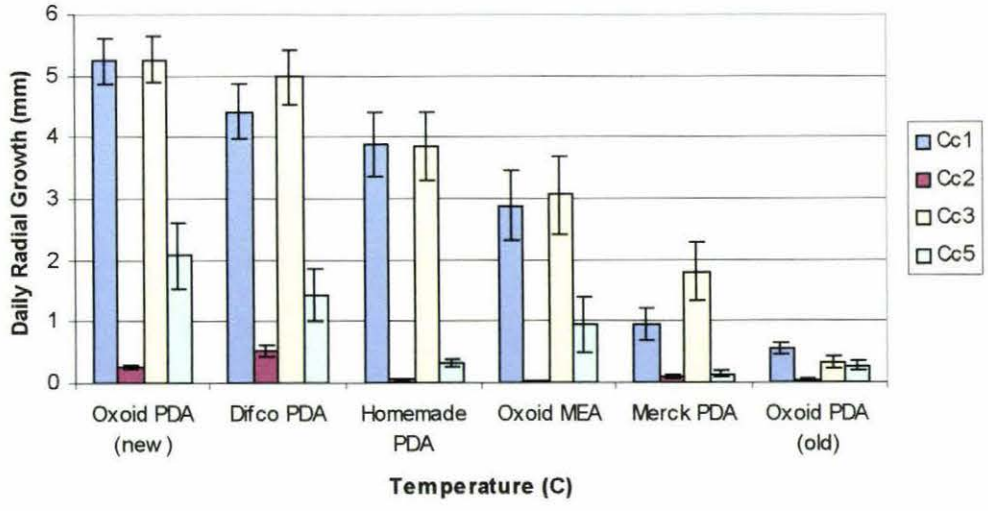

Figure 2.5
Mean isolate growth over all temperatures on each medium.

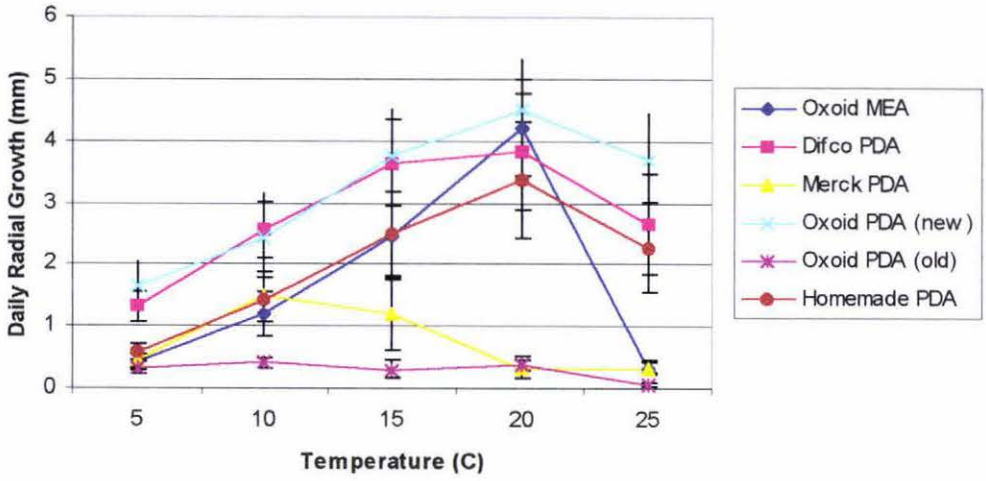

Figure 2.6
Mean isolate growth on each medium at each temperature.

2.4.3 Effect of Age of Agar on Growth of *C. camelliae*

2.4.3.1 Experiment 3 – Growth of two isolates on six agars of Difco PDA of different age

There was strong evidence for differences between isolates (F1, 90=97.45, P=0.0001) and media (F5, 90=4.07, P=0.0022), but no evidence that the difference between isolates depended on age of the media (F3, 90=2.24, P=0.0894). Isolate Cc1 grew consistently better than Cc3, growth was best on agar of 4 d and 7 d old (no significant difference). There was no significant difference between growth on agars of 0 d, 14 d, 21 d and 50 d old. The Duncan's groupings are shown in Table 2.1.

Table 2.1
Duncan's Test for Significant Differences between Isolates and Media of *C. camelliae* grown on Difco PDA of Six Ages

Duncan's Grouping†	**Mean**	**N**	**Isolate**
a	2.14	60	1
b	1.76	40	3
Duncan's Grouping†	**Mean**	**N**	**Media**
a	2.31	10	7 d
a	2.26	10	4 d
b	1.97	20	0 d
b	1.91	20	14 d
b	1.89	20	21 d
b	1.88	20	50 d

† within each section of the table numbers followed by the same letter are not significantly different by Duncan's test (P<0.05)

2.4.4 Effect of Camellia Petals or Iron Supplements on Growth of *C. camelliae*

2.4.4.1 Experiment 4 - Growth of four isolates on ten petal agars at one temperature

There was strong evidence for differences between isolates (F3, 88=688.06, P=0.0001), for differences between camellia agars and plain Difco PDA (F2, 88=19.57, P=0.0001), for differences between agar preparations (F4, 88=27.91, P=0.0001), that differences between isolates was dependent on camellia species (F6, 88=0.0001), that differences between isolates was dependent on agar preparation (F12, 88=9.25, P=0.0001), that differences between camellia species was dependent on agar preparation (F4, 88=3.82, P=0.0066) and that the difference between isolates was dependent on camellia species and agar preparation (F12, 88=2.74, P=0.0034).

On all camellia agars, the rate of growth of Cc5 was significantly less than the growth of Cc1, Cc2 and Cc3 (Figure 2.7). The difference in growth rate between the latter isolates depended on camellia agar and the method of preparation. The interaction between isolate and petal agar preparation and camellia species is shown in Figures 2.8 and 2.9. On *C. japonica* agars, isolate Cc1 and 3 grew significantly better on propylene oxide-sterilised petals than Cc2, but the situation was reversed on the control, autoclaved and boiled petal agars (Figure 2.8). The same pattern was found with agars incorporating petals of *C. sasanqua* (Figure 2.9).

Averaged over all isolates, the growth rate was generally greater on camellia-based agars than on non-supplemented Difco PDA (Figure 2.10). There was no significant difference between growth on *C. sasanqua* petal agars or on *C. japonica* petal agars because of the effect of averaging over all isolates. For isolates Cc2 and Cc3, growth was greater on *C. sasanqua* agar that had been prepared using the water in which the petals had been boiled than on *C. japonica* agar prepared in the same way. Similarly, Cc1 and Cc3 grew better on *C. sasanqua* petals that had been autoclaved than on autoclaved *C. japonica* petals.

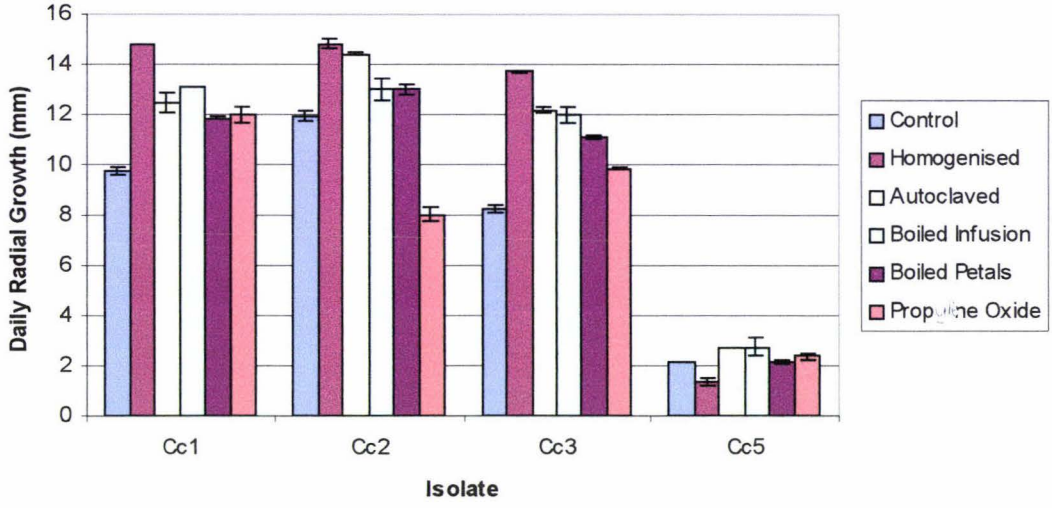

Figure 2.7
Mean isolate growth over both camellia species by isolate.

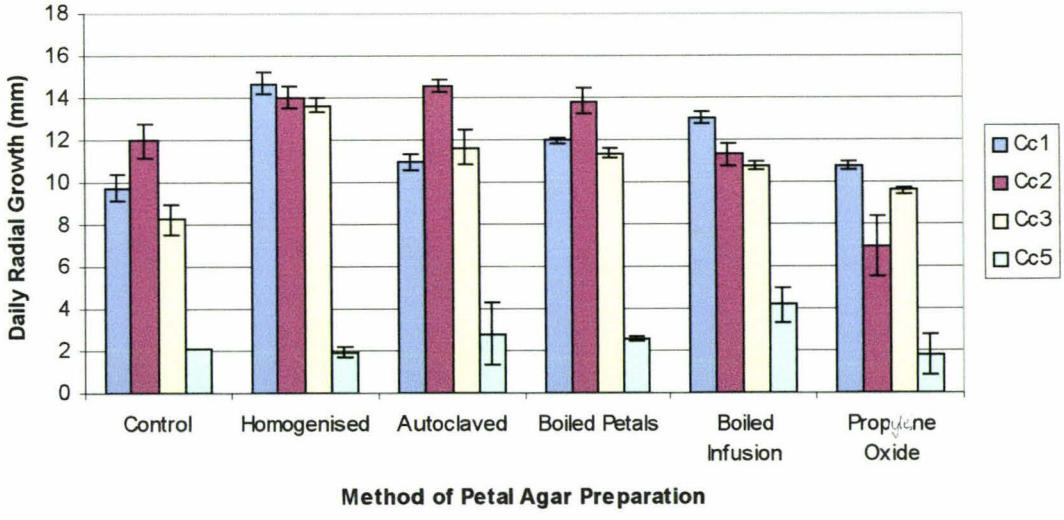

Figure 2.8
Mean isolate growth on petal agars of *C. japonica*.

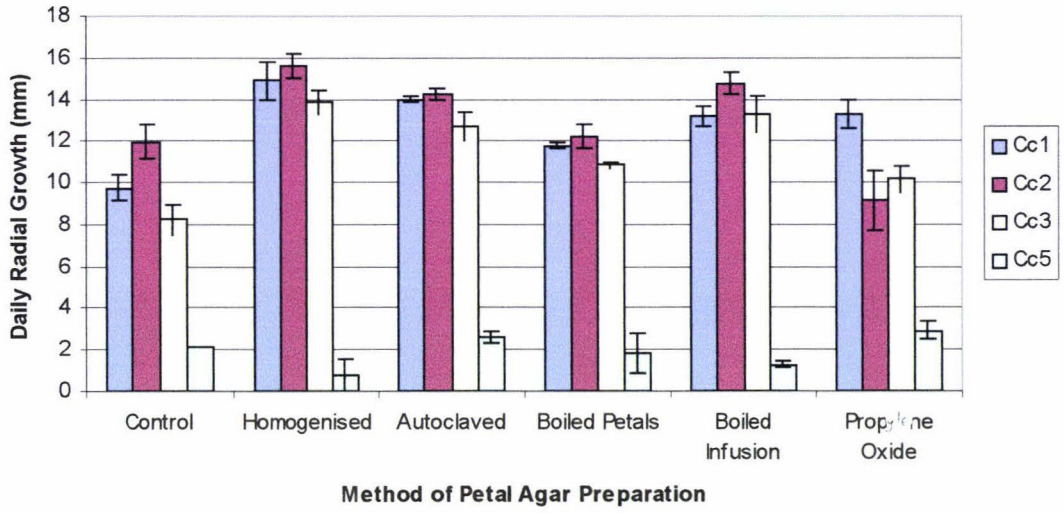

Figure 2.9
Mean isolate growth on petal agars of *C. sasanqua*.

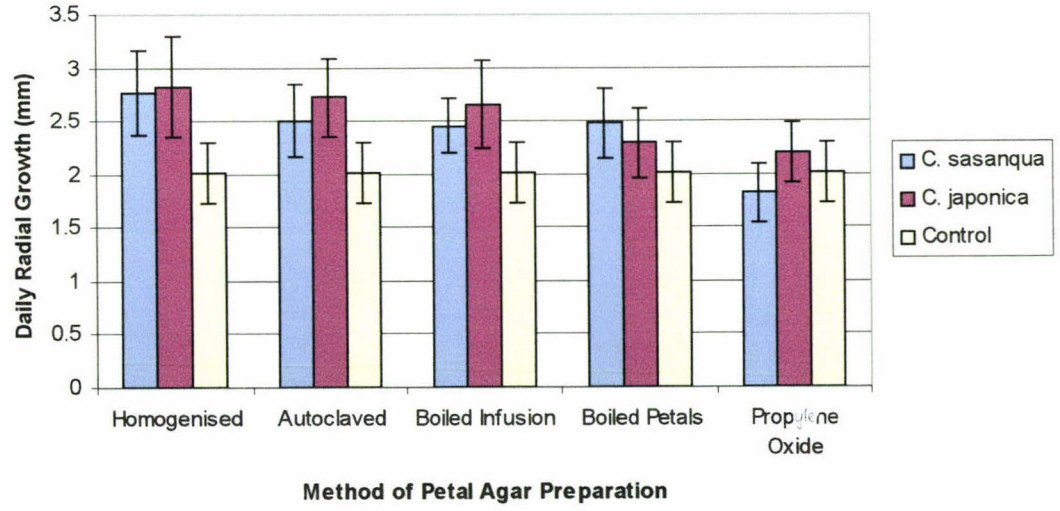

Figure 2.10
Comparison of mean isolate growth on camellia agars and control.

2.4.4.2 Experiment 5 - Growth of four isolates agars amended with two concentrations of iron

No consistent pattern of fluorescence was observed in these treatments. Many control and ferric iron-amended agars showed at least some fluorescence. Of these, most showed slight fluorescence (Figure 2.11), but two isolate/iron combinations (Cc3 on Difco PDA + 1.0 μm and Cc1 on Oxoid PDA + 0.1 μm) were strongly fluorescent. There was no apparent link between fluorescence and agar type (Table 2.2), but there may be a link between fluorescence and isolate, with Cc3 showing fluorescence in 10/11 samples but Cc5 on only 2/11.

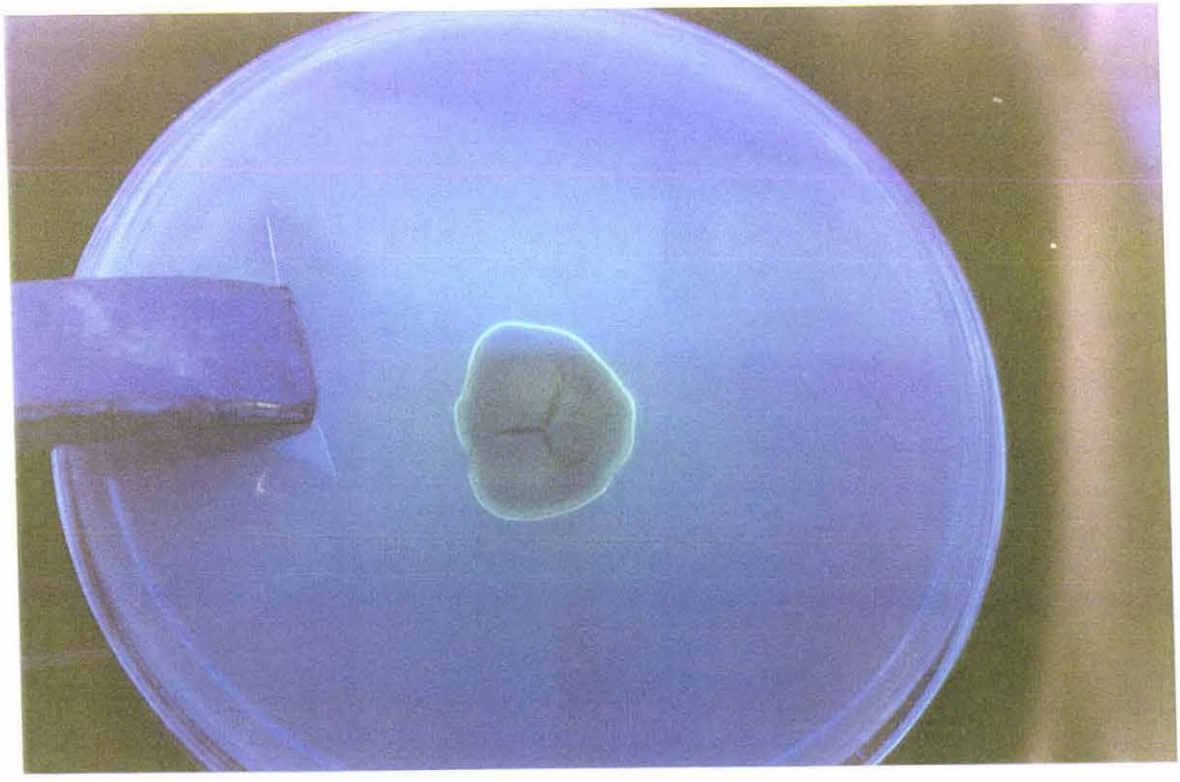

Figure 2.11
Isolate Cc1 on non-supplemented Difco PDA. Example of the'slight fluorescence' exhibited by many of the isolate/iron combinations.

Table 2.2
Fluorescence of *C. camelliae* Isolates by Agars Amended with Ferric Iron

	Difco PDA				**Merck PDA**				**Oxoid PDA**				**Homemade PDA**			
	Isolate				**Isolate**				**Isolate**				**Isolate**			
	1	**2**	**3**		**1**	**2**	**3**		**1**	**2**	**3**		**1**	**2**	**3**	
	5				**5**				**5**				**5**			
Control	0	0	1	0	1	1	1	0	1	1	0	0	0	0	1	0
0.1 μm Ferric Iron	1	0	1	1	0	1	1	0	2	0	1	0	0	1	1	0
1.0 μm Ferric Iron	0	1	2	0	0	1	1	1	1	1	1	0	-	-	-	-

0=no fluorescence
1=slight fluorescence
2=fluorescence
-no data

2.4.5 Effect of Repeated Sub-Culturing on Pathogenicity

2.4.5.1 Experiment 6 - Growth of a repeatedly sub-cultured isolate, a sub-cultured isolate recovered after infecting a petal, and three freshly isolated isolates on seven media at five temperatures

There was strong evidence for differences between isolates (F4, 350=168.69, P=0.0001), between media (F6, 350=578.11, P=0.0001), between temperatures (F4, 350=254.6, P=0.0001), that differences between isolates is dependent on media (F24, 350=46.27, P=0.0001), that differences between isolates was dependent on temperature (F16, 350=8.57, P0.0001), that the differences between media were dependent on temperature (F24, 350=37.74), P0.0001) and that the difference between isolates was dependent on media and temperature (F96, 350=13.54, P0.0001).

Growth was lowest at 5°C, progressively increased at 10°C, 15°C and 20°C and decreased at 25°C (Figure 2.12). The poor growth of isolate Cc6 at 25°C gave the temperature and isolate interaction.

All isolates grew well on Oxoid PDA (new) and Difco PDA and poorly on Oxoid PDA (old), LM and and Merck PDA (Figure 2.13). On Homemade PDA growth of

Cc3R was poor and on Oxoid MEA growth of Cc3R and Cc6 was poor.

On most media, growth was greatest at 20°C except Merck at 10°C. Media could be divided into three groups, with good growth on Oxoid PDA (new) and Difco PDA, moderate growth on Homemade PDA and poor growth on the remaining agars (Figure 2.14).

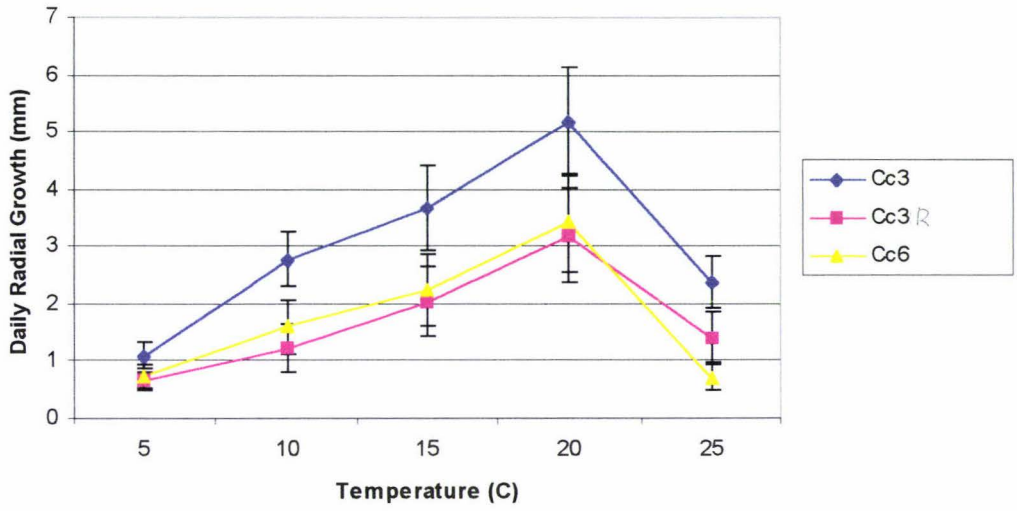

Figure 2.12
Mean isolate growth over all media at each temperature.

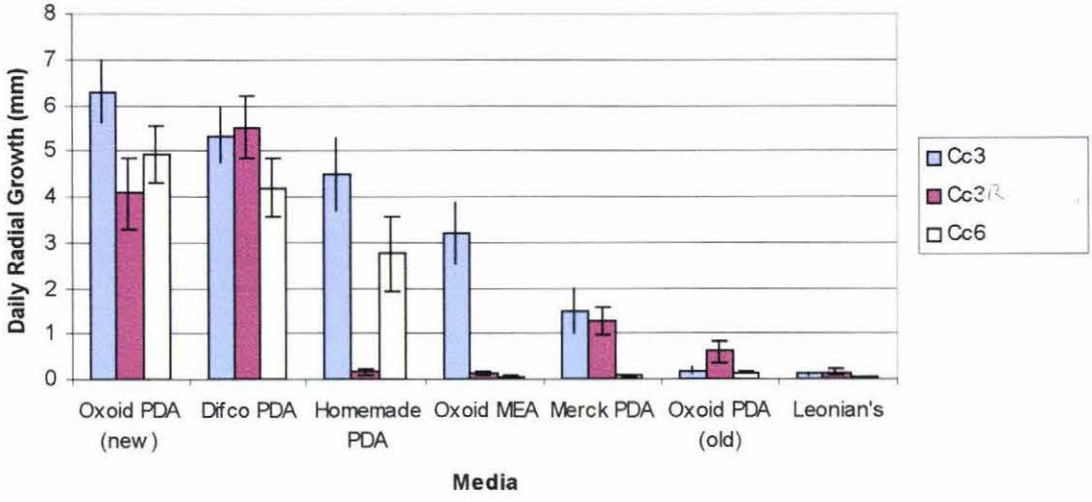

Figure 2.13
Mean isolate growth over all temperatures on each medium.

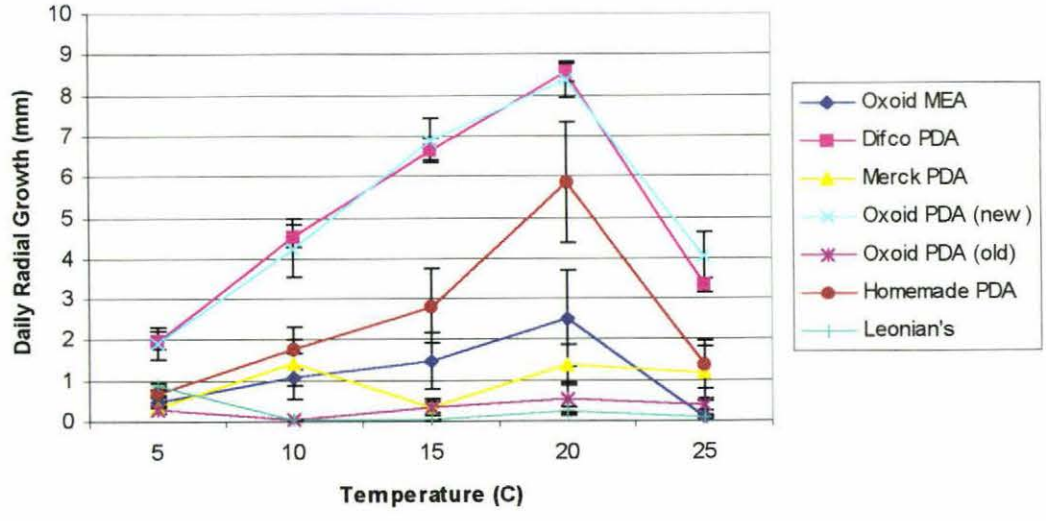

Figure 2.14
Mean isolate growth on each medium at each temperature.

Figure 2.15 shows an atypical colony grown on Difco PDA at 20°C.

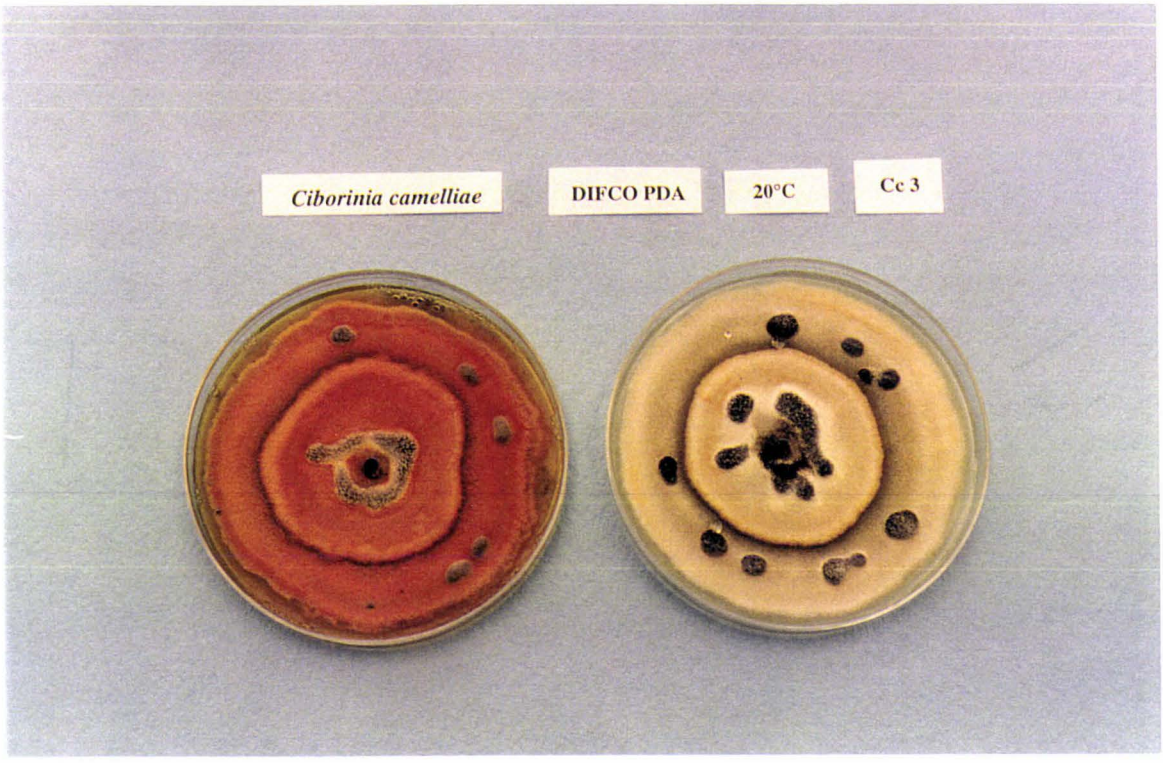

Figure 2.15
Atypical colony colour (left) of *C. camelliae* grown on Difco PDA at 20°C.

2.5 DISCUSSION

The fungus was successfully and consistently isolated from the sclerotial medulla only but isolations from sclerotial material that included rind were all contaminated, despite a harsh surface sterilisation with sodium hypochlorite. This indicates that the rind contains other bacterial and fungal populations that were not killed by the sterilisation procedures used, probably because they were protected within the rind. Fullerton et al (1988) reported that only "recently blighted flowers [should] be used for isolations" as older infections were associated with a range of other saprophytic fungi which overgrew *C. camelliae*. Nevertheless, *C. camelliae* was never isolated from diseased petals, regardless of sterilisation procedure or age of petal infection, in the current study. Plakidas (1950), Anzalone (1959), Alford (1961), Zummo (1960) and Alford & Sinclair (1962) did not specify the age of infected petals used for isolations.

The appearance of this fungus in culture agrees with that described by Hansen & Thomas (1940) and Kohn & Nagasawa (1984). Figure 2.16 shows the typical grey and white felty mycelium of a colony grown on Difco PDA. There was, however, variation both in colony form and colour, for example on Homemade PDA and Merck PDA growth was frequently wedge-shaped, mycelium of colonies grown on Homemade PDA was dark grey, sclerotia were not produced on oatmeal agar (data not cited) and, rarely, colonies were typical in every respect but the mycelium was orange in colour (Figure 2.15). *C. camelliae* had two distinct growth flushes. At ~30 mm diameter, the colony ceased to grow and sclerotia formed in a circular pattern about the inoculation plug. The colonies began their second growth period ~48 h later (quite variable), and stopped once the edge of the dish was reached. Sclerotia also formed in this outer ring, though they were often smaller in size than those formed in the centre and they rarely coalesced.

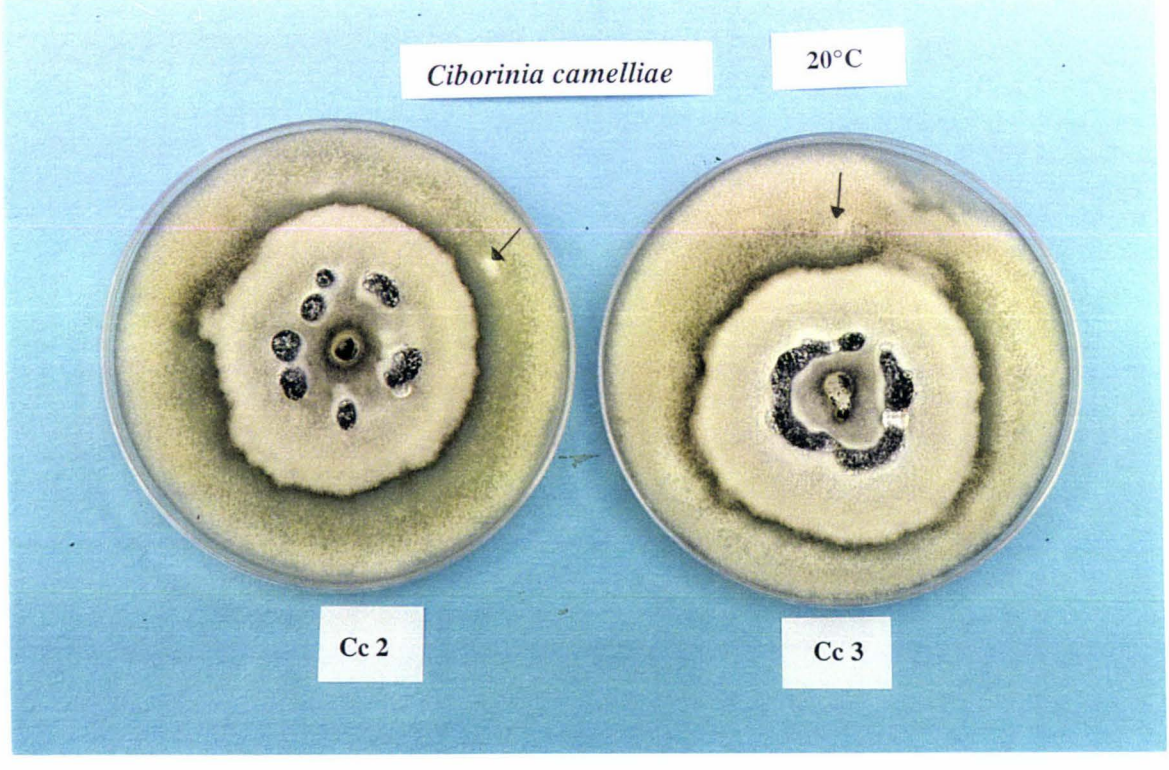

Figure 2.16
Typical features of colonies of *C. camelliae*. Isolates Cc2 and Cc3 grown on Difco PDA at 20°C for 16 d. Fully formed black sclerotia are seen in the centre of both colonies, while the sclerotial initials are just visible in the outer rings (black arrows).

Unlike Alford (1961), Anzalone (1959) and Zummo (1960) there was no difficulty in maintaining the fungus in culture nor did it lose the ability to form sclerotia as reported by Fullerton et al (1998). Thirteen months after they were first isolated (January 1998), Cc1 and Cc3 continue to grow well and form sclerotia and are the most frequently used isolates in this study. Isolate Cc2 tends to be more variable and sometimes establishes colonies, but sometimes fails to grow for no apparent reason when inoculated on to agar plates. Although it continues to form sclerotia, it is not used as frequently because of this capricious behaviour. The growth of Cc5 was consistently poor, sclerotia did not often develop, but it did form abundant microconidia.

Sub-culturing did not appear to affect the growth of Cc3 (Experiment 6). It out-performed Cc3R on several media (Oxoid PDA new, Homemade PDA, Oxoid MEA) and at most temperatures. Cc3 continues to be used in infection studies (Chapter 5) with no apparent loss of pathogenicity. Sub-culturing, however, does appear to have affected the ability of Cc5 to form typical mycelial colonies and sclerotia. In Experiment 1 (begun 19.2.1998), Cc5 formed typical colonies (Figure 2.17), but by Experiment 2 (begun 29.6.1998) colony growth and sclerotial formation was much reduced.

In Experiments 1 and 2, differences between growth of isolates, the effect of temperature on growth, effect of medium on growth and the interaction between these three factors. Overall, Cc1 and Cc3 had a greater growth rate than the other isolates examined. The growth of Cc5 was consistently poor when compared to the growth of other isolates, although on some media (e.g. LM and Merck PDA) growth of all isolates was poor. The greatest growth occurred on Oxoid PDA (new), Difco PDA, Homemade PDA and Oxoid MEA. Growth was greatest between 15°C and 20°C. At 25°C growth tended to be less regular in form, colour change was more frequent and fewer sclerotia formed. There is some variation in the conditions used for culture of *C. camelliae* reported by other workers. Barnett & Lilley (1948) report that the best mycelial growth occurred at 25°C; Johnson (1971) cultured it at 20°C and Hansen & Thomas (1940) between 15°C -18°C. The cultures of Baxter et al (1987) did not form sclerotia above 22°C.

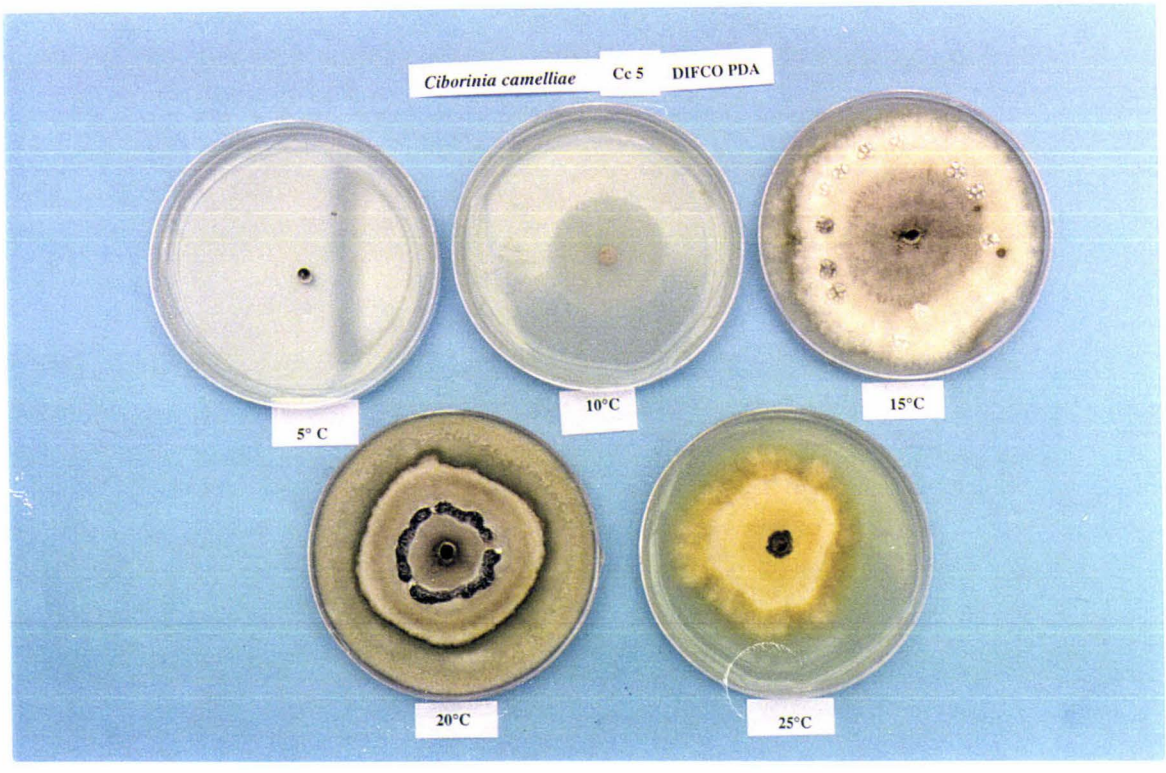

Figure 2.17
Colonies of *C. camelliae* isolate Cc5 grown on Difco PDA at five temperatures for 14 d. Growth at 5°C and 10°C is limited, at 15°C and 20°C mycelium has reached the plate edge and ceased growth. Colony form is typical at 20°C but at 25°C, the colony growth has become irregular and orange in colour.

Likewise, variation in growth on different media has been noted. Fullerton et al (1998) reported radial daily growth on Difco PDA as 2.7 mm (21°C) and Kohn & Nagasawa (1984) as 6 mm (18°C) compared with 6.5 mm (Cc3), 2.27 mm (Cc5) and 0.62 mm (Cc2). In Experiment 2 Fullerton et al (1998) found the fungus did not grow readily on MEA, but Oxoid MEA was one of the best media in the current study and Kohn & Nagasawa (1984) report a radial daily growth of 4.3 mm.

The variation in optimum conditions reported for the culture of *C. camelliae* indicates isolates of the fungus vary in their response to environmental conditions. When culturing the fungus for the first time, it may be necessary to establish optimum conditions (media and temperature) for each culture. It would be interesting to establish whether this variation could be correlated with the ecotype of the fungus – whether isolates from temperate populations grow better at higher temperatures than those from cold-winter populations. However this speculation requires much more information

about the fungus than is currently available.

Although significant interactions between the three factors occurred, these interactions were not consistent between experiments. Minor variation in external conditions between experiments may have occurred, but it is believed that it is more likely that these inconsistencies are due to natural biological variation in the performance of the fungus. During routine sub-culturing similar inconsistencies were also noted.

The age of the Difco PDA preparation has been shown to affect the growth of *C. camelliae*. Growth was greatest on preparations between 4 and 7 d old. This result must be considered when conducting further growth experiments and when reviewing these growth experiments. Because of the scale of some experiments, agars were prepared up to 10 d in advance, so that at inoculation, the age of the agars could vary between 0 and 10 d old. However, the difference between the best growth and least growth was very small and unlikely to have significantly altered the findings of the growth experiments. The effect of agar age may be more important when working with difficult-to-culture isolates.

Agars amended with a variety of petal preparations did increase the growth rate compared to Difco PDA but the growth rate on Difco PDA was also high compared with other experiments. On *C. sasanqua* preparations, for example, growth of Cc1 was 10.7 mm/d on propylene oxide-sterilised petal agar, 14.66 mm/d on homogenised petal agar and 9.75 mm/d on Difco PDA. The most rapid growth obtained by Fullerton et al (1998) was also on agars amended with a petal infusion base. Their method of petal agar preparation differed to those used in Experiment 4, and the greatest growth rate was 4.72 mm/d on an agar made with camellia petal infusion, dextrose and agar (2.7 mm/d Difco PDA). While there was some variation in growth between isolates on *C. sasanqua* and *C. japonica* agars, overall, either species would be acceptable for use in camellia agar preparation.

In the initial tests of growth on different media (Experiment 1) some colonies grown on Merck PDA showed varying degrees of fluorescence. Fluorescence has been demonstrated in a number of Pseudomonad bacteria which produce siderophores – iron-

chelating substances – where iron is a limiting factor (Bakker et al 1993). Four PDA media were amended with ferric iron and the growth of colonies tested for fluorescence. The production of siderophores by *C. camelliae* was not proven in this experiment. Higher levels of fluorescence would be expected in the control PDA preparations if siderophores were produced in response to low iron levels. However, the results of this experiment to do not support this theory. There was no apparent correlation between the occurrence of fluorescence and the level of iron in the PDA preparation and colonies on Merck PDA did not fluoresce more than colonies on other PDA preparations. However, there may be a link between the occurrence of fluorescence and isolate. This experiment did not have replicates; a larger experiment would be required to determine whether siderophores are produced by *C. camelliae* and if so whether this is in response to low iron levels. The issue of fluorescence and siderophores was not pursued because it did not appear to be of significance to the culture of *C. camelliae.*

These experiments demonstrate that isolate, media and temperature all influence variation in growth of *C. camelliae.* Optimum temperature for growth and sclerotial formation of these isolates was between 15°C and 20°C. *C. camelliae* appears to be sensitive to the medium on which it is grown. Camellia petal supplements increase the growth rate, but petals are not available year-round and the benefits do not outweigh the extra time involved in preparation of supplemented agar media. The brand of PDA is important. At the beginning of this work, Merck PDA was the standard PDA used and this accounted for the initial difficulty in establishing cultures of the fungus.

CHAPTER THREE

DISEASE SURVEY IN NEW ZEALAND

3.1 INTRODUCTION

Camellia flower blight was first identified in New Zealand in September 1993. It is not known whether sclerotia or infected flowers were the original inoculum source, but its introduction - accidental or not - was not prevented by quarantine regulations.

The initial identification was made in a Wellington suburban garden by Stewart & Neilson (1993). From the amount of disease in the garden, Stewart & Neilson (1993) thought it had been there at least two previous seasons and they also concluded that it was likely to have already spread.

A survey conducted by the Ministry of Agriculture and Fisheries (MAF) showed that the disease was widespread in Wellington (Hill 1993). Fifty-four properties were visited, 49 within Wellington, two in Upper Hutt, and one each at Paraparaumu, Shannon and Fielding. Of the 49 properties visited in Wellington, 33 were found to have camellia plants infected with *C. camelliae*. The disease was not found outside Wellington, although only five sites outside the city were examined.

By 1996, the disease was known to be present in Waikanae, Wanganui and Kauangaroa (Neall et al 1998). These outbreaks of infections were believed to be the result of transport of infected plant material or contaminated soil to each site.

In September 1997, a diseased flower was found in a garden in Palmerston North and in a limited survey of camellias in the city, diseased flowers were found at a further four sites, including the Arboretum at Massey University. The extent and amount of the disease indicated that it had been present, (but unobserved), more than one season.

Were these infections the result of introduced infected plant material or contaminated soil, or was the problem more pervasive than previously thought? To answer this question, surveys were conducted in 1997 and 1998 with the objective of determining the distribution of the disease in New Zealand and to assess the method(s) and rate of spread.

3.2 MATERIALS AND METHODS

Previous surveys for this disease have been conducted in the United States (Hansen & Thomas 1940; Richmond 1949; Gill & Ridley 1954; Winstead et al 1954; Plakidas 1955; Anzalone & Plakidas 1958; Alford 1961; Alford et al 1961; Cochran 1962), but the methods used in these surveys were not reported.

This survey was designed to determine the distribution of the disease in both North and South Islands. Surveys were conducted during the disease seasons in 1997 and 1998. The first survey was conducted in spring 1997 and covered the lower half of the North Island. The second survey was done in the spring of 1998, and covered the South Island and much of the North Island south of Auckland.

The number of camellia plants examined in each city or town varied depending upon size of the locality and availability of camellias. A subjective decision was made at each site when it was felt sufficient plants had been examined to fairly represent whether the disease was present or not. A review of the literature indicated that the disease distribution is often patchy, even in areas where it has been present for several years (Hill 1993). In view of this, and of my 1997 survey experience, the 1998 survey method was modified so that in the larger towns and cities, were sampled from each quadrant (north, south, east and west). This strategy had two benefits: first, it increased the chances of discovering the disease, especially if is was only present in small amounts in a restricted area and second, in places were the disease was found, the local distribution could be evaluated.

Survey routes were the main state highways in each Island and on some backroads. Stops were made at each town or city along the route to examine camellia plants. Rural properties were also checked where possible.

Camellia plants in public places such as road verges, churchyards, public parks and gardens were surveyed together with those in private gardens, where the plants could be examined without entering private property (e.g. where plants were adjacent to footpaths). In addition, many gardens of Camellia Society members, their friends and relations, were inspected.

Disease identification was made on the basis of signs and symptoms exhibited by the flower. Blight-infected flowers were easily distinguished by colour from other flower rots caused by pathogens, saprophytes and weather damage. Blight-infected flowers have a characteristic grey-brown rot and the symptoms spread upwards from the base of the flower. Browning symptoms caused by other factors are typically orange-brown, and the pattern of browning generally differs. The characteristic ring of grey or white mycelium around the base of the flower was also evident, except in petal-shattering camellia varieties. Positive identification of the disease was made by the presence of sclerotia in the petals. In all cases where the disease was identified, or suspected, samples were taken back to the laboratory and held under suitable conditions for sclerotial formation. Alford (1961) and Alford et al (1961) made positive identifications only if they found apothecia or sclerotia, but the sclerotia alone were considered sufficiently distinctive to be used for a positive identification here.

3.3 RESULTS

For reasons of scale the points marked on the maps illustrated in Figures 3.1, 3.2 and 3.3 are indicative only and not all sites could be individually marked. Full details of survey sites are given in Appendix III, while a summary of the number of camellia plants examined in each town or region and the number which were or were not found to be diseased is given in Appendix IV.

The distribution of the disease found in the 1997 survey of North Island is shown in Figure 3.1. The disease was found to be widespread in the lower, central and western North Island. From Wellington, the disease was found at frequent intervals up the west coast to New Plymouth. It was not found round the western side of Mt Taranaki, from Manaia in the south to New Plymouth in the north. The most northern point that the disease was found on State Highway 3 was at Urenui. From Wellington eastwards, the disease was found in Upper Hutt, Kaitoke, over the Rimutakas and into the Wairapapa, as far north as Paihiatua. It was not found in several towns (e.g. Ekatahuna). Inland it was found at several sites, including Fielding, Kimbolton, Marton, Mangaweka, a property 2 km south of Taihape, Turangi and Hatepe (on the eastern shore of Lake Taupo). Hatepe was the most northern point in the North Island at which the disease was found.

The disease was not found in the Hawkes Bay, or in the areas of Taumaranui, Te Kuiti, Hamilton, Tauranga, Rotorua or Tokoroa. The Auckland region was not surveyed.

The 1998 survey of the North Island showed the disease has spread further round Mt Taranaki to Opunake and Okato. It was also found in Woodville, Taihape and Taupo with an isolated outbreak detected in Mt Eden, Auckland. These new infection sites are shown in Figure 3.3.

The disease was not found in the Hawkes Bay, or in the areas of Taumaranui, Te Kuiti, Hamilton, Tauranga, Rotorua or Tokoroa.

The 1998 survey (Figure 3.3) showed that the disease was widespread in the northern half of the South Island. It was present in Picton, Blenheim, Nelson, Stoke, Richmond, Motueka and many sites in Golden Bay. It was found in six suburbs in Christchurch (Monavale, Sumner, Redcliffs, Heathcote, Fendalton and Bryndwyr) and a single infected flower was found at Kaiapoi.

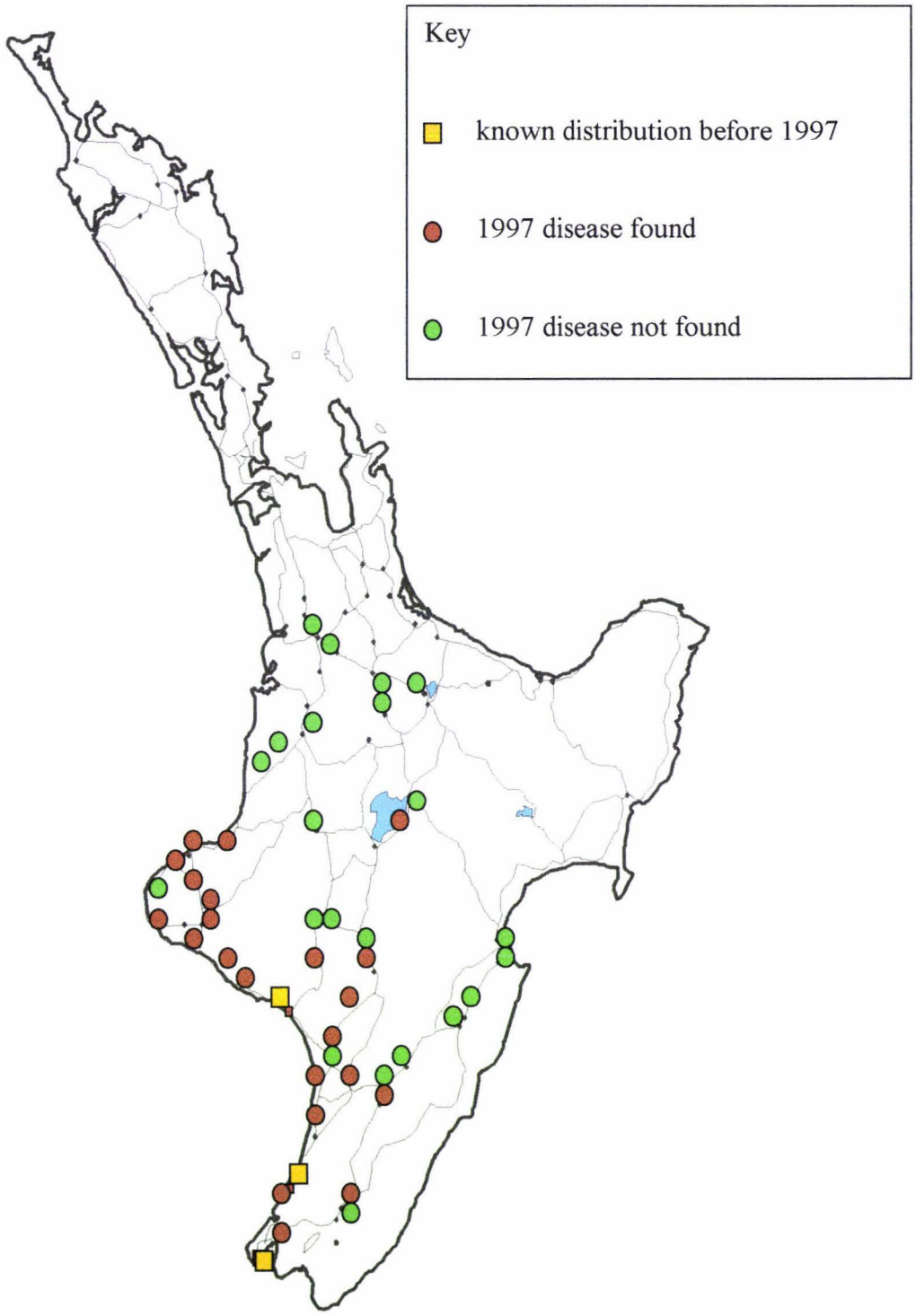

Figure 3.1 Distribution of *C. camelliae* in the North Island 1997.

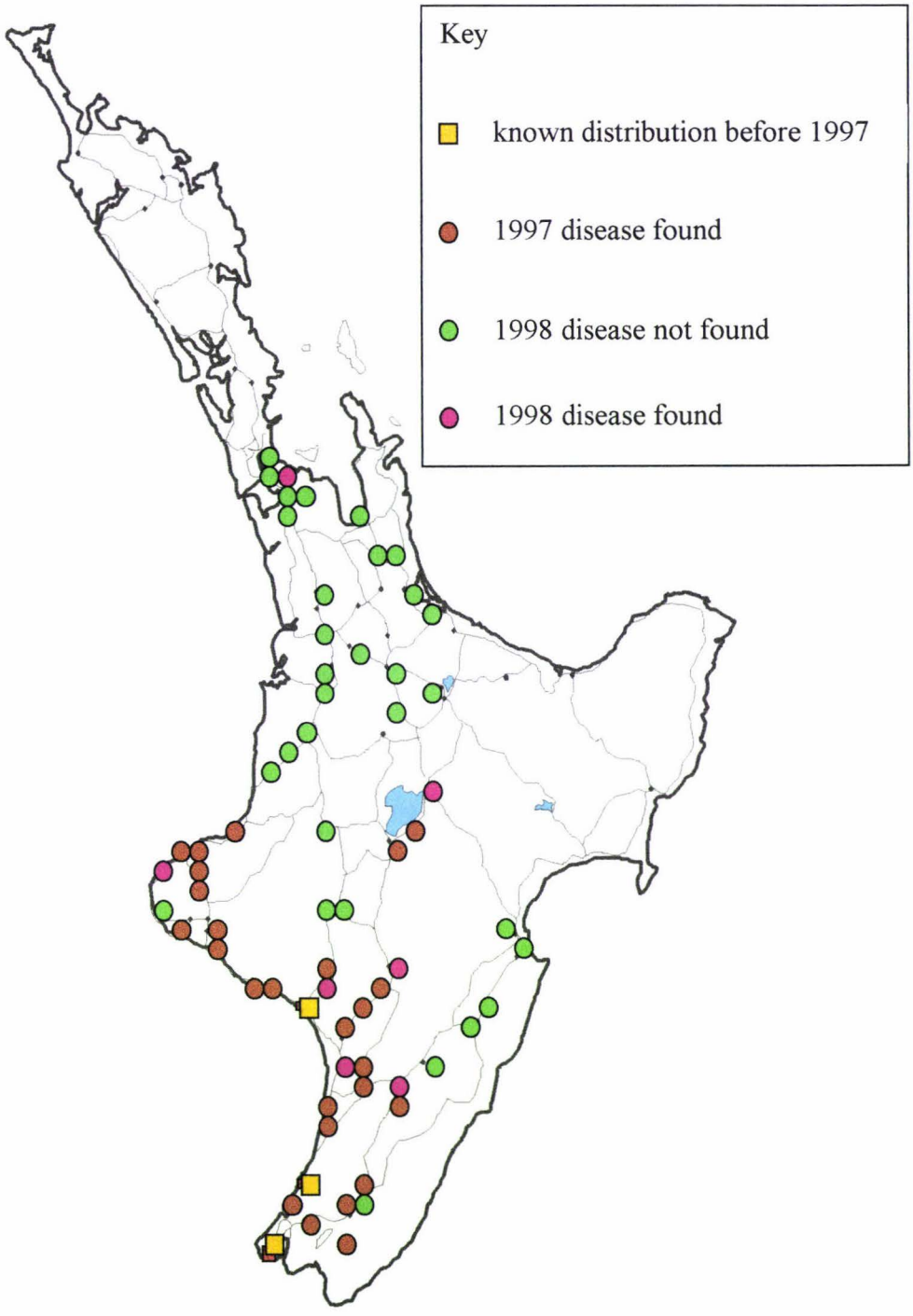

Figure 3.2 Distribution of *C. camelliae* in the North Island 1998.

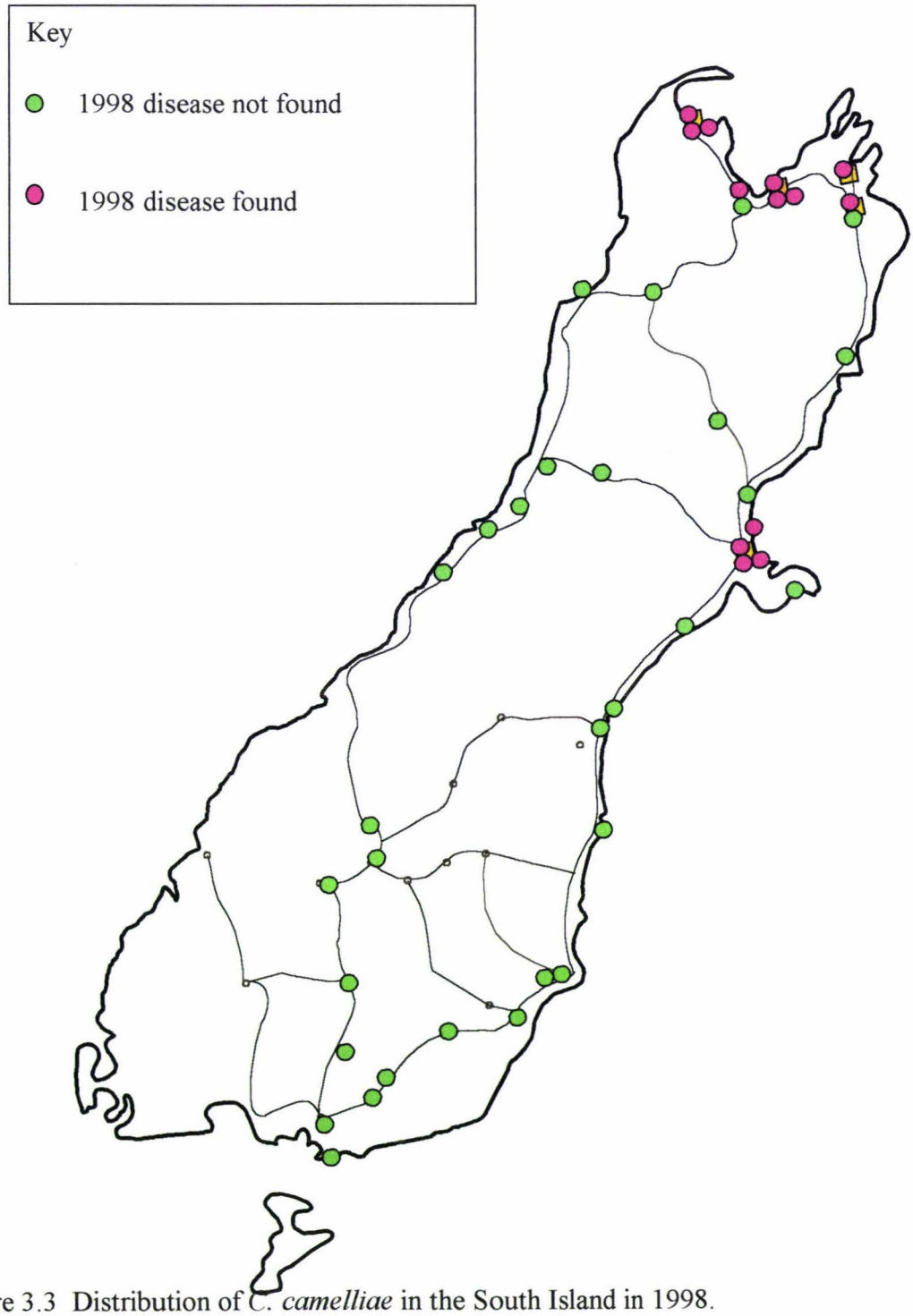

Figure 3.3 Distribution of *C. camelliae* in the South Island in 1998.

The disease was not found at any site on SH1 between Blenheim and Christchurch nor on SHs 6, 65, 69 and 7 between Nelson and Christchurch. It was not found on Banks Peninsula, or at any site on SH1 south of Christchurch. Flower blight was not found in Southland, Westland or Buller.

Many garden centres and nurseries were visited on the survey and several were found to have infected plants. These outlets were in regions where the blight appeared to have been established for one or more years and where a large amount of inoculum might be expected in the air. No diseased plants were found at outlets in areas free of the disease.

3.4 DISCUSSION

These surveys were limited by time and money and a thorough search was not possible in Christchurch and Auckland where the disease may be present in suburbs other than those in which it was found. A negative result from a site does not necessarily indicate that the disease is not present in an area, as not all flowers on all plants could be examined. However, the close proximity of infected locations and the large areas where the disease was not found give confidence that the overall pattern of distribution is correct.

As can be clearly seen in Figures 3.1, 3.2 and 3.3, the disease is now present in both the North and South Islands and is far more widely distributed than thought prior to these surveys. It is widespread in the lower, central and western North Island and in the northern part of the South Island and Christchurch. In the North Island the disease front appears to run from Urenui in the north-west, through Taupo to Woodville in the southeast. Few infected plants or flowers were found at this front, and none were located north of it (except Auckland). In the northern South Island, the disease front is delineated by mountain ranges to the south.

3.4.1 Methods of Spread

The widespread occurrence of camellia flower blight would indicate that the main method of spread has been through windborne ascospores, supplemented in part with the transfer of infected plant material/contaminated soil to disease-free sites. No other

dispersal method could account for its current widespread distribution.

At four infected sites - Hatepe, Mangaweka, Christchurch and Auckland - it appears that human activity has been responsible for the introductions. In the Christchurch and Auckland outbreaks, no infection sources were found within 280km, a strong indication that infected material was transported to these regions. Anecdotal evidence suggests that it is not uncommon for people to dig up various garden plants when moving house, and this would be an ideal method for transfering the disease to disease-free sites. Only one infected plant was found at Mangaweka and this was young (3-4 years old) and freshly planted specimen which was probably bought from an infected area. The origin of the Hatepe infections is less obvious. Four mature (>15 years old) plants were found to be heavily infected at a site with no other infections around it. Hatepe is a small, holiday bach-type settlement on the shores of Lake Taupo. Although no other diseased camellia flowers were found, it was not possible to check all properties in the area and I suspect that infected planting material was brought in several years ago, and that the disease spread to these four plants. Such isolated outbreaks, ahead of the disease front, demonstrate how the pathogen can 'leap frog' to new areas.

Windborne ascospores have been estimated to travel anywhere from 500 m to several kilometres (Zummo & Plakidas 1959; Zummo 1960; Zummo & Plakidas 1961; Baxter & Epps 1981; Baxter & Seagars 1989; Stewart & Neilson 1993; Stewart 1994; Baxter & Thomas 1995; Bond 1996) in a viable state. Only Zummo (1960) and Zummo & Plakidas (1961) appear to have based their estimate on experimental evidence. Brown (1983) reports anecdotal evidence which suggests that ascospores may be blown 8-10 km and up to 25 km. The evidence from this survey supports the longer distances suggested by Brown (1983). Infected plants were found at Kakatahi, on SH4, 20km north of the nearest infected plants. This portion of SH4 between Wanganui and Raetihi lies in a river valley in rugged terrrain and is sparsley populated. While this may be direct evidence for the long-distance dispersal of ascospores, it would seem likely, from the distribution of farms and towns, that this is not an unusual case.

The majority of ascospores released by an apothecium would not be carried great distances from the source and the spore load would become more diluted with distance and only a very small percentage of ascospores would land on camellia flowers in a viable state. Ascospores of *Sclerotinia sclerotiorum* may remain viable for one to five months under suitable conditions (Stevens & Hall 1911; Moore et al 1949; Hungerford & Pitts 1953; Partyka & Mai 1962). Given a windspeed of 50km/hr, a not unusual event in New Zealand, an ascospore could be carried large distances quickly and remain viable. At two sites 500 m distant from the inoculum source, Zummo & Plakidas (1960; 1961) found that the incidence of blight was still considerable (37% and 63%), indicating that the spore load was still high. One or two spores only could cause a flower infection which develops sclerotia from which the apothecia could produce thousands of ascospores the next season. A rapid explosion of disease could result in only one or two years from very few initial infections. This theory is difficult to test without isolates carrying a physiological, morphological or genetic marker or without introducing it to new sites. Relative rates of spread and distribution may give some information in New Zealand: in the more populous North Island, towns and small settlements are more closely spaced than in the South Island and the farm size is generally smaller. Thus, there are many 'staging posts' for airborne inoculum. In the South Island, where settlements are fewer and more widely spaced, farm size is larger and there are extensive tracts of native vegetation, windborne ascospores may not be as important in its spread.

3.4.2 Rate of Spread

The distance from Wellington to New Plymouth by road is 355 km (AA mileage guide) and Wellington to Taupo is 380km (AA mileage guide). Assuming that the disease began spreading northwards in 1992, then over seven years it has covered an average of 50-60 km each year. However, I believe that the disease has been in Wellington much longer than previously thought, perhaps ten to thirteen years. Assuming it had been present eleven years then it has spread and average of 32-35 km per year. Although direct evidence is lacking for such large increments by windborne ascospores alone, in combination with leap frogging on infected plant material, such dispersal is possible. In

the Wairarapa, where the disease is less well established, the Rimutaka and Tararua ranges may have formed a partial barrier to its spread. Wellington to Woodville is 184 km (AA mileage guide), giving an average yearly increment of 26 km over seven years.

3.4.3 New Zealand and United States Experience Similar

These surveys have revealed that the disease is more widely distributed than previously thought. The disease had been present (in some cases for several seasons), but unidentified, at many sites, indicating a lack of public awareness. Even Camellia Society members, who were alert for signs of the disease, failed to identify it in their own communities. This experience is typical of that reported in the United States where the disease was present several years at each property before its eventual identification (Gill & Ridley 1954; Winstead et al 1954; Baxter & Berly 1956; Plakidas 1957).

3.4.4 Conclusion

These surveys have shown that the disease is present in both North and South Islands. It is widespread in the lower, central and western North Island, within Christchurch and in the northern region of the South Island. Windborne dispersal appears to be the main method of spread, but spread on infected material has occurred, and may become more important in the future. It would appear that viable ascospores may be blown considerable distances (~20 km), but scientific testing of this has not been done. The spread of the disease within New Zealand mirrors that within the United States.

CHAPTER FOUR

SCLEROTIAL GERMINATION

4.1 INTRODUCTION

Sclerotia are the survival structures of many fungi in the Subdivision Ascomycotina. In the absence of a host or unfavourable conditions compact masses of mycelium form a sclerotium, by which the fungus remains in a dormant but viable state until conditions are suitable for germination. When the sclerotium germinates, it may produce vegetative hyphae (myceliogenic germination – hyphal and eruptive), asexual spores (sporogenic germination) or fruiting bodies that produce sexual spores (carpogenic germination). The sclerotia of some fungi are able to germinate by more than one method. Successful infection of a host (by sclerotial means) requires germination be not a random event, but governed by endogenous and/or exogenous factors which ensure effective contact with a host. The major physiological change occurring during germination is the conversion of insoluble storage material into soluble carbohydrates (Coley-Smith & Cooke 1971). However, the factors involved in triggering sclerotial germination, are complex.

The following is a summary of some of the environmental conditions known to be important for sclerotial germination. There is a large amount of literature on the subject, although very little work has been done on the genus *Ciborinia*. This summary, therefore, refers mainly to work on other genera, especially on *Sclerotinia*, which could be relevant to *Ciborinia*. The sclerotia of *C. camelliae* are described in relation to their part in the life cycle of this pathogen and the need to be able to carpogenically germinate sclerotia of this pathogen is discussed.

4.2 FACTORS INVOLVED IN SCLEROTIAL GERMINATION

The aim is to recognise the conditions that stimulate germination, allowing the fungus to be manipulated in the laboratory. With many sclerotial plant pathogens, both sclerotia produced in culture and those formed naturally are capable of germinating and there is a wide range of literature on the subject. However, this information is sometimes confusing and frequently conflicting, even for work on the same species. In some fungi, artificial stimulation of the sclerotia to produce the sexual stage is relatively simple, and sclerotia germinate profusely (e.g. *S. sclerotiorum*). For others, discovering the conditions that trigger germination has been more difficult (e.g. *B. cinerea*), and for still others, artificial germination of sclerotia has not been achieved (e.g. *C. camelliae*).

4.2.1 Dormancy

Germination of sclerotia requires the breaking of sclerotial dormancy. Dormancy is a rest period, a reversible interruption in the infection cycle of the fungus (Sussman & Halvorson 1966). Two types of dormancy are recognised – constitutive and exogenous. Constitutive dormancy depends on the internal development of the sclerotium. It is a period of maturation during which the internal physiological state of the sclerotium prevents germination and there is some evidence that the sugar-mannitol pathway is involved in this inhibition (Cooke & Mitchell 1970). Sclerotia may be activated to germinate during this period by specific environmental stimuli that affect the physiological processes (Coley-Smith & Cooke 1971). Exogenous dormancy depends on unfavourable external conditions. When these conditions change in favour of the fungus, sclerotial germination can occur. Germination, therefore, may be a two step process, as both types of dormancy must be broken separately and the relevance to each type of dormancy of the factors considered below may vary.

4.2.2 Temperature and Temperature-Cycling

In addition to the temperature at which sclerotia are incubated, the temperature at which sclerotia are formed may also influence subsequent germination, thus the impact of temperature on sclerotial germination appears to be a complex issue. For example, Keay (1939) found that apothecia of *S. sclerotiorum* did not develop from sclerotia

grown at 25°C, but did so at 5°C, 10°C, 15°C and 20°C. Bedi (1962) found that 25°C was the optimum temperature to grow sclerotia for subsequent apothecial formation and that below 15°C did not produce apothecia. Purdy (1956) found that apothecia were produced on sclerotia formed at most temperatures (4°C, 12°C, 15°C, 18°C, 21°C, 24°C and 27°C). Willets & Wong (1980) conclude that the optimum temperature for growing sclerotia of *Sclerotinia* spp. for germination is between 10°C and 20°C.

Low temperatures or temperature cycling have been found to be effective for germination of sclerotia, but once again, the issue appears to be complex. A chilling period of 0°C-10°C is required by *Claviceps purpurea* for activation (Mitchell & Cooke 1968) and the longer the chilling period, the higher the proportion of sclerotia which germinated (Mitchell & Cooke 1968). However, too long a chilling period may reduce germination and chilling may not be required if the sclerotia are placed on a damp substrate instead (Mitchell & Cooke 1968). Sproston & Pease (1957) and Hawthorne (1973) have found that temperature cycling is effective for the sclerotial germination of *S. trifoliorum* and *S. minor* respectively.

In conclusion, the conditions required to stimulate germination of sclerotia vary, both between and within species. This variation may be due to differences in the life cycle of the pathogens or may reflect ecotypic variation, where isolates from different climatic regions behave dissimilarly.

4.2.3 Light

Light is not thought to affect the early stages of germination, although work by Letham (1975) and Bedi (1958) with *S. sclerotiorum* showed that it might play a part. Germination experiments frequently use a combination of light and temperature so that it is not possible to determine the relative importance of each (e.g. Sproston & Pease 1957; Fuentes et al 1964).

The intensity of the light may be important, although this has been much less studied. Ikegami (1959) found that light intensity was important in apothecial formation of *S. trifoliorum*, with no formation below 100 Lux and 78-100% germination at 600-1400

Lux.

More studies are required to determine the relationship of photoperiodicity, light intensity and quality on sclerotial germination.

4.2.4 Water

Free water or water potentials of around 0 kPa are required for sclerotial germination (Phillips 1987), possibly indicating a water-soluble inhibitor (Casale & Hart 1983). They found that germination was quicker if sclerotia were leached, and that the germination rate was higher if the water was changed regularly. Batra & Korf (1959) found leaching by water was a prerequisite to germination of cultured sclerotia of *C. erythronii*. Leaching does not appear to be necessary for the germination of *C. wisconsinensis* (Batra 1960) and *C. pseudobifrons* (Groves & Bowerman 1955).

4.2.5 Exogenous Nutrients or Signals

The sclerotia of *Sclerotinia* spp. do not require an external source of nutrients in order to germinate (LeTourneau 1979). Exudates from roots, however, have been shown to stimulate germination in several species, but this response may be more important in myceliogenic germination and infection of host roots.

4.2.6 Other Factors

There are a number of other factors that have been shown to influence the germination of sclerotia. For example, Bedi (1963) has shown that the nutrient status of the substrate on which the sclerotia are grown can affect germination and Coley-Smith (1960) that gentle abrasion of the rind can break dormancy.

4.3 SCLEROTIA OF *C. CAMELLIAE* AND THE IMPORTANCE OF PRODUCING THE SEXUAL STAGE

Sclerotial formation is an integral part of the life cycle for *C. camelliae*. Once the

infection has established, the sclerotia begin forming in the base of the petals, taking 15-30 d to form depending on temperature and moisture (Brown 1983). During the months of lying in debris under the plant, petal and other tissue rot away, leaving behind the sclerotia. These lie dormant in the soil for at least nine months and may survive for at least 4 years (Baxter & Thomas 1995). Thus, for the majority of its life cycle, the pathogen exists only as sclerotia.

The current distribution of *C. camelliae* indicates that sclerotia are able to survive a wide range of environmental conditions, from light winter snows through to hot, dry summers. It has been said, however, that the disease is more severe following mild, wet winters (Tourjé 1958) than very cold and/or very dry winters (Baxter & Epps 1981). Baxter & Epps (1981) stated that sclerotia "may cease any physiological activity" during a cold and/or dry winter and believed that the very low incidence of blight in South Carolina in 1981 was the result of a cold, dry winter in 1980/81. Brown (1983) reported that an unusually cold Georgian winter, when temperatures got as low as 5°F (-15°C) and did not rise above freezing for several days, did not kill the sclerotia, though both the appearance of blight and camellia flowers was delayed that year.

Sclerotia of *C. camelliae* germinate to produce apothecia in early spring and the infection season continues for three-four months. Only carpogenic germination has been observed in this species, as would be expected of a flower and/or foliage-infecting pathogen (Garrett 1956). The sclerotia are larger than those in species that germinate myceliogenicly because carpogenic germination is energetically more costly and requires larger food reserves (Coley-Smith & Cooke 1971).

Ascospores are the only natural infective propagule of *C. camelliae*, as this fungus does not produce asexual spores or vegetative mycelium for infection. To study the infection process, plant resistance or fungicide control, it is necessary to imitate as closely as possible the natural infection conditions. This requires that ascospores be the infective inoculum rather than mycelial plugs. However, ascospores are only produced for about three months a year, seriously limiting the type of work that can be conducted for the majority of the year. In order that studies can be conducted year-round, a year-round supply of ascospores must be available and this can only be achieved through the

artificial induction of sclerotial germination. Artificial germination of sclerotia of *C. camelliae* has not been reported, although naturally formed sclerotia have germinated in the laboratory during the normal infection season (Thomas & Hansen 1946).

4.4 AIMS

The aims of this work were to elucidate the conditions which a) induce sclerotial germination of *C. camelliae* and b) prolong the shelf life of germinated sclerotia.

4.5 MATERIALS AND METHODS

The sclerotial germination experiments were divided into two parts, according to whether established germination methods were trialled or whether novel germination methods were used. Untreated controls were not always prepared, either due to low numbers of sclerotia available or to lack of available space in the laboratory. A combination of cultured sclerotia and naturally formed sclerotia were used, depending on what material was available. Treatments are numbered from 1 through to 47 to avoid confusion when referring to a particular treatment of an experiment.

4.5.1 Adaptation of Hawthorne's (1973) Method for *S. minor*

Treatment 1

The basic method of Hawthorne (1973) was used but was modified to accommodate the vagaries of *C. camelliae* and available equipment. Cultures of *C. camelliae* grown on wholemeal agar formed abundant microconidia but failed to form sclerotia. Consequently sclerotia were obtained from isolates of Cc3 grown on Difco PDA for four weeks at 20°C. The sclerotia were collected and agar scraped off the lower surface before air-drying for four days in the laboratory (~12°C-18°C). Sclerotia were not sieved as few small sclerotia were produced. Sclerotia (86 g and 97 g) were placed in each of two 90 mm glass petri dishes containing 20 ml of tap water and were incubated for seven weeks at 15°C 12 h/10°C 12 h in constant darkness. Stipes were not observed at the end of this period when they were transferred to 10°C 12 h light/12 h dark under near-UV, tungsten and fluorescent lights.

4.5.2 Adaptation of Sansford & Coley-Smith's (1992) Method for *S. sclerotiorum*

Treatment 2

Autoclaved (121°C/15 min) 250ml conical flasks containing 5 g perlite, 10 g whole wheat grains and 25 ml ROW were inoculated with 3, 5 mm diameter plugs of actively growing mycelium from Cc3. They were incubated at 20°C for four weeks in constant darkness and were shaken vigorously at 2, 4, 6, 10 and 21 days.

4.5.3 Adaptation of Batra & Korf's (1959) Method for *C. erythronii*

Treatments 3-9

Batra & Korf (1959) do not give detailed instructions for their germination method (for sclerotia formed in culture), but they identified two essential factors before sclerotia could be induced to germinate:

1. Sclerotia must be well washed.
2. Sclerotia must be overwintered.

After the dormancy period (unspecified), they placed sclerotia on moist sand at 15°C in constant darkness for 8-10 d to form stipes. The chambers were then transferred to a north-facing window (Northern Hemisphere) where apothecial discs formed and apothecia matured in 8-12 d.

Seven treatments were based on this method.

Treatment 3

Three to four month old sclerotia of isolate Cc1 were grown and stored on Difco PDA at 20°C. Sclerotia were washed and soaked for six weeks in SROW at room temperature then rinsed and placed on moist filter paper in 90 mm glass petri dishes. Sclerotia were cold-conditioned (simulated overwintering period reported by Dillard et al (1995) to be effective for breaking dormancy of *S. sclerotiorum*) for 8 weeks at 5°C in constant darkness. At eight weeks, sclerotia were transferred to chambers containing moist sand and incubated at 15°C for 21 d. Although no stipes formed, the chambers were transferred to a glasshouse and placed under a bench to give partial shade.

Treatment 4

As for Treatment 1 but using a combination of 4 and 6 week old sclerotia of isolates

Cc1 and Cc3 and sclerotia were shaken in ROW for 5 min.

Treatment 5

Naturally formed sclerotia that had been stored at room temperature in the laboratory for 12 months were cleaned in ROW and given the same treatment as those in Treatment 1.

Treatment 6

As for Treatment 3 except that sclerotia were maintained at 5°C throughout the course of the experiment.

Treatment 7

As for Treatment 4 except that after 11 weeks at 5°C sclerotia were placed in a chamber containing moist sand under a bench in a glasshouse to give partial shade.

Treatment 8

As for Treatment 3 except sclerotia were kept at 20°C in constant darkness throughout the course of the experiment.

Treatment 9

As for Treatment 3 except sclerotia were kept at 20°C in constant darkness for 8 weeks before transfer to sand at 15°C for 21 d.

4.5.4 Adaptation of Groves & Bowerman's (1955) Method for *C. pseudobifrons*

Treatment 10

Isolates Cc1 and Cc3 were grown on Difco PDA for 5 d at 20°C to establish growth , then at either 5°C or 15°C for six weeks. Sclerotia were collected and the agar removed from the lower surface. Sclerotia were washed for 12 h in ROW and placed on moist sand at 0°C for 4 weeks. Microconidia from isolates Cc1, Cc2, Cc3 and Cc5 were combined and sprayed onto the sclerotia using a De Vilbis sprayer. Sclerotia were incubated for 3 weeks at 5°C, transferred to 15°C for 10 d and then placed in a glass house under a bench to give partial shade.

Treatment 11

Naturally formed sclerotia which had been stored at room temperature in the laboratory for 12 months were cleaned in ROW, incubated on moist sand for 6 weeks at either 5°C

or 15°C then were transferred to 0°C for 4 weeks. Microconidia from isolates Cc1, Cc2, Cc3 and Cc5 were combined and sprayed onto the sclerotia using a De Vilbis sprayer. Sclerotia were incubated for a further 3 weeks at 5°C, transferred to 15°C for 10 d and then placed in a glass house under a bench to give partial shade.

4.5.5 Novel Overwintering Conditioning Treatments (1)

Treatments 12-18

Current season sclerotia were collected from the Wellington Botanic Gardens in November 1997. Sclerotia were thoroughly washed, air-dried at 25°C for 3 d and placed in 200 ml squat plastic pots (No.6074, LilyPak Industries Ltd., Auckland, New Zealand) containing ~1 cm moist coarse sand (except Treatments 17 and 18). There were 18 pots with ~7 sclerotia per pot (135 per treatment) of assorted sizes

Treatment 12

Sclerotia were incubated at 1°C in constant darkness for 36 weeks (12.12.1997-19.8.1998) to simulate overwintering. They were then collected and separated into 4 aliquots of ~25 sclerotia each. These 4 aliquots were each given a different incubation environment to stimulate germination.

Treatment 12A sclerotia were reburied in perlite and incubated at 10°C/15°C 12 h light/12 h dark.

Treatment 12B sclerotia were reburied in perlite and held at 10°C in constant darkness.

Treatment 12C sclerotia were reburied in perlite and held at 15°C in constant darkness.

Treatment 12D sclerotia were sewn into a 12 cm^2 mesh (insect screen) bag and staked out under a camellia plant in the Massey Arboretum.

Treatment 13

Sclerotia were incubated under a bench in the laboratory for 50 weeks from 12.12.1997-23.11.1998 to simulate conditions under a bush in a very sheltered place.

Treatment 14

Sclerotia were alternately incubated at 1°C for 4 weeks in constant darkness, 15°C for 4 weeks in constant darkness for 50 weeks to simulate seasonal changes.

Treatment 15

Sclerotia were incubated as for Treatment 14 but with a 48 h incubation at –5°C to -10°C in the middle of the 1°C period to simulate frost conditions.

Treatment 16

Sclerotia were incubated at 15°C for 4 weeks in constant darkness, 5°C for 4 weeks in constant darkness, then transferred to 10°C under 12 h near-UV, tungsten and fluorescent lights for 42 weeks (adaptation of method described by Faretra & Antonacci 1987; Faretra et al 1988).

Treatment 17

Sclerotia were sewn into 14 cm^2 mesh (insect screen) bags and staked on the soil surface under a camellia plant in the Massey Arboretum (where flower blight had already been found) for 49 weeks.

Treatment 18

Sclerotia were sewn into 14 cm^2 mesh (insect screen) bags and buried ~2.5 cm deep under a camellia plant in the Massey Arboretum for 49 weeks.

Sclerotia were watered at intervals but drying out was a problem at 15°C for Treatments 14 and 15. At 12 weeks, 5 marked pots from each Treatment (except Treatments 17 and18) were sprayed with a mixture of microconidia from isolates Cc1, Cc2, Cc3 and Cc5 (2.4 x 10^6/ml). After 13 weeks incubation the sclerotia in all Treatments (except Treatments 17 and 18) were covered by 0.5-1 cm of sand to prevent rapid drying out. At 23 weeks all sclerotia were removed from their containers and rinsed. Sand was removed from the pots, drainage holes burnt into the bottom, and the sclerotia reburied in ~2 cm of perlite. The pots were placed in larger containers containing felt and ROW.

4.5.6 Novel Overwintering Conditioning Treatments (2)

Treatments 19-25

These experiments were a repeat of those in Section 4.5.5 Novel Overwintering Conditioning Treatments (1) except that:

1. The sclerotia were collected from Harcourt Park, Upper Hutt on 22.12.97 and were not cleared of attached petal tissue in case this tissue contributed to the nutrition or maturation of the sclerotia.

2. There were seven pots containing ~16 sclerotia each (115 total) per treatment.

Treatment 19

Sclerotia were incubated at 1°C in constant darkness for 32 weeks (7.1.1998-19.8.1998). They were then collected and divided into 4 piles, each of ~25 sclerotia of assorted sizes.

Treatment 19A sclerotia were reburied in perlite and incubated at 10°C/15°C 12 h light/12 h dark.

Treatment 19B sclerotia were reburied in perlite and held at 10°C in constant darkness.

Treatment 19C sclerotia were reburied in perlite and held at 15°C in constant darkness.

Treatment 19D sclerotia were sewn into a 12 cm^2 mesh (insect screen) bag and staked out under a camellia plant in the Massey Arboretum.

Treatment 20

As for Treatment 13 but for 46 weeks from 7.1.1998-23.11.1998.

Treatment 21

As for Treatment 14, for 46 weeks.

Treatment 22

As for Treatment 15, for 46 weeks.

Treatment 23

As for Treatment 16.

Treatment 24

As for Treatment 17.

Treatment 25

As for Treatment 18.

As for Treatments 14 and 15, drying out was a problem for Treatments 21 and 22. All Treatments except 24 and 25 were sprayed with the same microconidial suspension used in Treatments 12-16, except this was done at 8 weeks and four pots were sprayed. As

for Treatments 12-16, Treatments 19-23 were covered with sand after 9 weeks and at 19 weeks were reburied in perlite and place in containers containing felt and ROW.

4.5.7 Naturally Conditioned Sclerotia

Treatments 26-33

Previous seasons sclerotia were collected from Wellington Botanic Gardens on 17.8.1998. They were sorted into 7 treatments of ~45 sclerotia per treatment. Sclerotia were buried in 2-3 cm of perlite in 200 ml squat plastic pots (No.6074, LilyPak Industries Ltd., Auckland, New Zealand) with holes burnt into the bottom and placed in larger containers with felt and ROW. These experiments ran from 19.8.1998-23.11.1998. An eighth treatment (33) was added 16 d after this experiment was begun using 8 sclerotia taken from each of Treatments 26-32.

Treatment 26

Sclerotia were incubated at 10°C/15°C in constant darkness.

Treatment 27

Sclerotia were incubated at 10°C/15°C in 12 h light/12 h dark.

Treatment 28

Sclerotia were incubated at 10°C in constant darkness.

Treatment 29

Sclerotia were incubated at 15°C in constant darkness.

Treatment 30

Sclerotia were incubated at 20°C in constant darkness.

Treatment 31

Sclerotia were incubated at 1°C in constant darkness.

Treatment 32

Sclerotia were incubated at 5°C in constant darkness.

Treatment 33

Sclerotia were incubated outside the laboratory window in natural light and temperature conditions.

4.5.8 Immature Apothecia

Treatments 34-36

Immature apothecia were collected from Wellington Botanic Gardens in August 1998. They were stored in 190 x 120 x 40 mm containers (airtight lids) with moist paper towels and wire mesh to prevent the stipes from directly contacting the towels. Each container held 20-30 germinated sclerotia and containers were stored at either 1°C or 5°C. In the first 10 d 3 mature apothecia were removed from containers stored at 1°C and 4 from containers stored at 5°C. Thereafter the remaining stipes withered and appeared to die. After 11 weeks storage, stipes were observed to have recommenced growth and new stipes had appeared (Figures 4.1 and 4.2). To determine whether light was required for formation of the apothecial disc, these sclerotia and stipes were subdivided into one of three treatments.

Treatment 34

Three sclerotia with multiple stipes were placed in a clear 190 x 120 x 100 mm container with moist paper towels. Wire mesh prevented stipes from directly contacting the towels and the container was wrapped in Gladwrap. Sclerotia were incubated at 10°C/15°C 12 h light/12 h dark and misted at regular intervals to prevent them drying out.

Treatment 35

Three sclerotia with multiple stipes were incubated as above but aluminum foil was wrapped outside the Gladwrap to prevent entry of light. Sclerotia were incubated at 10°C/15°C in constant darkness and misted at regular intervals to prevent them drying out.

Treatment 36

One sclerotium with multiple stipes was placed in a 200 ml squat plastic pot (No.6074, LilyPak Industries Ltd., Auckland, New Zealand) with moist filter paper. The pot was wrapped in Gladwrap and incubated on a laboratory windowsill and misted at regular intervals to prevent it drying out.

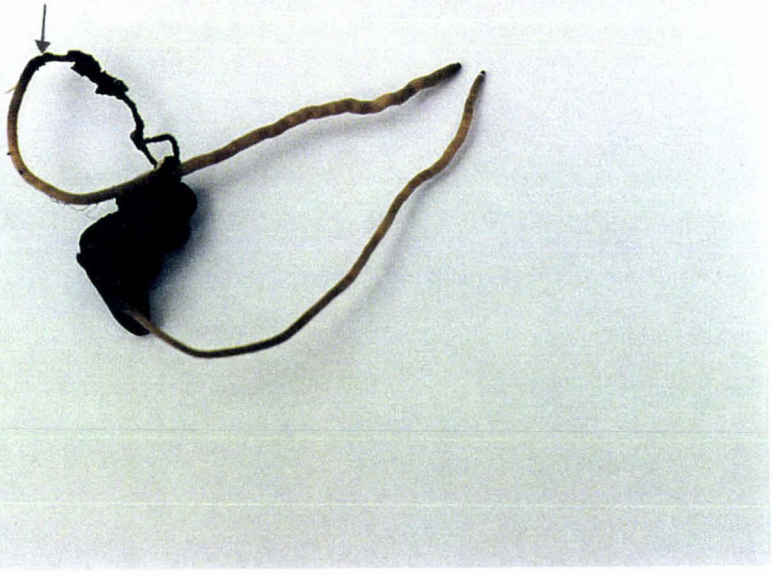

Figure 4.1
C. camelliae stipes after 11 weeks storage at 5°C (Section 4.5.8). A withered stipe (black arrow) has recommenced growth at the tip to produce a healthy stipe.

Figure 4.2
Newly formed stipes from sclerotia stored at 5°C for 11 weeks (Section 4.5.8).

4.5.9 Natural Conditioning of Cultured Sclerotia

Treatments 37-47

Sclerotia of isolates Cc1, Cc2, Cc3 and Cc5 were grown on Difco PDA and camellia petal agars as used in Section 2.3.4.1 (Appendix I) at 20°C (sclerotia from camellia-based agars were bulked for this work). At collection, sclerotia grown on Difco PDA were 2-4 weeks old, sclerotia grown on camellia agars were 3 weeks old. Sclerotia were soaked in ROW for three days. The water was changed three times a day and the bottles were shaken 1-2 h each day on a Griffin Flask Shaker (Speed 2). Agar did not separate from the sclerotia during this treatment, so was scraped off the lower surface with a scalpel. About 50 sclerotia per isolate per treatment were divided between 5 narrow-necked McCartney bottles (25 ml). Each McCartney bottle received 2 ml SROW and either 1 ml microconidial suspension (a mixture of all isolates totaling 6.8 x 10^6spores/ml) or 1 ml SROW and were incubated on their sides with a loose cap to allow gas exchange. Sclerotia were cold-conditioned at 5°C for 6 weeks. Bottles were placed in a random order on a tray in clear 190 x 120 x 100 mm containers (5 per container) and 1-2 cm tap water added (Figure 4.3). The containers were wrapped in net curtain to prevent insects entering and incubated (beginning 21.7.1998) for 25 weeks under camellia bushes at 70 Church St, Palmerston North. Treatments are shown in Table 4.1.

Table 4.1 Germination Experiment using Sclerotia Cultured on various Agars

Treatment	Agar	Isolate	Microconidia	Cold-Conditioning
Treatment 37	Difco	1,2,3,5	-	+
Treatment 38	Difco	1	+	+
Treatment 39	Difco	2	+	+
Treatment 40	Difco	3	+	+
Treatment 41	Difco	5	+	+
Treatment 42	Camellia	1,2,3,5	-	+
Treatment 43	Camellia	1,2,3,5	+	-
Treatment 44	Camellia	1	+	+
Treatment 45	Camellia	2	+	+
Treatment 46	Camellia	3	+	+
Treatment 47	Camellia	5	+	+

4.6 RESULTS

4.6.1 Hawthorne's (1973) Method for *S. minor*

Treatment 1
Stipes of *C. camelliae* were not produced during incubation at 15°C 12 h/10°C 12 h in constant darkness as they were for *S. minor*, nor did they form after transfer to diurnal conditions under near-UV, tungsten and fluorescent lights.

4.6.2 Sansford & Coley-Smith's (1992) Method for *S. sclerotiorum*

Treatment 2
The experiment was discontinued because sclerotia failed to form in the perlite-wheat-water medium.

4.6.3 Batra & Korf's (1959) Method for *C. erythronii*

Treatments 3-9
Neither stipes nor apothecia were formed at any temperature treatment combination.

4.6.4 Groves & Bowerman's (1955) Method for *C. pseudobifrons*

Treatments 10-11
Neither stipes nor apothecia were formed in either treatment, regardless of the temperature at which the sclerotia were formed or incubated.

4.6.5 Novel Overwintering Conditioning Treatments (1)

Treatments 12-18
Neither stipes nor apothecia were produced in any Treatment except Treatment 18 where two mature apothecia developed together with one stipe, which failed to mature because it dried out.

4.6.6 Novel Overwintering Conditioning Treatments (2)

Treatments 19-25
Neither stipes nor apothecia were produced in any treatment except Treatment 25 where two apothecia were produced.

4.6.7 Naturally Conditioned Sclerotia

Treatments 26-33

Neither stipes nor apothecia were produced in any treatment except Treatment 28, where sclerotia were held at 10°C in constant darkness. Of the 40 sclerotia, 14 had produced stipes and some more than one stipe (Figure 4.3). None of the stipes was visible above the surface of the perlite.

Figure 4.3
Stipes produced by sclerotia stored at 10°C (Treatment 28).

4.6.8 Immature Apothecia

Treatments 34-36

Apothecial discs were observed in Treatment 34 after 7 d incubation. Mature apothecia were observed after 16 d. Apothecial discs did not form in Treatment 35. The stipes in Treatment 36 dried out quickly and died despite regular misting.

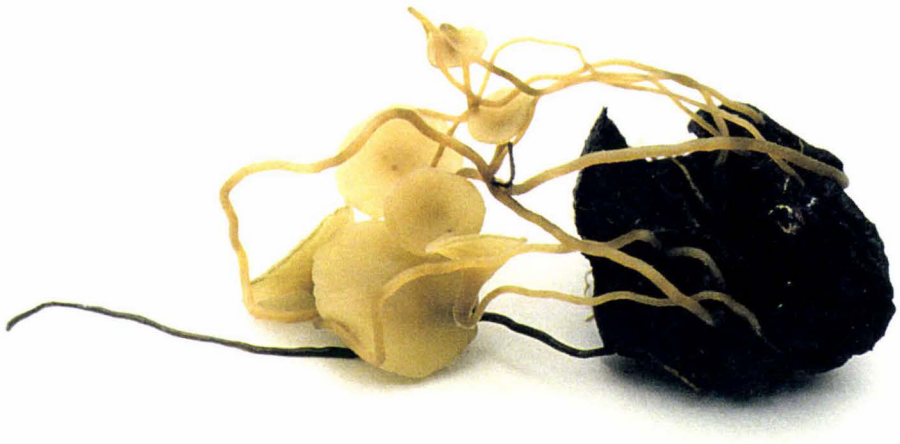

Figure 4.4
Stipes matured into apothecia after 10 d in 10°C/15°C 12 h light/ 12 h dark (Treatment 34).

4.6.9 Natural Conditioning of Cultured Sclerotia

Treatments 37-47
Neither stipes nor apothecia were produced in any treatment.

4.7 DISCUSSION

Ascospores are essential for studies of infection, host resistance and fungicide efficacy. A reliable, year-round supply of ascospores would allow studies to be conducted over a much longer time period (April-December) than at present when ascospore production occurs only during August, September and part of October.

Since the ascospores are only produced over a 2-3 month period in spring, the most likely triggers would appear to be temperature and temperature fluctuations and/or daylength. Similar conditions to those in spring are also found in autumn and the absence of ascospore production in autumn suggests that a winter chilling period may be required to mature the sclerotia and prime them for germination. The most likely treatments for inducing sclerotial germination would therefore appear to be those that

simulate winter chilling in short days or dark followed by incubation at higher temperatures with light or alternatively, methods that are successful for other, related fungi.

Methods used for apothecial production in *S. minor* (Hawthorne 1973) and *S. sclerotiorum* (Sansford & Coley-Smith 1992) were unsuccessful when applied to *C. camelliae*. Even those used for other species of *Ciborinia*, e.g. *C. erythronii* (Batra & Korf 1959) and *C. pseudobifrons* (Groves & Bowerman 1955) were unsuccessful. The descriptions given in Batra & Korf (1959) and Groves & Bowerman (1955) for *C. erythronii* and *C. pseudobifrons* respectively were not sufficiently detailed to be sure that the method was followed exactly but they fitted the descriptions given.

A range of experimental conditions that simulated winter chilling and spring conditions were devised but all failed to stimulate apothecial formation.

One possibility is that the materials used to hold the sclerotia (plastic pots, perlite, or water) might have inhibited germination. However, naturally stimulated sclerotia incubated at 10°C in these materials did germinate (Treatment 28) but the sclerotia were in these materials for only 14 weeks compared to 36-50 weeks for other experimental treatments. The suitability of these materials, therefore, should be considered further. The plastic pots (No.6074, LilyPak Industries Ltd., Auckland, New Zealand) are sold commercially and are intended for food storage. These pots were chosen because of their size and shape, but it is possible that the plastic emits volatiles or other antifungal substances that inhibit sclerotial germination. Perlite is an inert medium and it, or vermiculite (a similar medium), is routinely used to hold sclerotia for germination of other fungi (Sansford & Coley-Smith 1992; Delclos & Raynal 1995; Dillard et al 1995). Sand has been used (e.g. Smith & Boland 1989; Huang 1991; Gerbrandy 1992; Huang & Kozub 1993) but it dried out quickly in the current work and consequently was replaced by perlite medium. Water quality may also be an issue. Sclerotia were rubbed clean and regularly watered using ROW, except for one week when ROW was not available and tap water was used. It is possible that nutrients and metabolites contained in tap water, rainwater, or untreated water are required for the stimulation of germination. Hawthorne (1973) specifies tap water, Mylchreest & Wheeler (1987),

Smith & Boland (1989) and Sansford & Coley-Smith (1992) used distilled water. Gaunt (Pers. Comm.) considered that water quality was critical for apothecial formation of *S. fructicola* mummies.

Neither washed nor unwashed sclerotia germinated, so the significance of leaching from the sclerotium, materials broken down from rotting petal tissue, or the presence of a large microflora is unknown.

Naturally formed sclerotia, with and without petal tissue, air-dried for several days in the laboratory and buried ~2.5 cm deep in soil under a camellia plant, had a germination rate of <2%. This could indicate that:

1. The percentage of sclerotia germinating each year is very small, and the number of sclerotia used in each experiment was too few to detect this percentage.
2. Some part of the collection and/or initial storage procedure prevents/inhibits/delays germination.
3. Some part of the burial process induced germination, e.g. host-root exudates, microflora of the rhizosphere.
4. The relative size of the sclerotium is a measure of its length of dormancy and longevity. The naturally formed sclerotia used in these experiments were biased towards larger sclerotia (>0.5 cm) rather than small sclerotia, simply because they were easier to handle. It was observed that large sclerotia tend to form in blighted flowers that remain whole, whereas small 'pip' sclerotia tend to form in blighted petals that are separated from the flower mass (Figure 4.4). Thomas & Hansen (1946) reported that while both large and small sclerotia germinated in the year following formation, apothecia were produced in greater numbers and over a longer period from large sclerotia than from small sclerotia. While the large sclerotia germinated again the second year after formation, the small sclerotia failed to germinate. It may be that having smaller food reserves, the small sclerotia germinate the following disease season because they would have insufficient food reserves to survive to the second season and then produce apothecia. By contrast, larger sclerotia may remain dormant or germinate in more than one disease season. The leaching process may be a factor. Nutrients or inhibitors would be leached more rapidly from small sclerotia than those from larger sclerotia because of the

greater surface/volume ratio.

Sclerotia that were left to condition naturally after the previous blight season and were collected just prior to the infection season failed to germinate, except for those stored at 10°C. This indicates that 10°C may be the critical temperature for these isolates and establishes that this temperature should be a component in further sclerotial germination experiments. The stipes grew through, but were not visible above, the perlite medium (Figure 4.3), suggesting that light was not required to initiate germination but was required to orientate growth.

Sclerotia failed to form on the wheat-perlite-water medium recommended by Sansford & Coley-Smith (1992). Sclerotia also failed to form on wholemeal agar (see Section 4.5.1) and wheat, although Holcomb (1980; 1994) does not appear to have had the same problem. Sclerotia are routinely grown on wheat and water at Lincoln University (50 g wheat, 50 ml ROW and 0.0025% chlorophenol; autoclave twice, incubate 18°C for several weeks), although contamination by *Penicillium* spp. is a problem (Judith Pay Pers. Comm). However, they were consistently obtained on Difco PDA.

None of the cultured sclerotia germinated in any treatment and while cultured sclerotia of *B. cinerea* and *S. sclerotiorum* are capable of carpogenic germination, it remains to be seen whether cultured sclerotia of *C. camelliae* are likewise capable. Unlike sclerotia of *S. sclerotiorum*, sclerotia of *C. camelliae* incorporate host material within the sclerotium. This may be essential for germination although Kohn (1982) reports that sclerotia of *C. hirsuta* grown on MEA produced apothecia. Thus, host material incorporated into the sclerotial medulla may not be essential for apothecial production by some (or all) members of this genus.

Experiments that followed established methods were based on the most successful techniques, for example, Hawthorne (1973) incubated sclerotia of *S. minor* at five temperature regimes, but only the 15°C/10°C 8 h light/16h dark was successful and this was the regime trialled here.

Novel methods evolved from the assumption that there was a long period of constitutive dormancy that could be broken by simulated winter temperatures (cold-conditioning) followed by simulated spring temperatures. The assumption that constitutive dormancy was at work follows from the observation that sclerotia do not germinate in autumn, when temperature and light cycles are similar to those of spring, and when susceptible flowers are present (Haasis 1953; Frampton 1994). Sclerotia were cycled between 1°C and 15°C, but whether these temperatures, the combination of temperatures or the duration of each temperature was significant is unknown. Batra & Korf (1959) report that dormancy of *C. erythronii* was not broken during incubation (1-12 weeks) at 0°C, Brix & Zinkernagel (1992) showed that freezing and thawing of sclerotia of *S. cepivorum* decreased their ability to germinate (myceliogenic) whereas sclerotia of *S. sclerotiorum* germinate myceliogenically when stored below 0°C but carpogenically when stored above 0°C (Huang 1991). Once it became apparent that sclerotia in the 1°C control treatments were not going to germinate, the sclerotia were assigned to new experiments, but germination still did not occur.

A few sclerotia from Treatments 18 and 25 buried under a camellia bush produced apothecia but none was formed by sclerotia on the soil surface (Treatments 17 and 24). Burial may be a significant factor in survival or germination, but whether burial prevents desiccation, allows more microbial contact or has some other effect, is unknown. These treatments were not fertilised with microconidia but this does not indicate that the fungus is homothallic since large sclerotia could form from multiple infections on a flower and thus contain genetic material from more than one source.

The results of experiments with germinated sclerotia (Section 4.6.8) show that sclerotia and stipes may be stored at 1°C-5°C for up to 11 weeks. During this period, the stipes may wither and appear to die, but new growth can occur and more stipes germinate. Iriyama (1980) has observed similar behaviour with *C. camelliae*. Sclerotia with stipes transferred to 15°C/10°C 12 h light/12 h dark formed apothecia with viable spores, while stipes held at 15°C/10°C in constant darkness failed to differentiate. This confirms that light is not necessary for stipe formation but is required for apothecia to form as in other apothecial fungi (e.g. Delclos & Raynal 1995). Baxter (Pers. Comm. to

Terry Stewart) states that stipe elongation ceases as soon as light strikes the apothecial stalk.

These sclerotia will be stored until the next disease season, in an attempt to induce apothecial formation from the same sclerotium in more than one season. Williams & Western (1965) report that sclerotia of *Sclerotinia* spp. buried 15 cm deep produced stipes of up to 6.5 cm and then ceased to grow. Energy reserves had not been fatally depleted as the same sclerotia formed apothecia the next year when removed from that depth. Repeated germination has been reported in *C. purpurea* (Cooke & Mitchell 1967), *S. sclerotiorum* and *S trifoliorum* (Williams & Western 1965). Dingley (1993) reported that *C. camelliae* sclerotia could germinate in more than one season, though this is not reported elsewhere. In the experiment with sclerotia reported by Thomas & Hansen (1946), large sclerotia were reported to have germinated in two successive seasons although it is not clear whether the same sclerotia germinated in both seasons, or whether it was different sclerotia in the same batch that germinated in each season. The definition of what constitutes a 'sclerotium' needs to be clarified in respect to this pathogen, as a large sclerotium is often an amalgamation of several smaller sclerotia. When sectioned, large sclerotia may, or may not, be found to be internally divided by rind. Since it is not known whether these smaller sclerotia are genetically identical, it is not certain whether a large sclerotium is a single entity or a collection of sclerotia.

Determination of the conditions that trigger sclerotial germination in *C. camelliae* remains a priority and although the experiments reported here were largely negative, it is important to record procedure in detail until a successful method is developed or future researchers could waste valuable time repeating techniques that do not work

There is now a considerable amount of information on techniques that have not been successful in stimulating sclerotia to germinate. The one firm conclusion is that light is not essential for stipe initiation but is required for apothecial disk formation. Other factors that should be considered are:

1. Water and Leaching Ultrapure water is not found in camellia groves but rainwater does not have chemicals such as the chlorine found in tap water which could inhibit stipe formation. On the other hand, soil contains many nutrients and micro-

organisms which could stimulate germination. These factors should be considered in future work.

2. Temperature The temperature and temperature cycling used in this work were unsuccessful but there are so many temperature combinations and cycling times that it is possible the correct combination was not tested. There are, therefore, ample combinations to be tested in future work.
3. Winter Chilling Simulated winter chilling by cold-conditioning sclerotia prior to temperature/temperature cycling/light regime treatments would appear to remain an important factor as previously discussed. Assuming it is required, then what is the critical temperature? There is some evidence that 0°C may be inhibitory but that 5°C may be effective. Also, the duration of this conditioning period may be critical, and I suggest that 8 weeks be the minimum period since 4 weeks did not work in these experiments and the months of June and July give approximately 8 weeks of cold weather before sclerotial germination in August-October.
4. Chemical Stimulation The conversion of insoluble storage materials into soluble carbohydrates that occurs during germination (Coley-Smith & Cooke 1971) is accompanied by a decrease in the dry mass of the sclerotium (Saito 1977). In *S. sclerotiorum*, this reduction in dry mass is due to the degradation of β-1,3 glucans by the increased activity of β-1,3 glucanase enzymes (Saito 1974). In future experiments, stimulation of *C. camelliae* sclerotia by soaking them in glucanase (and other similar enzymes) or soluble carbohydrates could be investigated.
5. Containers and Medium Whether the pots and perlite used in these experiments affected germination is not known, but in future experiments, glass containers could be substituted for plastic. Perlite, sand and soil should be considered for the medium, although sclerotia may be camouflaged in soil.

C. wisconsinensis (Batra 1960), *C. hirsuta* (Kohn 1982) and *C. allii* (Leu & Wu 1985) have also been germinated and the methods used for these fungi should be trialled in further experiments with *C. camelliae*. Sclerotia of *C. camelliae* form apothecia readily during a well-defined infection season so theoretically, it should be possible to induce germination in the laboratory.

CHAPTER FIVE

INFECTION STUDIES

5.1 INTRODUCTION

Plant disease is the result of a chain of events involving both the plant and the plant pathogen. These events occur in a preordained sequence, where successive events are determined by prior outcomes. This process is called the disease cycle and it involves the inoculation of the pathogen onto a host, penetration of the host by the pathogen, establishment of infection in the cells or tissues of the host, the colonisation of these, reproduction by the pathogen (asexual, sexual, or a form/stage capable of reproducing later) and dissemination of these propagules (Agrios 1988).

The study of infection centres on pre-penetration, penetration and the immediate post-penetration interactions and it is considered here from the perspective of aerial infection of plant parts by airborne spores in a compatible (disease-producing) interaction.

5.1.1 The Phylloplane and the Infection Process

Infection is a process that begins when inoculum contacts the surface of a host plant. The spore actively attaches itself to the surface of the host through the secretion/production of an extracellular matrix (Paus & Raa 1973; Hamer et al 1988; Deising et al 1992; Clement et al 1993; Braun & Howard 1994; Cole et al 1996). Adhesion occurs before the spore germinates, and in the case of *Magnaporthe grisea* (blast of rice) and *Cochliobolus heterostrophus* (southern corn leaf blight) adhesion has been shown to become effective 20 min after inoculation (Hamer 1988; Braun & Howard 1994). The spore germinates to produce a germ tube, which is also attached to the surface by the extracellular matrix (Beckett et al 1990; Clement et al 1993; Braun & Howard 1994; Cole et al 1996). The germling penetrates the plant tissue through the

formation of appressoria, through natural openings (stomata, hydathodes etc.) or wounds. An apressorium is a specialised infection structure, and its formation is essential for many (fungal) pathogens in order to penetrate their host (Emmett & Parbery 1975; Staples & Hoch 1987; Stapes & Macko 1980). The appressorium adheres tightly to the substratum and builds up and maintains a high internal osmostic potential (Howard et al 1991a; Howard et al 1991b). This pressure allows the penetration peg on the underside of the appressorium to break through the plant cuticle. As well as penetration by direct mechanical force, enzymatic degradation of the cuticle under the appressorium may also play a role for some fungi (Sweigard et al 1992a; Sweigard et al 1992b; Kolattukudy 1985). Once inside the plant, hyphae ramify through the tissue and in successful infections, symptoms of disease will develop.

A spore landing on the plant surface, however, is not alone and it must interact with the abiotic and biotic environment of the phylloplane. These interactions with the surface, the phylloplane microflora, the exudates from plant and microflora, and sundry pollen grains and dust, can affect the germination and growth of the spore.

5.1.2 Stimulation of Germination

Spores can be induced to germinate by the addition of exogenous nutrients or exudates. Amino acids, sugars and other water-soluble substances have been shown to increase growth or per cent germination. For example, Chou (1972) and Blakeman (1975) showed that fructose promoted germination of *B. cinerea* macroconidia more than other simple sugars. Similarly, appressorium formation in *Colletotrichum gloeosporoides* (anthracnose of avocado) is induced by the long-chain fatty alcohols found in avocado wax (Podila et al 1993).

5.1.3 Inhibition of Germination

Substances on the phylloplane can also effect inhibition of spore germination. Exudates from microflora, especially bacteria, have been shown to inhibit the germination of *B. cinerea* macroconidia (Rossall & Mansfield 1980) and self-inhibition of spore germination has been shown to occur where spore concentrations are dense (Cochrane 1960), particularly with rust fungi (Allen 1955; Yarwood 1956).

5.1.4 Effect of Temperature, Humidity and Free Water on Germination and Growth

The optimum temperature for germination and growth of spores varies and generalisation is difficult but generally, germination is delayed by temperatures lower or higher than the optimum. Germlings appear less sensitive to sub-optimal temperatures (Cochrane 1960).

Moisture is usually an essential requirement for germination and/or growth of spores, and it is this requirement that is utilised in disease forecasting methods (Royle & Butler 1986). Relative humidity (RH) and the presence of free water on plant surfaces are often interrelated. For example, macroconidia of *B. cinerea* germinate in water droplets, or on dry surfaces where RH is high (Harrison 1984; Cole et al 1996). Jhorar et al (1998) found that infection by conidia of *Didymella rabiei* (Ascochyta blight of chickpea) requires at least 95% RH and Semeniuk (1993) reports the same for *Leptotrochila medicaginis* (yellow leaf blotch of alfalfa). Beckett et al (1990) found that the spores of *Uromyces viciae-fabae* (rust of legumes) can adhere to surfaces under dry conditions, but high RH and/or free water improves this adhesion. Thus is would appear that one reason moisture is required for infection is that without adhesion, pre-penetration and penetration events are compromised.

Duration of free water on the surface also affects germination. Jhorar et al (1998) found that germination and penetration by germlings of *D. rabiei* increased linearly with increasing wetness periods. Once penetration has taken place, however, RH and/or free water become irrelevant, as the fungus is maintained in the constant internal environment of the leaf/petal tissue (Brown & Tanner 1981).

5.1.5 Plant Resistance

A range of mechanisms exist in plants which enable them to defend themselves against potential pathogens. Assuming host/pathogen compatibility, plant resistance (or susceptibility) can occur at any stage once the inoculum arrives on the infection court. Various mechanisms exist which kill/inhibit the pathogen on the surface, prevent pathogen penetration, or kill the pathogen inside the plant host.

Preformed, constitutive resistance mechanisms may include the wax/cuticle or epidermal boundary, the structure of the stomata, or phytoanticipans (antifungal substances). The cuticle of the papaya has been shown to be an effective barrier against infection by *Nectria haematococca* (Dickman et al 1989). *N. haematococca* requires a wound for infection, but Dickman et al (1989) demonstrated that the insertion of a cutinase gene (cutinase degrades cuticle) from another fungus, enabled *N. haematococca* to infect intact papaya fruit. McLean (1921) demonstrated that the morphology of the stomata determined susceptibility of *Citrus grandis* to *Pseudomonas citri* (bacterial canker of citris). The stomata of *C. grandis* hold droplets of water, whereas the stomata of *C. nobalis*, a resistant fruit, do not. The soil fungus *Gaeumannomyces graminis* causes take-all of wheat and other grasses. Oats are resistant to *G. graminis* because they secrete avenacin, a toxic, antifungal substance. *G. graminis* var. *avenae*, however, is able to infect oats because they produce avenacinase which deglucosylates avenacin to less toxic forms (Turner 1961; Crombie et al 1986).

Structural and chemical resistance mechanisms that are induced by contact with the pathogen also exist. For example, reed canary grass forms papillae opposing the site of penetration from an appressorium (Sherwood & Vance 1976). Hypersensitive response (HR) describes the rapid cell death that occurs after the host cell has been penetrated by a pathogen. This localised cell death (and other events) limits the spread of the pathogen and only occurs in resistant (incompatible) varieties (Agrios, 1988; Goodman & Novacky 1994). The pathogen may also induce host production of a range of phenolic compounds and phytoalexins (Matern & Kneusel 1988; Nicholson & Hammerschmidt 1992; Strange 1992). The role of phytoalexins in host resistance has been intensively studied for *Phytophthora megasperma* f.sp. *glycinea* (root and stem rot of soybean). Glyceollin I, a phytoalexin, has been shown to accumulate in response to *P. megasperma* f.sp. *glycinea* infection (Keen & Horsch 1972) and it accumulates to inhibitory concentrations in the vicinity of the fungus at the time growth of *P. megasperma* f.sp. *glycinea* is halted (Hahn et al 1985).

Disease is the exception rather than the rule, as plants are naturally resistant to the vast majority of pathogens. The interaction between host and pathogen results in either a

compatible or incompatible reaction. The reaction depends on whether the pathogen is virulent and the host is susceptible to that pathogen. This virulent/susceptible reaction may exist at the genus or species level, where the basic host/pathogen interaction is incompatible (e.g. *B. cinerea* cannot infect members of the genus *Quercus*, yet is a common pathogen on vegetables, ornamentals and fruit (Agrios 1988)) or at the race/cultivar level, where races of a pathogen differentially infect cultivars of the same species (e.g. stem rust of wheat caused by *Puccinia graminis tritici*). Flor (1942), (Flor 1946) and Flor (1971) developed the gene-for-gene hypothesis of resistance based on his work with *Melampsora lini* (flax rust). This hypothesis states that for every gene for virulence in the pathogen, there is a corresponding gene for susceptibility in the host.

5.1.6 Infection and Resistance to *C. camelliae*

C. camelliae infection occurs on petals of the genus *Camellia*, but nothing is known about the infection (pre-penetration, penetration, post-penetration events) process of this pathogen or other *Ciborinia* species. Similarly, there has been no study of resistance in the genus *Camellia*. No list of cultivars or species known to be susceptible (or resistant) is currently available and no camellia varieties are known to be resistant (Baxter & Segars 1989). Hansen & Thomas (1940) reported that more than 50 varieties of camellia (in a nursery) appeared to be equally susceptible and Baxter & Berly (1956) reported more than 100 varieties (in private gardens) which showed no resistance to *C. camelliae*. Presumably these are the more common cultivars and species (i.e. *C. japonica*, *C. reticulata*, *C. saluenensis*, and hybrids) that are widely distributed. Baxter (unpublished, cited in Baxter & Epps 1981) reported that 'Betty Sheffield' and 'Rev. John G. Drayton' did not appear to be quite as susceptible as other cultivars, although they could be infected by *C. camelliae*. Opinions differ as to whether the autumn flowering *C. sasanquas* are susceptible to the disease. Both Frampton (1994) and Haasis (1953) state that *C. sasanqua* is susceptible, whereas Matsumoto (1995) states that it is immune. He also states that wild japonicas (in Japan) are not "as damaged as other cultivars" by this pathogen.

5.2 AIMS

The aim of this section of work was to develop a method for the inoculation of camellia petals with airborne ascospores to determine whether camellia species/varieties were equally susceptible to this pathogen. Since ascospores were only available for part of the year, agar plug inoculations of petals were also conducted, both to gain experience and to examine susceptibility in petals of different physiological maturity. Minor experiments on collection and storage of ascospores, on whether microconidia could initiate petal infections and on whether camellia leaves could be infected were also carried out.

5.3 MATERIALS AND METHODS

Most camellia specimens were collected from the grounds of Massey University (Tiritea) Campus or the Victoria Esplanade, Palmerston North. Where the cultivar is unknown, it is referred to by number, e.g. 'Tree 7' (*C. sasanqua* and autumn flowering species) or letter, e.g. 'Tree A' (*C. japonica*, *C. reticulata*, hybrids and spring flowering species). A list of unknown species, with a brief description of each, is given in Appendix V. All flowers and petals collected for testing were in as good condition as possible, i.e. no bruises, insect holes etc.

For mycelial plug infections, all isolates were grown on Difco PDA at 20°C 12 h light/12 h dark. Preliminary experiments showed that actively growing mycelium was required for infection; colonies were ~6 days old and mycelial plugs (3 mm diameter) were taken from the leading edge.

A method was developed for inoculating petals with airborne ascospores. The apparatus consisted of an enclosed 330 x 280 x 360 mm container, an enclosed 80 x 120 x 65 box mm modified to fit inflow and outflow tubes, and an aquarium pump (1 Litre/min) and Drechel bottle. The assembled apparatus is shown in Figure 5.1.

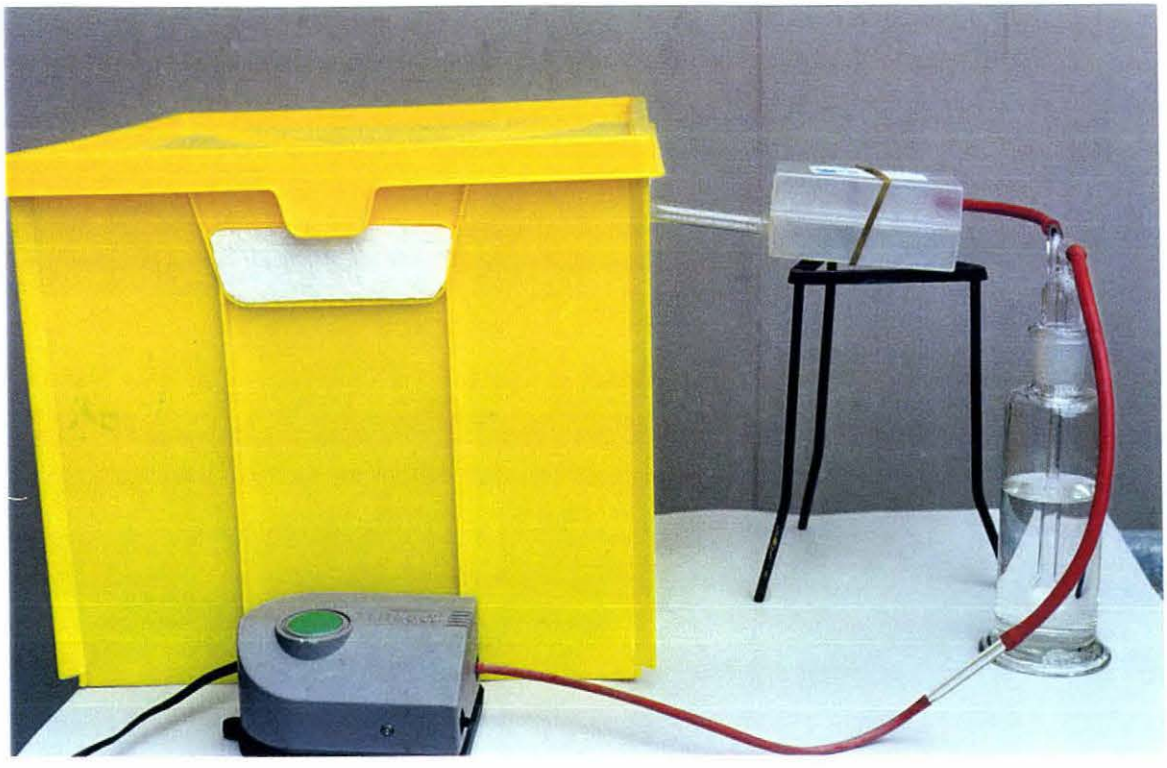

Figure 5.1
Ascospore inoculation apparatus. The aquarium pump blows air through water to saturate it, then over sclerotia with apothecia in the translucent box. Ascospores are blown into the yellow box and settle out on petals laid in the bottom.

Deposition of ascospores onto the floor of the settling chamber was assessed using glass slides and petals from varieties known to be susceptible to *C. camelliae* infection. The results of a single test using glass microscope slides (26 x 76 mm) found the number of ascospores/cm^2 to vary between 20 and 62 and distribution within the settling chamber was fairly even. Two tests with camellia petals showed similar results although the number of infections/cm^2 was not counted. Because the release of ascospores could not be controlled, the number of ascospores landing per cm^2 could vary considerably. To ensure infection of each batch of petals, 3 apothecia and a 4 h blow time appeared to be the optimum condition. Apothecia were viable for ~3 days and were changed in rotation. To ensure that ascospores had actually been released, two petals of 'Desire' (a susceptible variety) were placed with each batch of petals to indicate the presence or absence of viable ascospores.

5.3.1 Possible Non-Ascospore Sources of Infection

5.3.1.1 Microconidia

Objective: to determine whether microconidia can cause infections.

Experiment 1 Inoculation of petals by *C. camelliae* microconidia.

Isolate: Cc3

Camellia: Tree 3

Tree 7

'Crimson King'

'Shishigashira'

Fifteen petals in good condition were collected from each of the plants. Petals were laid on the surface of insect screen mesh supported by bottle tops to prevent them contacting the wet paper towels that lined a 190 x 120 x 100 mm container.

Treatment 1
Five detached but intact, uninoculated petals (control).

Treatment 2
Five petals with two pinpricks inoculated with a 10µl droplet of SROW over both holes.

Treatment 3
Five petals with two pinpricks inoculated with a 10µl droplet of microconidial suspension (1.7 x 10^6spores/ml) over both holes.

The containers were covered with Gladwrap and incubated at 20°C in 12 h light/12 h dark for 6 d after which petals were assessed for browning symptoms characteristic of *C. camelliae* infection.

5.3.1.2 Petal-to-Petal Infection

Objective: to determine whether infected petals can transmit infection to healthy petals or flowers.

Experiment 2 Infection petal-to-flower.

Inoculum: naturally infected petals of 'Leonora Novick' and 'Desire'

Camellia: 'Madame Hahn'
'Rendezvous'
'Debutante'
'Roger Hall'

Treatment 4
Two intact flowers of each variety.

Treatment 5
Two flowers each of 'Madame Hahn' and 'Rendezvous' were interleaved with four infected petals from 'Desire', and two flowers each of 'Debutante' and 'Roger Hall' were interleaved with four infected petals from 'Leonora Novick'. All flowers were place in a humid chamber and incubated at 20°C in 12 h light/12 h dark for 6 d. Flowers were assessed daily for symptoms of *C. camelliae* infection.

Experiment 3 Infection petal-to-petal, effect of wounding and water on infection.

Inoculm: naturally infected petals of 'Dixie Knight', 'Leonora Novick', 'Hawaii' and 'San Dimas'

Camellia: 'Roger Hall'
'E. G. Waterhouse'
'R. L. Wheeler'

The 8 treatments are shown in Figure 5.2 and the variety and number of petals used in each Treatment are given in Table 5.1.

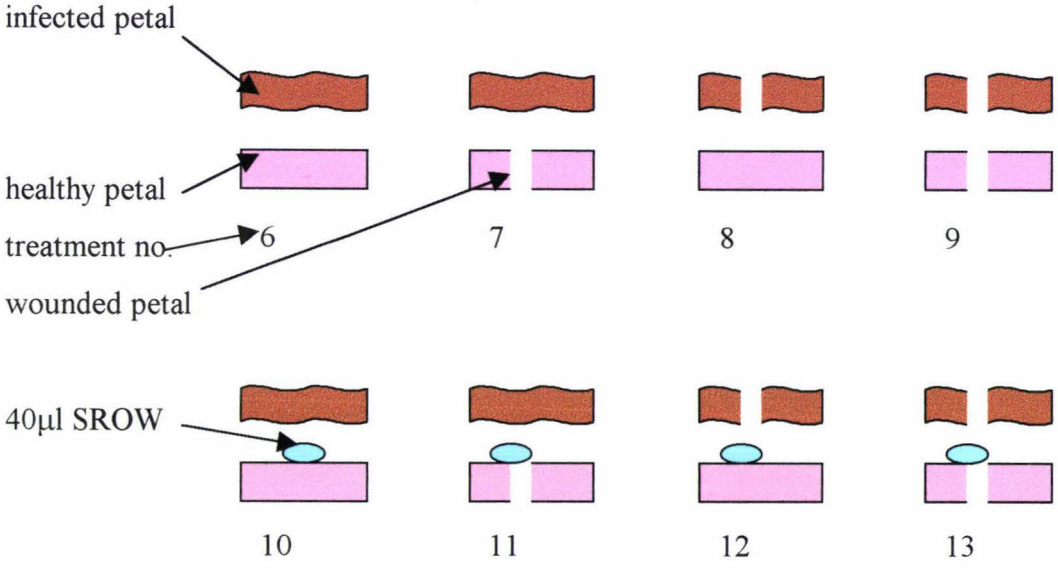

Figure 5.2

Diagramatic representation of the 8 petal-to-petal infection treatments. Neither petals in Treatment 6 nor those in Treatment 10 were wounded. Treatments 6-9 were the four combinations of wounded and unwounded healthy and diseased petals and Treatments 10-13 were the corresponding treatments with water added.

Table 5.1
Camellia Varieties Used for Assessing Petal-to-Petal Infection

Date	Healthy Petal	Infected Petal	Number of Petals in Each Treatment							
			6	7	8	9	10	11	12	13
17.9.1998	'Roger Hall'	'Dixie Knight'	3	3	3	3	3	3	3	3
17.9.1998	'Roger Hall'	'Leonora Novick'	4	4	4	4	4	4	4	4
25.9.1998	'E. G. Waterhouse'	'Hawaii'	3	3	3	3	3	3	3	3
21.10.1998	'E. G. Waterhouse'	'San Dimas'	3	3	3	3	3	3	3	3
21.10.1998	'R. L. Wheeler'	'San Dimas'	3	3	3	3	3	3	3	3

Diseased and healthy petals were pressed together, placed in a humid chamber and incubated at 20°C in 12 h light/12 h dark for 4 d before examination for symptoms consistent with infection by *C. camelliae*.

5.3.1.3 Leaves

Objective: to determine whether leaves of camellia can be infected after finding *C. camelliae*-like sclerotium in a camellia leaf.

Experiment 4 Infection of camellia leaves by mycelial plug.

Leaves were surface sterilised 1 min in 1:5 Janola (42 g/Litre sodium hypochlorite), rinsed in ROW an air-dried. A set of five pin holes was made in each side of the leaf (Figure 5.3) so that a 4 mm plug of agar would cover each set of holes.

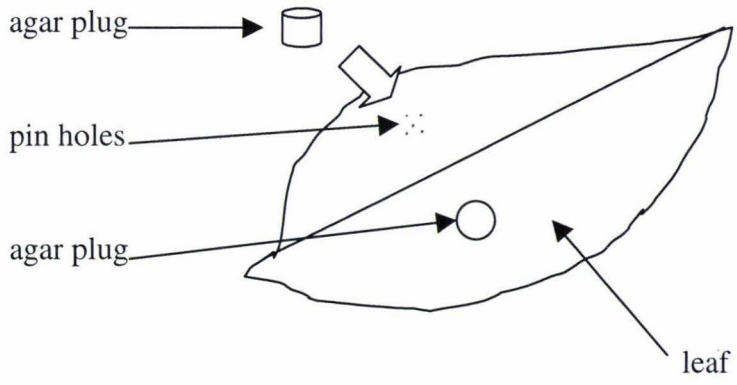

Figure 5.3
Inoculation method of camellia leaves with agar plugs.

On each leaf, one set of holes was covered by a plug of Difco PDA and the other with a plug of mycelium, inverted. Leaves were placed in a humid chamber and incubated at 20°C in 12 h light/12 h dark for 11 days. On 7.5.1998, treatments using infected and non-infected camellia petals were also done. Details of experiment dates, cultivar inoculated, inoculum type and number of leaves inoculated are shown in Table 5.2.

Table 5.2
List of Materials and Treatments Used in Agar Plug Inoculation of Leaves

Date	Camellia	Isolate/Inoculum	No. Leaves Inoculated
7.5.1998	'Crimson King'	agar	8
		agar + Cc1	8
		agar	8
		agar + Cc2	8
	'Crimson King'	non-infected petal	8
		infected petal + Cc1	8
		non-infected petal	8
		infected petal + Cc2	8
19.5.1998	Tree 7	agar	10
		agar + Cc2	10
	Tree 14	agar	10
		agar + Cc2	10
19.5.1998	'Kanjiro'	agar	10
		agar + Cc5	10
	Tree 15	agar	10
		agar + Cc5	10
18.1.1999	'Chang's Temple'	agar	8
		agar + Cc1	8
		agar	8
		agar + Cc2	8
	'Hulyn Smith'	agar	8
		agar + Cc1	8
		agar	8
		agar + Cc2	8
	'Leonora Novick'	agar	8
		agar + Cc1	8
		agar	8
		agar + Cc2	8
	'Desire'	agar	8
		agar + Cc1	8
		agar	8
		agar + Cc2	8

5.3.2 Stages in Bud Burst of Camellia Flowers

5.3.2.1 Bud Burst

Objective: to determine whether stages in bud development can be identified in order that each stage can be checked for susceptibility.

Experiment 5 Identification of stages in bud development of camellia flowers.

Camellia: Tree 2
Tree 6
Tree 7
'Kanjiro'
'Crimson King'
'Moonlight'

Ten unopened buds on each plant were marked with coloured wool. Each bud was examined daily until the bud had fully opened.

5.3.3 Mycelial Plug Infection

5.3.3.1 Inoculation of Bud Burst Stages

Objective: to inoculate stages of bud burst to determine whether there are differences in susceptibility.

Experiment 6 Inoculation of bud burst stages using agar plugs (preliminary test).

Inoculum: Cc3

Camellia: Tree 7
'Crimson King'
'Kanjiro'

Buds at each of the six stages identified in Experiment 4 were collected off each plant. The petals were prepared for inoculation by removing sepals and any damaged outer petals. Petals were peeled off the bud (without damaging the petal) and laid out in columns of five, so that there were five petals at each stage from each variety in each Treatment. The Treatments are shown in Table 5.3. Petals were wounded twice by needle, so that the holes could be covered by the agar plug.

Table 5.3
Inoculation of Bud Burst Stages

Treatment No.	Treatment of Petals at each Bud Burst Stage			
	non-wounded	wounded	agar plug	agar + Cc3 plug
Treatment 14	+	-	-	-
Treatment 15	-	+	-	-
Treatment 16	+	-	+	-
Treatment 17	-	+	+	-
Treatment 18	+	-	-	+
Treatment 19	-	+	-	+

The petals were incubated in humid chambers at 20°C in 12 h light/12h dark for 4 d and then assessed for symptoms of infection caused by *C. camelliae*.

Experiment 7 Inoculation of bud burst stages from a range of camellia varieties using agar plugs (routine testing).

Routine testing of camellia species and varieties was carried out using Treatments 18 (nonwounded/agar + Cc3) and 19 (wounded/agar + Cc3) only. Cc3 was used in all inoculations. This experiment was carried out throughout the year, from May to November, as each camellia variety became available. Preparation and inoculation of the full six treatments on each variety was too time consuming to be practical, and sometimes insufficient buds at each stage were available.

Petals were prepared, incubated and assessed as described in Experiment 6.

Data were not statistically analysed, as replicate treatments were not done for the majority of species or varieties tested since the objective was to carry out an initial screening of a large number of varieties with further tests of any that appeared promising. Where replicates were completed, the number of infections was quite variable between replicates and more replicate treatments were required in order to assess variability. A Chi square was performed on each treatment to determine whether the number of infections at each stage could occur by chance. The results of this

experiment are compared with those in Experiment 8.

Experiment 8 Inoculation of bud burst stages from a range of camellia varieties using ascospores.

Testing of camellia species and varieties was carried out as described in Experiment 7, except that:

1. Ascospores were used as inoculum.
2. Petals were not wounded (Treatment 19).
3. Five petals of each bud burst stage were treated in the ascospore chamber and five petals were not.
4. Petals were prepared, incubated and assessed as described for Experiment 8.

5.3.4 Ascospore Infections

The following experiments were carried out during the disease season when ascospores were available. Collecting trips to Wellington and Wanganui were made as necessary to collect apothecia. These were stored in a container on moist paper towels at 5°C until required or until they expired and were discarded.

Experiment 9 Effect of wounding and water on infection by ascospores.

Petals of 'Dreamboat' and 'Desire' were collected and treated as described in Table 5.4.

Table 5.4
List of Petal Wounding and Water Treatments used in Ascospore Infection.

Treatment	Ascospore Inoculation	Nonwounded	Wounded (2 pinpricks)	Water Droplet (20µl)
Treatment 20	-	-	-	-
Treatment 21	+	+	-	-
Treatment 22	+	+	-	+
Treatment 23	+	-	+	-
Treatment 24	+	-	+	+

Ten petals of each variety were prepared for each treatment. Treatments 21-24 were placed in the ascospore inoculation chamber for 3 h then incubated in humid chambers at 20°C in 12 h light/12 h dark for 3 d. Petals were assessed for symptoms of infection

by *C. camelliae* and whether infections appeared to be associated with water and/or wounds.

Experiment 10 Resistance screening of camellia varieties to detect variation in susceptibility.

A variety of camellia species and varieties was tested (9 *C. reticulata*, 29 *C. japonica*, 18 hybrids, 4 unknown and 19 other species).

Twenty petals of each variety or species were placed in the ascospore inoculation chamber and blown for 4 h. Ten control petals were placed in a humid chamber without inoculation. After 4 h, the inoculated petals were placed together with the control petals, and incubated at 20°C in 12 h light/12 h dark for 3 d. Petals were assessed for symptoms of *C. camelliae* ascospore infection. Infected controls were not considered a problem, but if inoculated petals did not develop symptoms, few infections resulted, or atypical symptoms were observed, the species or variety was tested again with a fresh collection of petals. Each variety or species was scored as either 'S' (susceptible) or 'R' (resistant) on the basis of the number of petals infected by the ascospore treatment. Infection of five or fewer petals and/or atypical symptoms was classed as 'R' provided this also agreed with observations in the field.

5.4 RESULTS

5.4.1 Possible Non-Ascospore Sources of Infection

5.4.1.1 Microconidia

Experiment 1 Inoculation of petals by *C. camelliae* microconidia.

Four control petals (Treatment 1) exhibited browning symptoms consistent with aging and/or infection by other microbes. All petals in Treatments 2 and 3 exhibited localised browning around the pin holes, but nothing to indicate infection by *C. camelliae.*

5.4.1.2 Petal-to-Petal Infection

Experiment 2 Infection petal-to-flower.

Two flowers in Treatment 4 exhibited small brown lesions at the end of 6 d. These flowers were incubated a further 5 d, when sporulation of *B. cinerea* was observed. All flowers in Treatment 5 exhibited sporulation of *B. cinerea* at the end of 6 d. Petals in contact with the *C. camelliae*-infected petals were examined for signs of *C. camelliae* infection, but none was apparent.

Experiment 3 Infection petal-to-petal, effect of wounding and water on infection.

The results of Experiment 3 are shown in Table 5.5. Infected petals did infect other petals and wounding and free water was not always required for this to occur. The number of petals infected by each treatment varied within and between varieties but it is not clear whether this is a factor of the inoculation date, the healthy variety or the donor infected variety.

Table 5.5
Infection of Camellia Varieties Petal-to-Petal.

Date	Healthy Petal	Infected Petal	No. of Petals Infected in Each Treatment (/3)†							
			6	7	8	9	10	11	12	13
17.9.1998	'Roger Hall'	'Dixie Knight'	3	3	2	3	3	2	3	3
17.9.1998	'Roger Hall'	'Leonora Novick'	3	4	2	4	3	2	1	3
25.9.1998	'E. G. Waterhouse'	'Hawaii'	0	0	0	0	1	0	2	1
21.10.1998	'E. G. Waterhouse'	'San Dimas'	0	1	0	0	0	2	1	0
21.10.1998	'R. L. Wheeler'	'San Dimas'	2	0	0	0	2	1	2	1

† results out of 3 petals except 'Roger Hall' x 'Leonora Novick' where results out of 4 petals.

5.4.1.3 Leaves

Experiment 4 Infection of camellia leaves by mycelial plug.

Two leaves from 'Crimson King' inoculated with Cc1 developed browning symptoms that exceeded those associated with wounding only. Slivers cut from the margin of the infection were cultured on Difco PDA at 20°C in 12 h light/12 h dark. The resulting colonies appeared to be *C. camelliae* colonies, however, when inoculated onto wounded 'Yuletide' petals only *Botrytis*-type sporulation was observed.

5.4.2 Stages in Bud Burst of Camellia Flowers

5.4.2.1 Bud Burst

Experiment 5 Identification of stages in bud development of camellia flowers.

Six stages in the development of the unopened bud through to fully opened flower were identified. These were:

Stage 1 – unopened bud

Stage 2 – splash of petal colour visible as sepals separate

Stage 3 – whole tip of petals emerged clear of sepals

Stage 4 – elongation; petals remain tightly furled

Stage 5 – unfurling; petals relaxing

Stage 6 – fully opened flower

These stages are illustrated in Figure 5.4.

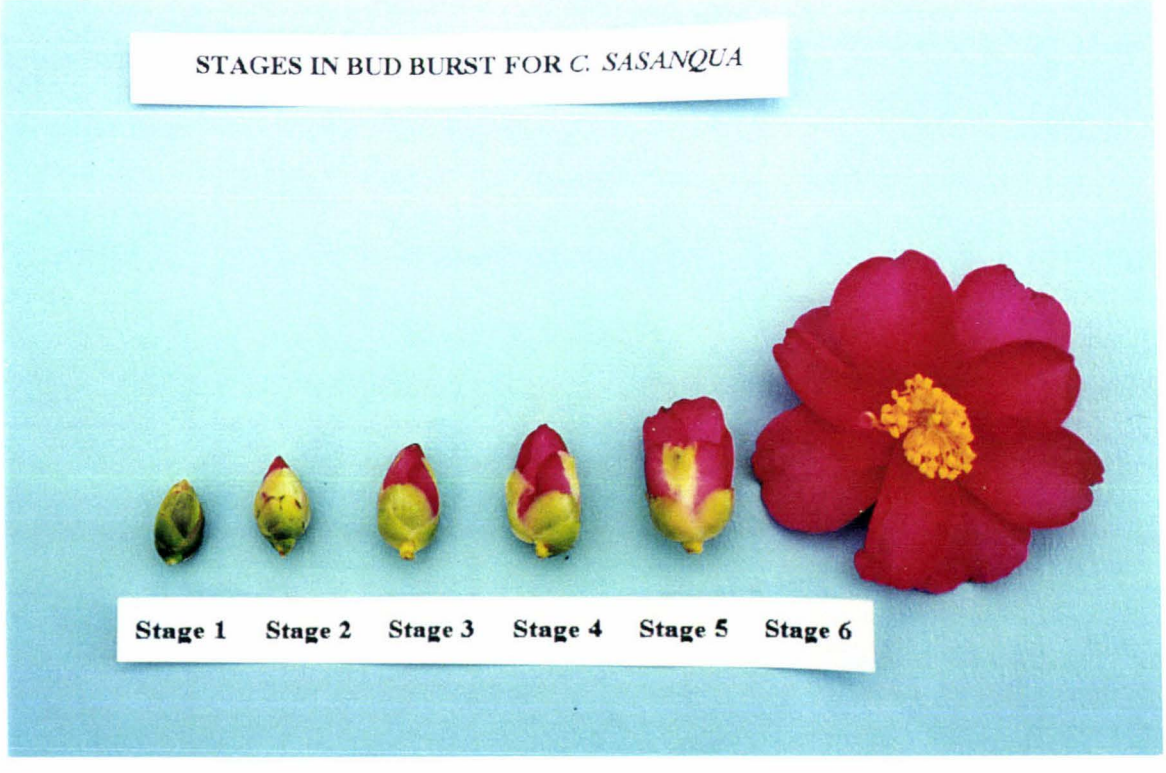

Figure 5.4
Stages in bud development of *C. sasanqua* 'Kanjiro'.

These stages are guidelines only and the time from unopened bud through to fully opened flower was highly variable (between different varieties and within flowers from the same plant) as was the length of time each bud remained at each stage. This key was developed for *C. sasanqua* flowers, but it was found to be equally applicable to *C. japonica, C. reticulata*, hybrids and other species examined, although the size/elongation judgement must be made relative to buds of the same variety. Stage 5 is difficult to identify on some spring flowering varieties and the smaller-flowered species (e.g. *C. forrestii*) appear to move rapidly through several stages.

5.4.3 Mycelial Plug Infections

5.4.3.1 Inoculation of Bud Burst Stages

Experiment 6 Inoculation of bud burst stages using agar plugs (preliminary test).
The results of the inoculations of camellia petals at each stage of bud burst are shown in Table 5.6.

All petals that were not inoculated with Cc3 failed to develop *C. camelliae* infections. Infections occurred on petals that were inoculated with Cc3 but the results indicate that wounding is usually required for infection to take place from agar plugs on *C. sasanqua* petals. A total of 3 infections occurred in nonwounded/agar + Cc3 petals compared with a total of 47 infections in wounded/agar + Cc3 petals. They also indicate that younger buds may be more resistant to infection as fewer infections occurred in Stages 1-3 (3 infections total) compared with Stages 4-5 (44 infections total).

Table 5.6
Infection of Petals at Each Bud Burst Stage

Camellia	**Treatment**	**No. Petals Infected with *C. camelliae* at each Bud Burst Stage (/5)**					
		1	2	3	4	5	5
'Kanjiro' (replicate 1)	nonwounded/uninoculated	0	0	0	0	0	0
	wounded/uninoculated	0	0	0	0	0	0
	nonwounded/agar plug	0	0	0	0	0	0
	wounded/agar plug	0	0	0	0	0	0
	nonwounded/agar + Cc3	0	0	0	0	0	0
	wounded/agar + Cc3	0	0	0	3	2	4
'Kanjiro' (replicate 2)	nonwounded/uninoculated	0	0	0	0	0	0
	wounded/uninoculated	0	0	0	0	0	0
	nonwounded/agar plug	0	0	0	0	0	0
	wounded/agar plug	0	0	0	0	0	0
	nonwounded/agar + Cc3	0	0	0	0	0	1
	wounded/agar + Cc3	0	1	0	0	4	4
Tree 7	nonwounded/uninoculated	0	0	0	0	0	0
	wounded/uninoculated	0	0	0	0	0	0
	nonwounded/agar plug	0	0	0	0	0	0
	wounded/agar plug	0	0	0	0	0	0
	nonwounded/agar + Cc3	0	0	0	0	0	0
	wounded/agar + Cc3	0	0	1	1	3	4
'Crimson King'	nonwounded/uninoculated	0	0	0	0	0	0
	wounded/uninoculated	0	0	0	0	0	0
	nonwounded/agar plug	0	0	0	0	0	0
	wounded/agar plug	0	0	0	0	0	0
	nonwounded/agar + Cc3	0	0	0	0	0	2
	wounded/agar + Cc3	0	0	0	2	2	5
'Crimson King'	nonwounded/uninoculated	0	0	0	0	0	0
	wounded/uninoculated	0	0	0	0	0	0
	nonwounded/agar plug	0	0	0	0	0	0
	wounded/agar plug	0	0	0	0	0	0
	nonwounded/agar + Cc3	0	0	0	0	0	0
	wounded/agar + Cc3	0	1	0	1	4	5

Experiment 7 Inoculation of bud burst stages from a range of camellia varieties using agar plugs (routine testing).

Of the 38 species and varieties tested, five were tested twice and one thrice. The list of species and varieties tested and the raw data are given in Appendix VI. Overall totals of infections which occurred in each Treatment at each stage are shown in Table 5.7.

Table 5.7
Total Number of Petals Infected by *C. camelliae* in Each Stage of Bud Burst

Total Number of Bud Burst Tests (including replicates)	**Total Number of Nonwounded/agar + Cc3 (/225)**						**Total Number of Wounded/agar + Cc3 (/225)**					
	Stage of Bud Burst (1-6)											
	1	2	3	4	5	6	1	2	3	4	5	6
45	4	4	5	6	11	37	115	139	166	167	193	209

The results indicate that wounding was required for infection by mycelial plugs of *C. camelliae* and the χ^2 performed on nonwounded (F5, χ^2=74.79, P<0.001) and wounded treatments (F5, χ^2=23.96, P<0.001) shows strong evidence that the number of petal infections in each stage was not randomly distributed. More infections occurred in the latter stages of bud burst. With nonwounded/agar + Cc3 there was no significant correlation between stage of bud development and number of infections but with wounded petals + Cc3 the correlation was significant (R^2=0.97, P<0.0003).

The few infections that occurred in the nonwounded treatments indicate that either the fungal mycelium has limited ability to penetrate the petal surface or that the petal surface was compromised in someway, either before collection or during the preparation and inoculation process.

Petal wounding increased the number of infections in all bud burst stages. Fifty one percent of the buds inoculated at Stage 1 developed *C. camelliae* symptoms compared with 93% at Stage 6. This indicates that physiological changes during bud development may affect the resistance of the petal to infection.

Experiment 8 Inoculation of bud burst stages from a range of camellia varieties using ascospores.

Other experiments (Section 5.4.4) have shown that wounding is not required for ascospore infection so only nonwounded treatments were carried out.

Total numbers of infected petals for each stage and treatment are shown in Table 5.8. The list of species and varieties tested and the raw data are given in Appendix 6.

Table 5.8
Total Number of Petals Infected by *C. camelliae* Ascospores in Each Stage of Bud Burst.

Total Number of Bud Burst Tests	**Total Number of Uninoculated Controls (/75)**						**Total Number of Ascospore Inoculations (/75)**					
	Stage of Bud Burst (1-6)											
	1	2	3	4	5	6	1	2	3	4	5	6
15	0	0	0	0	3	7	3	9	22	41	44	65

The total number of petals infected in the uninoculated controls was low compared to the inoculated treatments. Chi square tests gave strong support that the distribution of infected petals between bud burst stages within uninoculated (F5, χ^2=39.94, P<0.001) and inoculated (F5, χ^2=90.44, P<0.001) treatments did not occur by chance. Control infections probably occurred prior to collection. More infections occurred in petals in the latter stages of bud burst (Stages 4-6). At Stage 1, only 4% of petals were infected, at Stage 4 55% were infected, and at Stage 6 87% of petals became infected with *C. camelliae*. There was a significant correlation (R^2=0.97, P<0.0003) between the stage of bud development and the number of infections. Control petal infections were not analysed because they were uninoculated and a few infections occurred prior to collection.

5.4.4 Ascospore Infection

Experiment 9 Effect of wounding and water on infection by ascospores.

All petals of each variety in Treatments 21-24 showed multiple infections by *C.*

camelliae ascospores. Neither wounding nor water appeared to affect the number and site of infection. Two control petals of 'Desire' also developed symptoms of infection by *C. camelliae.* These would have occurred in the field prior to collection and were not considered to have compromised the validity of the results of the experiment.

Figure 5.5 shows the effect of wounds on infection by ascospores.

Figure 5.5
Effect of wounding camellia petals on infection by ascospores. Three days after inoculation on 'Brian' (left and centre) and 'Prestons Rose' (right). Ascospores do not require wounds for infection. Most of the wound sites can be seen separate from the disease lesions.

Experiment 10 Resistance screening of camellia varieties to detect variation in susceptibility.

The summary of results of ascospore infection of petals to detect differences in susceptibility are shown in Table 5.9. Full data are given in Appendix VI.

Table 5.9
Summary of Resistance Screening of Camellia with Ascospores.

Camellia Variety or Species	Susceptible/Resistant
all C. japonica varieties	S
all C. reticulata varieties	S
all hybrids except 'Spring Mist'	S
'Spring Mist'	R
unknown camellia varieties	S
C. chekiangoleosa	S
C. cuspidata*	R
C. euphlebia	S
C. forrestii*	R
C. fraterna	S
C. gigantocarpa	S
C. grijsii*	R
C. longicarpa*	R
C. lutchuensis	R
C. pitardii var. pitardii	S
C. polydonta	S
C. rosiflora	S
C. transarisanensis*	R
C. transnokoensis	R
C. yuhsienensis	R
C. yunnanensis*	R

* atypical symptoms

The results indicate that the majority of *C. japonica, C. reticulata* and hybrids are susceptible to infection by ascospores of *C. camelliae.* The number of petals infected during the inoculation procedure was not an absolute indication of the level of susceptibility of each variety as the number of ascospores per batch of petals was variable. The important point was to distinguish between susceptible varieties and varieties which may have some resistance. The petals of several species (e.g. *C. forrestii* and *C. longicarpa*) were not infected after repeated inoculation tests and these petals often showed atypical symptoms such as small brown lesions that failed to develop and pitting of the leaf surface (Figure 5.6). Petals that developed atypical symptoms were returned to incubation conditions to await sclerotial formation as

confirmation of *C. camelliae* infection. Symptoms rarely enveloped the entire petal and sclerotia were never formed.

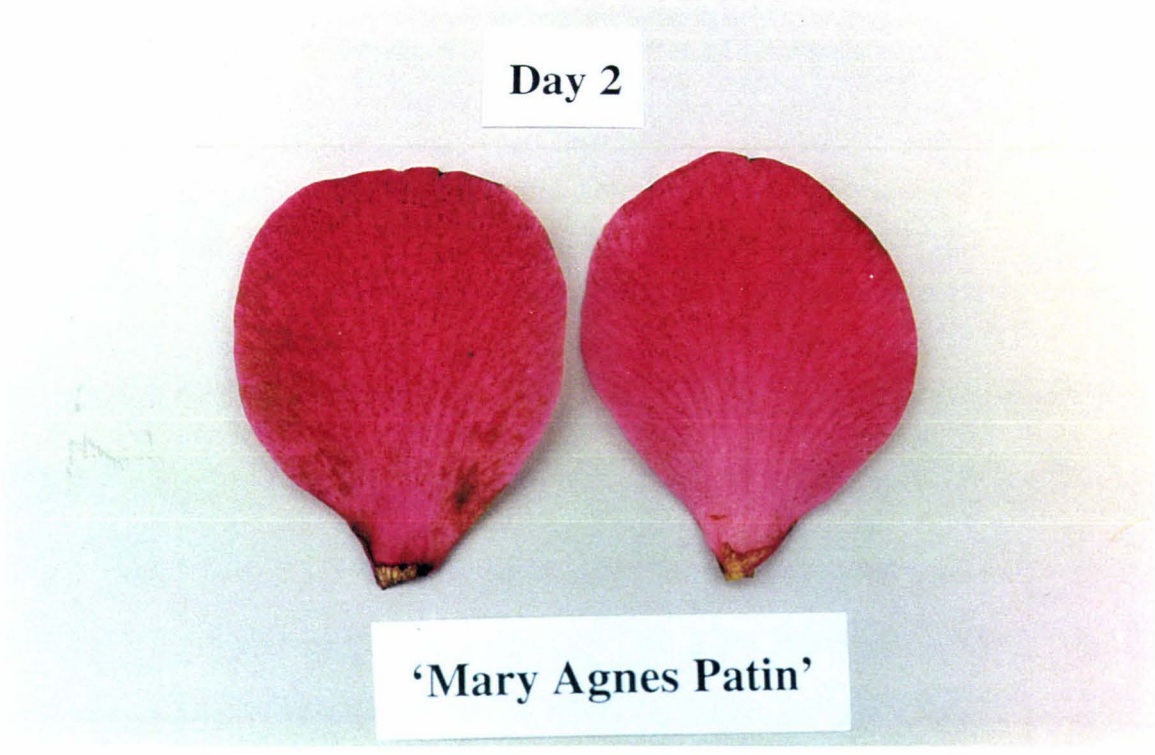

Figure 5.6
Pitting of the petal surface in susceptible *C. japonica* 'Mary Agnes Patin'. Although this variety was susceptible, the pitting symptoms show up more clearly on the red petals than on the white petals of species that developed pitting but no infection.

5.5 DISCUSSION

The infection studies utilised several inoculum sources and techniques, examined the infection of petals at different stages of development and the potential for spread by other means (microconidia and petal-to-petal), as well as initiating a screening programme for resistance.

5.5.1 Other Means of Spread

Ascospores are the only known inoculum source of camellia flower blight, but because microconidia are produced in great numbers during the infection season, they have the potential to play a significant role in the infection of flowers. The debate continues as to whether microconidia are sexual in function, act as infection propagules or are

redundant. Dodge (1932; 1935) and Drayton (1934) demonstrated that microconidia acted as fertilising agents in *Neurospora tetrasperma*, *N. sitophila* and *Sclerotinia gladioli*. Ultrastructural studies by Urbasch (1985) showed that the microconidia of *B. cinerea* had all the essential organelles required for germination and Harrison & Hargreaves (1977) germinated microconidia of *B. fabae* after a cold-conditioning period.

In the experiment reported here, microconidia did not cause infection in petals of *C. sasanqua*. Further experiments would be necessary, using other camellia species and cold-conditioned microconidia before this could be confirmed. Field experience suggests that microconidia do not need to act as infection propagules, as the ascospore inoculum load appears to be very high in areas where the disease is present.

The transfer of infection from infected to healthy petals is possible (Experiment 3) without wounding or free water. Infection transfer is quite variable, however possibly due to variations in the virulence of isolates, in the resistance of petals or variations in petal-to-petal because of the curved nature of some petals. Petal-to-petal infection is unlikely to be a major method of spread within a season except within a flower, by contact of adjacent flowers or possibly by diseased petals falling onto an adjacent flower. Ascospores would still appear to be the main source of inoculum and spread of the pathogen.

5.5.2 Mycelial vs. Ascospore Inoculum

Experiments using mycelial plug infection of petals showed that wounding was usually required for infection to occur. Ascospores, however, do not require wounding and appressoria have been observed on germinated ascospores (Peter Long Pers. Comm.). It is therefore assumed that *C. sasanqua* flowers are susceptible to ascospore infection, but avoid the pathogen through autumn-flowering. Both types of inoculum readily infect the later stages of bud development, but it would appear that the physiological state of petals in buds limits infection. During the disease season, it is known that flowers can become infected as soon as the petal tips emerge, but this was not observed.

5.5.3 Resistance Screening

Experiment 10 was the first known attempt to document susceptibility of camellia species and varieties to *C. camelliae*. The majority of commonly grown camellias – the *C. japonicas, C. reticulatas* and hybrids – all appear to be susceptible to *C. camelliae* infection (with one exception). A number of camellia species appear to have some level of resistance to the pathogen, however, these are species are not commonly grown and are only available from specialist nurseries. This is the first report that such resistance may exist and has important consequences for future work with the *C. camelliae* pathogen and plant breeding. Table 5.10 shows the distribution of resistant and susceptible varieties within the Genus Camellia.

Species with resistant characteristics were found in the Subgenera Metacamellia, Protocamellia and Camellia. The parentage of all hybrids tested was checked in the International Camellia Register. All parents were of susceptible origins (e.g. *C. japonica* x *C. saluenensis*) except for one hybrid 'Spring Mist' a *C. japonica* 'Snow Bells' (S) x *C. lutchuensis* (R). The results of one test of 'Spring Mist' are shown in Figure 5.7.

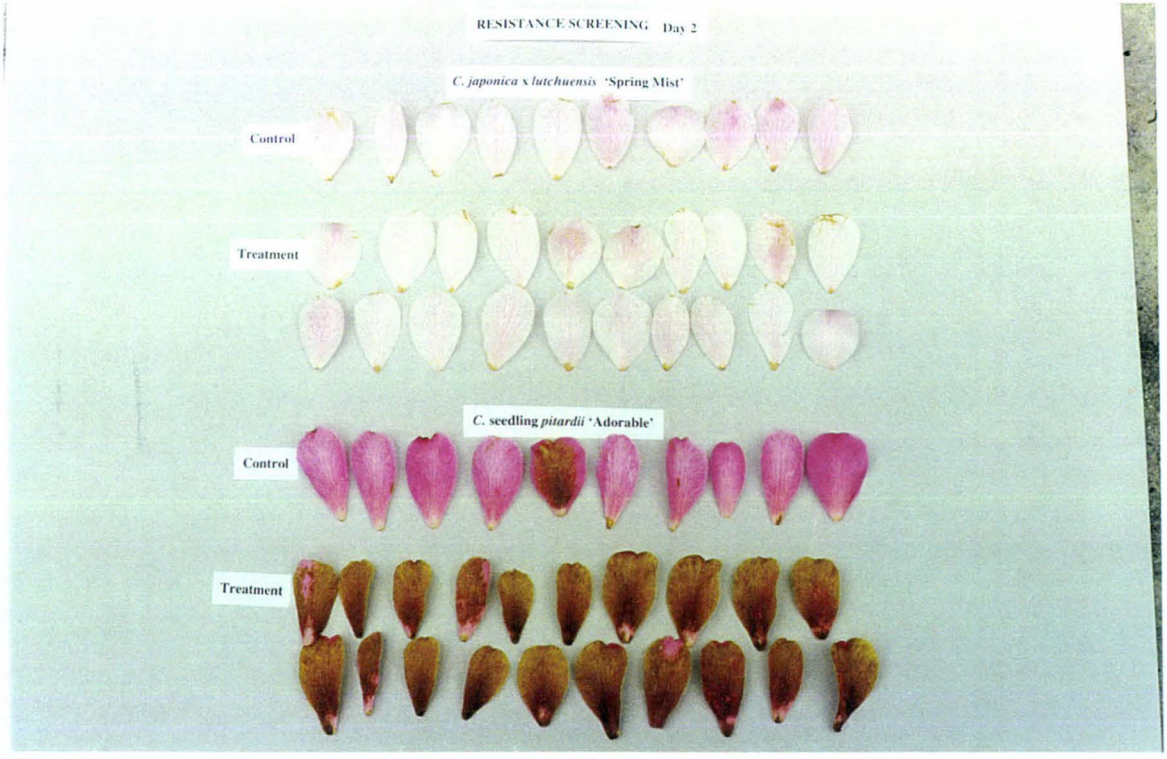

Figure 5.7
A resistance test of 'Spring Mist' (top) which was resistant and 'Adorable' (bottom) which was susceptible. Photograph taken 2 d after inoculation.

Table 5.10
Distribution of Resistance and Susceptibility in the Genus Camellia (Phylogeny According to Chang & Bartholomew 1984, Species According to International Camellia Register).

Subgenus	Section	Species	Resistant/Susceptible
Protocamellia	Stereocarpus	C. yunnanensis	R
	(Archeocamellia)		
	(Piquetia)		
Metacamellia	Theopsis	C. cuspidata	R
		C. forrestii	R
		C fraterna	S
		C. longicarpa	R
		C. lutchensis	R
		C. rosiflora	S
		C. transkoenoensis	R
		C. transarisanensis	R
	(Eriandria)		
Thea	Chrysantha	C. euphlebia	S
	(Longipedicellata)		
	(Calpandria)		
	(Longissima)		
	(Thea)		
	(Glaberrima)		
	(Corallina)		
	(Brachyandra)		
Camellia	Oleifera	C. sasanqua	
	Paracamellia	C. yuhsienensis	R
		C. grijsii	R
	Camellia	C. chekioangaleosa	S
		C. japonica	S
		C pitardii var pitardii	S
		C. polydonta	S
		C. reticulata	S
		C. saluenensis	S
	(Furfuraceae)		
	(Luteoflora)		
	(Tuberculata)		
	(Pseudocamellia)		

( ) no species tested in Section

There were a number of drawbacks to the testing procedure and the results cited here can only be considered provisional. For example, in the settling chamber the number of ascospores per cm^2 could not be controlled so inoculum levels varied between each batch of petals. Similarly, petals, particularly small petals, were frequently curled, thus affecting the aerodynamic flow and possibly ascospore deposition. Before further work is planned, the testing procedure needs to be modified to provide quantitative and qualitative data about levels of resistance in each variety. Ascospore suspensions, where the inoculum level can be controlled, would provide this consistency, and the resistance levels in the species and varieties could be ranked (Figure 5.8). Ascospore suspensions were trialled using ascospores stored on membrane filters (Hunter et al 1982) but the ascospores were not viable. Obviously, the results of this season's work need to be validated in order for the field of resistance work to be explored.

Figure 5.8
Petals from 'Zhangjia Cha' (left) and 'Warwick Berg' (right) two days after inoculation using the ascospore settling chamber. Both were inoculated in the same batch, however, symptoms are more pronounced on 'Zhangjia Cha'. An inoculation technique that allowed resistance levels between camellia species and varieties to be quantified could be used to qualify issues such as the speed with which *C. camelliae* spreads within host tissue.

These experiments show that resistance to *C. camelliae* almost certainly exists in certain species and future research should be directed towards understanding the mechanisms of this resistance and how it could be applied to the development of camellia varieties resistant to *C. camelliae.*

CHAPTER SIX

PRELIMINARY BIOLOGICAL CONTROL INVESTIGATION

6.1 INTRODUCTION

The use of biological organisms to control pests is not a new concept, but in the second half of the twentieth century it has undergone a renaissance. This renewed interest is driven (in part) by the increasing cost of pesticides, the rise of pathogen resistance to existing pesticides and the desire for environmentally safer alternatives.

6.1.1 Advantages of Biocontrol

Biological control is seen as a safer alternative to chemical pesticides. In general, the control organism is very specific in its mode of action and thus there are few side effects for other beneficial organisms in the environment (although there are some notable exceptions such as the introduction of the mongoose into Fiji for rat control: it preferred chickens). In ideal circumstances, biological control agents are self-sustaining, and will persist. In practice, however, it is often necessary to manipulate the environment or biocontrol agent in order to attain effective control. The potential for the pest to develop resistance (either behaviourally or physiologically) is also much reduced. Although resistance to *A. radiobacter* by *A. tumefaciens* has been documented (through the transfer of the plasmid carrying the resistance gene) (Stockwell et al 1996; Vicedo et al 1996), in general, the development of resistance to biocontrol agents is considered unlikely (van Emden 1989).

6.1.2 Disadvantages of Biocontrol

While biocontrol may offer a safer alternative to chemical pesticides, it is not without limitations. In general, biocontrol agents are used to curb the population of the target pest so that the pest is controlled, but is not eliminated from the environment and at low levels it may still cause economic damage. Control may be slow initially, as the

biocontrol agent takes time to spread from its release site or build up to populations that are effective against the pest. The efficacy and consistency of control is also affected by the variability of the system (e.g. extremes in weather), so that results may be unpredictable. The use of a biocontrol agent limits the subsequent use of chemical pesticides against other pests in the system because of the danger to the biocontrol agent. This problem spawned the integrated control concept of "applied pest control which combines and integrates biological and chemical control" (Stern et al 1959).

6.2 BIOLOGICAL CONTROL OF PLANT PATHOGENS

The worldwide loss to economic crops caused by plant pathogens alone is estimated to be between 13-30% of the annual production (James 1981). In the United States there are about 2000 plant diseases common on 31 principal crops (Lewis & Papavizas 1991). Of these 2000, about 90% are soilborne pathogens (Wilson 1968). There are seven commercial biocontrol products registered for use against plant pathogenic organisms (Lewis & Papavizas 1991) and these all target soilborne pathogens. While fewer in number, foliar pathogens, such as *B. cinerea,* also cause significant crop losses, but biological control in the phyllosphere has been less successful than in the rhizosphere. The phyllosphere presents quite a different environment to that of the rhizosphere and it is believed that this difference explains why biocontrol in this area has been less successful than for pathogens of the rhizosphere (Andrews 1990; Andrews 1992).

6.3 AIMS

The objective of this study was to trial a new method for the isolation of potential biocontrol agents effective against *C. camelliae.* This method was developed by Dr Darryl Cook at Massey University to isolate potential biocontrol agents effective against *B. cinerea* Pers., a common pathogen on a variety of glasshouse, fruit, flower, ornamental and vegetable crops (Agrios, 1988). In contrast to other screening techniques, this method sought antagonists which attached to the hyphae and macroconidia of *B. cinerea.* Using the attachment assay as part of a screening programme, he isolated 20 bacteria and yeast that adhered to *B. cinerea.* Fifteen of these were subsequently shown to have high antagonistic activity on glasshouse tomato

plants and postharvest kiwifruit (Cook 1997).

6.4 MATERIALS AND METHODS

The method followed is that taken from Cook (1997), Cook et al (1997a) and Cook et al (1997b) but adapted in several places where petals were substituted for leaves. Soil samples were not taken.

6.4.1 Collection of Potential Biocontrol Agents

Samples were collected from Massey University Arboretum on the 25 June 1998, Harcourt Park, Upper Hutt and the Botanic Gardens, Wellington on 17 August 1997. At each site, eight leaves (each from a different camellia plant) were removed and placed in Plastic Clip Seal bags (100 x 150 mm), four to each bag. Eight petals (each from a different camellia plant) were removed and placed in Plastic Clip Seal bags (100 x 150 mm), four to each bag. Each leaf or petal was handled only by its base while wearing new, nonsterile latex medical examination gloves.

6.4.2 Extraction of Potential Biocontrol Agents

In the laboratory, 20 ml of SROW was added to each bag. The bags were resealed expelling as much air as possible. Each bag was shaken and massaged gently for 2 min. The washings were poured into a 47 mm diameter filter holder and vacuum filtered through a 0.22 μm pore filter (Millipore GSWP 047 00). Potential biocontrol agents were washed from the filters and resuspended in 10 ml SROW. These were stored at 4°C until required.

6.4.3 Co-Incubation of Potential Biocontrol Agents and *C. camelliae*

A 50 mm diameter disc of camellia petal ('Zhangjia Cha') was laid at the bottom of a sterile glass petri plate. A 500 μl drop of SROW was placed in the centre of the camellia disc and this was overlaid with a 50 mm diameter disc of cellophane (Jam & Preserve Covers, Caxton Ltd., Christchurch). From the washings stored at 4°C, 950 μl of extract was placed in the centre of the cellophane and 50 μl of *C. camelliae* mycelial

scrapings were added to this. The *C. camelliae* mycelial scrapings were prepared from isolate Cc3 grown on Difco PDA at 20°C 12 h light/12 h dark. Mycelium was scraped from the surface of the culture, suspended in 50 µl of SROW and mashed into smaller pieces. Plates were incubated at 15°C for 16 h in the dark.

Four replicate plates were prepared from each sample.

After incubation, the cellophane containing the washings and *C. camelliae* mycelium was removed and placed in 25 ml Universal bottles containing 5 ml of McIlvaines Buffer (pH6.0) (Appendix I). The cellophane was agitated gently with a glass hockey stick to remove any colonies.

6.4.4 Wash Procedure

The wash procedure followed that described in Cook (1997), Cook et al (1997a) and Cook et al (1997b).

Flasks were incubated at 15°C only, as a 10°C incubator was not available at the time.

6.4.5 Culture of Potential Biocontrol Agents

A 500ul aliquot from each sample flask was pipetted onto either Nutrient Agar (NA) or Nutrient Yeast Dextrose Agar (NYDA) (depending in which medium they were originally incubated) spread with a sterile loop and incubated at 15°C for 72 hours. Colonies were reisolated until uniform colonies were obtained. Twenty-one bacteria and two yeast isolates were obtained. The source of these isolates is shown in Table 6.1.

Table 6.1
Site and Source of Potential Biocontrol Agents Obtained in Wash Procedure.

Site (Massey, Harcourt, Botanic)	**Sample (Leaf or Petal)†**	**Organism Isolated‡**
Wellington	leaf bag 1	bacterium WL1a
Wellington	leaf bag 1	bacterium WL1b
Wellington	leaf bag 2	bacterium WL2a
Wellington	leaf bag 2	bacterium WL2b
Wellington	leaf bag 2	bacterium WL2c
Wellington	petal bag 1	bacterium WP1a
Wellington	petal bag 1	bacterium WP1b
Wellington	petal bag 2	bacterium WP2a
Wellington	petal bag 2	yeast WP2b
Upper Hutt	leaf bag 1	bacterium UPL1a
Upper Hutt	leaf bag 1	bacterium UHL1b
Upper Hutt	leaf bag 1	bacterium UHL1c
Upper Hutt	leaf bag 2	bacterium UHL2a
Upper Hutt	leaf bag 2	bacterium UHL2b
Upper Hutt	leaf bag 2	bacterium UHL2c
Upper Hutt	petal bag 1	bacterium UHP1a
Upper Hutt	petal bag 1	bacterium UHP1b
Upper Hutt	petal bag 1	bacterium UHP1c
Massey	leaf bag 1	bacterium ML1a
Massey	leaf bag 1	yeast ML1b
Massey	petal bag 2	bacterium MP2a

† sample bag 1 or 2

‡ a, b and c represent the different organisms isolated from each sample bag

6.4.6 Spore Suspensions

Cell suspensions were made in SROW for each potential biocontrol agent. Cells were dislodged from the colony using a jet of SROW from a pipette tip. Repeated pipetting increased cell density. Suspensions were transferred to a 1.5 ml Eppendorff tube and centrifuged at 10 000 rpm for 5 min using a Biofuge A (Heraeus, Sepatech). The liquid was decanted off and the pellet resuspended in another 1.5 ml of SDW. A Petroff-Hauser Counter (MNK-780-T, Weber Scientific International Ltd., England) was used to count cells. A minimum of 600 cells was counted per sample (Meynell & Meynell

1970). Cell counts varied between concentrations of $2.5x10^9$ and $1.2x10^{10}$.

Ascospores of *C. camelliae* were collected on 3.0 µm or 5.0 µm polycarbonate filters (Poretics Products, California) by suspending a single apothecium above a filter for 36-48 h. The filters were stored separately in 25 ml Universal bottles half filled with calcium chloride and nonabsorbent cotton wool and stored at 4°C (Hunter et al 1982). Ascospores were scraped off the filter and resuspended in 1.5 ml SROW. Suspensions were transferred to a 1.5 ml Eppendorff tube and centrifuged at 10 000 rpm for 5 min using a Biofuge A (Heraeus, Sepatech). The liquid was decanted off and the pellet resuspended in another 1.5 ml of SROW and vortexed (~5 sec) to break up clumps. Ascospore concentration was determined using a haemocytometer and varied between $2.6x10^5$ and $8.4x10^5$.

6.4.7 *In vivo* Assay for Biocontrol

The assay was carried out in two parts because of the time taken to prepare samples. Petals were not sterilised. Eight petals of the cultivar 'Spring Festival' were laid out on the lid of a glass petri dish and 1 ml aliquot of the potential antagonist was applied to the petals using the Potter Tower with a nozzle pressure of 100 mm Hg. The same petals were immediately challenged with a 1 ml aliquot of *C. camelliae* ascospores. Controls of petals only, petals and 1 ml SROW and petals, water and *C. camelliae* ascospores were made for each part of the experiment. The petals were incubated at 20°C in sealed containers lined with moistened paper towels. Each sample of eight petals was assessed daily for 14 d for symptom development.

6.5 RESULTS

The results are shown in Table 6.2. Symptoms at Day 10 after inoculation are shown as many petals began withering after this time although there was plenty of moisture in the containers.

Table 6.2
In vivo Assay for Biocontrol on Camellia Petals Browning Symptoms Consistent with *C. camelliae* Infection 10 d after Inoculation.

Site and Sample† **Part I**	**No. Petals (/8)**
petals only	0
petals + water	0
petals + water + ascospores	0
Wellington L2a	1
Wellington P1a	1
Wellington P2b	0
Upper Hutt L1a	0
Upper Hutt L1b	0
Upper Hutt L1c	0
Upper Hutt P1a	0
Massey L1a	0
Massey P2a	0
Site and Sample† **Part I**	
petals only	0
petals + water	0
petals + water + ascospores	0
Wellington L1a	0
Wellington L1b	0
Wellington L2b	0
Wellington L2c	0
Wellington P1b	0
Wellington P2a	0
Upper Hutt L2a	0
Upper Hutt L2b	0
Upper Hutt L2c	0
Upper Hutt P1b	2
Upper Hutt P1c	0
Massey L1b	0

† sample bag 1 or 2; a, b and c represent the different organisms isolated from each sample bag

Neither controls nor treatments were consistently infected with *C. camelliae*. Of the three treatments where symptoms typical of infection by *C. camelliae* were found, only one appears to have been naturally infected before the experiment began because it appeared the day after inoculation whereas the other two did not appear for a further two days. The viability of the spores stored on filter papers was not tested at the time of this experiment since prior experience did not indicate that spore viability was affected by storage. However, subsequent experience has shown that spore viability can become much lower.

6.6 DISCUSSION

Because of time constraints (the disease survey) this experiment was not repeated before the end of the ascospore production season. Since infection by *C. camelliae* did not occur in the control treatments, it is likely that the viability of the ascospores was in someway compromised during the experiment although symptoms of *C. camelliae* infection appeared after 3 d incubation in two treatments (UHP1b and WL2a) indicating that at least some of the ascospores were viable. The ascospores were stored for up to 6 d by the method of Hunter et al (1982) who successfully stored viable ascospores of *S. sclerotiorum* for 24 mo. The ascospores were applied to the petals at high pressure (100 mm Hg) using the Potter Tower, and it is possible that this high-pressure application had a detrimental effect on the spores although Cook (1997), Cook et al (1997a) and Cook et al (1997b) used the technique successfully. Future experiments should be done with fresh ascospores until storage conditions have been further investigated.

It should also be noted that this experiment was very costly in terms of ascospores. Each sample was treated with ascospores from at least one apothecium, making it more time consuming to collect, prepare and count compared with the macroconidia of *B. cinerea* used by Cook (1997), Cook et al (1997a) and Cook et al (1997b). To conduct a full experiment, applying ascospores before and after inoculation with the potential biocontrol agent would require considerable time and ascospores.

The cultivar 'Spring Festival' was chosen for this experiment because it was known to be susceptible to *C. camelliae* and the small petals allowed eight to placed on a petri

dish (making one treatment sample). However the petals are small and thin and they dried out more quickly than petals of other cultivars with which I have worked. In future experiments of this type, a cultivar such as 'Roger Hall', which has small, but fleshy petals would be more appropriate.

The development of a foliar biocontrol agent that is effective against the ascospore and germling stage of *C. camelliae* is desperately needed. Since flowers open progressively during the flowering season, any spray programme requires regular applications (every 7-14 d) to be effective. A chemical spray programme would be expensive and gardeners may be reluctant to use chemical pesticides, especially in such quantities. Chemical sprays have also proved less than effective (see Tables 1.2 and 1.3). A foliar biocontrol agent, (even if it is only as effective as the best chemical pesticides available) would be a significant advance.

Current control measures have been largely ineffective in controlling this pathogen. Many concentrate on eliminating sclerotia in the soil, or preventing them from forming apothecia. Because ascospores are airborne, however, they make *C. camelliae* a community disease rather than an individual garden disease. While a gardener may control the disease in his or her garden, it does not prevent the ascospores from blowing in from neighbouring properties. Therefore, flower infection control is critical. A biocontrol agent active in the infection court would be a breakthrough, not only in the control of this pathogen, but for control of pathogens of aerial plant parts in general.

CHAPTER SEVEN

GENERAL DISCUSSION

7.1 OVERVIEW

This project was initiated to investigate the basic biology of the camellia flower blight pathogen (*C. camelliae*) and to provide a scientific basis for future research developments. A range of aspects, of both the pathogen and the host, have been studied, including growth in culture, distribution and spread within New Zealand, conditions for inducing sclerotial germination, the process of infection, the development of a method to screen camellia for resistance, the identification of sources of resistance and attempts to isolate a biocontrol agent effective against the ascospore stage of the pathogen.

While the sclerotial germination and biocontrol isolation work failed to achieve their ultimate objectives, minor goals were achieved and experience was gained in working with this pathogen. As Baxter (Pers. Comm. to Terry Stewart) stated: "do not underestimate this fungus". Other research areas have been more productive however, and discoveries that may lead to the development of camellia varieties that are resistant to the pathogen have been made.

The results obtained in these studies on *C. camelliae* indicate several new research directions.

7.2 DISEASE DISTRIBUTION AND SPREAD

7.2.1 New Zealand

The surveys conducted in 1997 and 1998 in the North and South Islands of New Zealand found that the disease was more widely distributed than previously thought. Evidence suggests that spread by windborne ascospores is the main source of spread of the pathogen, but that transfer on infected planting material may become more important as it establishes itself within communities.

The arrival of this pathogen in New Zealand provides an opportunity for the study of the spread of a disease within New Zealand. Successive surveys each disease season would allow the progression of the disease to be monitored, and predictions made about the likely rate of spread and pattern of distribution (e.g. effect of mountain barriers or Cook Strait) for other newly introduction organisms. From the perspective of the camellia grower in regions where the disease is not yet found, annual surveys would also be desirable. However, the cost in time and money is high and not a practical proposition without funding. On a smaller scale, an organised postal survey using Camellia Society members would be a less costly method of monitoring its spread. Previous surveys were not effective due to a low response (Terry Stewart, Pers. Comm.) rate. Disease-recognition skills by members and the general public would need to be improved.

An education programme is also desirable, for the public, for nurseries and for garden centre staff. Such a programme would increase general awareness about the disease, suggest control measures and lessen the risk of the disease being transferred by infected material. Despite the benefits of publicity, the disease will continue to spread and this must be weighed against the cost of such education programmes.

7.2.2 *C. camelliae* in Australia and Europe

Whether *C. camelliae* is present in Australia is unknown. As yet there are no special quarantine restrictions to ensure the disease is not imported from New Zealand on camellia plants, although as found in the surveys (Chapter 3), New Zealand nurseries appear to be doing a good job of controlling it. A survey to establish whether the pathogen is present would clarify whether special restrictions need to be imposed on the import of New Zealand camellias, but this matter requires some diplomacy and delicacy after the recent apple fire blight incident.

There have been reports that *C. camelliae* is present in Europe and is widespread in Portugal, Spain and France but this has not been confirmed by the scientific community. If the pathogen is present in France and control measures are not taken, then it is also likely to be present in the United Kingdom, as French nurseries export camellias to the United Kingdom.

7.3 STUDY OF RESISTANCE THROUGH INFECTION

Different levels of resistance to *C. camelliae* infection were identified (but not quantified) in camellia species. The existence of varying levels of resistance indicates that there is potential for new research directions, in particular:

1. The study of the mechanisms of resistance in camellia species and varieties with the ultimate aim of breeding camellias for resistance.
2. The study of the mechanisms of infection by *C. camelliae.*

These studies would overlap.

In order to begin this work, it is essential to have a method for the standardised inoculation of camellia petals for determining levels of resistance. One approach is the use of ascospores in suspension, where the concentration of ascospores can be controlled. However, the results of infection by ascospore suspensions and by airborne ascospores must be compared to ensure that the results of inoculation by suspension is comparable to those of the natural inoculum. If inoculation by suspension is shown to be a valid alternative, then screening of species and varieties can be instigated, levels of resistance quantified and conclusions drawn about the distribution of resistance in the Genus Camellia. The preliminary results reported in Chapter Five indicate that there may be a link between the level of resistance and Subgenus or Section within the Genus and this may have consequences for breeding programmes or grafting. It is also important at this point to differentiate between aggressiveness of *C. camelliae* isolates, so that aggressive isolates are used in the initial screening programme to avoid the experience of Dutch Elm Disease (DED), where a more aggressive isolate (*Ophiostoma novo-ulmi*) wiped out the screening elms that resisted the first isolate (*Ophiostoma ulmi*) (Smalley & Guries 1993).

7.3.1 Development of Resistant Camellia

Because no resistance was known to *C. camelliae*, breeding for resistance has not been a viable option for plant breeders. The potential now exists for a breeding programme for resistance. The techniques that would be required for such a project have not been developed for the Genus Camellia, although Haldeman (1995) has considered some of the prospects for genetic engineering in this genus. Callus production (Shults & Haldeman 1995) and infection of camellia using *Agrobacterium tumafaciens* (Haldeman & Locker 1996) have both been trialled.

Breeding for resistance in camellia, however, has similar problems to those encountered by those breeding for resistance to DED. American elms were those most widely grown, having the architectural qualities and adaptability required by the public; they have, however, low resistance to the pathogen (compare *C. japonica* and *C. reticulata*). Eurasian elms, which are highly resistant to the pathogen, are not considered aesthetically pleasing and have other undesirable traits (compare *C. forrestii* and *C. lutchuensis*) (Smalley & Guries 1993). Camellias differ in that any breeding programme would be inherently more difficult, as each variety is a result of specific breeding, and the flower form, colour and size would have to be retained.

Resistance may be able to be conferred by transformation, the insertion of a gene from another organism, thus avoiding problems associated with breeding. Because the host/pathogen relationship has not been investigated in camellia/*C. camelliae*, it is not known what type of gene or its origin would be required. Work in other host/pathogen relationships has concentrated on Pathogenicity Related (PR) proteins (e.g. chitinase), detoxifying enzymes, antimicrobial proteins and phytoalexin pathway changes (Higgins et al 1998). Grafting would be the perfect solution for camellia if it were found that susceptible scions could be grafted onto resistant rootstocks. Such a system has been shown to be effective against *Phialophora gregata* which causes brown stem rot of soybean. Resistance is conferred by the rootstock, so that a resistant rootstock x susceptible scion graft confers resistance on the scion, but a resistant scion x susceptible rootstock is susceptible (Bachman & Nickell 1999).

7.4 ARTIFICIAL PRODUCTION OF ASCOSPORES

All of the above studies depend on the availability of ascospores and this is currently limited to about three months over the disease season. Working February to April in the Northern Hemisphere would effectively double the period over which infection/resistance studies could be carried out, but this would still total less than six months each year. The basic problem is lack of ascospores. Infection studies of camellia using mycelial and ascospore inocula have shown the importance of working with the natural inoculum. Infection using mycelial plugs of the fungus grown in culture currently allows the conditions to be more closely controlled and repeated and

can be conducted year-round, but the results may not necessarily reflect the natural infection process.

It is therefore essential that techniques for induction of sclerotial germination be ascertained so that ascospores can be produced as required. Sclerotial germination experiments (Chapter Four) must be continued and expanded to include more variables. Once a method for sclerotial germination has been discovered, it can be refined to determine which conditions are critical.

7.5 MOLECULAR ASPECTS

Molecular techniques would be a useful tool in the study of *C. camelliae*. Currently, identification of the pathogen is made on the symptoms expressed by the flower and signs of the disease such as sclerotia and apothecia. During the course of this work, however, there were many instances where definitive identification of the disease could not be made, either because of anomalous symptoms or unreported infection of leaves or twigs. For example, Figure 7.1 shows a camellia leaf collected from Wellington Botanic Gardens. What appears to be a sclerotium, of the size and shape consistent with those of *C. camelliae* formed in petals, has formed in the leaf. No other disease is reported to produce these signs and we suspect that it may be an aberrant manifestation of *C. camelliae* infection. Likewise, *C. camelliae* is able to grow at least a few mm into the twig from the flower (Peter Long, unpublished). A molecular probe would be useful for tracking hyphae in plant tissue, for confirming petal and leaf infections and in quarantine work where there may be no visible symptoms of the disease.

Figure 7.1
Camellia leaf collected in the Wellington Botanic Gardens with a suspected *C. camelliae* sclerotium embedded in leaf tissue.

DNA analysis can also be used to examine population dynamics/biology and it may be possible to determine its worldwide spread, i.e. whether the population in New Zealand is derived from North America or Japan and establish its origins. The exact origins are uncertain as Japanese literature does not mention it until 1919 although camellias are important in both Japan and China so one would expect some literature on the problem. However, access to Chinese and Japanese literature on the subject is difficult because of language and, until recently, the political situation.

Molecular studies of *C. camelliae* have not been done, although some work on other members of the genus has been carried out (Holst-Jensen et al 1997a; Holst-Jensen 1997b). These studies investigated the phylogeny of various members of the Family SCLEROTINIACEAE based on a comparison of ribosomal DNA sequences. These studies showed that *C. erythronii* was less closely related to *C. foliicola* and *C. whetzelii* than various other members of the Family, although the relationship changed according to the DNA sequence compared (i.e. Internal Transcribed Spacer (ITS), 5.8S rDNA and 18S rDNA) and parsimony and tree analyses used. In a comparison of the ITS1 sequence of *C. ciborium* and *C. erythronii*, Carbone & Kohn (1993) concluded that the

genus *Ciborinia* was a heterogeneous grouping of species and in "need of mongraphic revision". One consequence of these findings is that if *Ciborinia* is a heterogeneous genus, then sclerotial germination treatments that work in other genera may be more relevant for stimulation of apothecial formation in *C. camelliae* than work on other *Ciborinia* species.

7.6 CONCLUSION

This project has investigated aspects of the biology of *C. camelliae* and its relationship with the camellia host. The most important finding in this work has been the discovery of resistance to the pathogen, where none was previously recorded. In order to exploit this, the host/pathogen interaction must be studied in more detail, so that effective control of the pathogen can be achieved through resistant varieties. Future work will require the development of new techniques and modification of existing methods to elucidate the host/pathogen interaction. Molecular tools specific to *C. camelliae* offer a means of studying the pathogen in detail and furthering our understanding of this pathogen and its life cycle.

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APPENDIX I

SOLUTIONS AND MEDIA

SOLUTIONS

McIlvaine Buffer

21.01 g/Litre citric acid monohydrate (0.1 M)

28.40 g/Litre Na_2HPO_4 (0.2 M)

To obtain pH 6.0, mix 36.85 ml citric acid with 63.15 Na_2HPO_4

From McKenzie & Dawson (1969).

MEDIA

Camellia Petal Agars

Agars were prepared using a) *C. sasanqua* petals and b) *C. japonica* petals. All petals were collected at Massey University and a mixture of cultivars was used in each preparation

Autoclaved Petals

Petals (60 g/500 ml) were added to Difco PDA prior to autoclaving (15 min at 121°C).

Boiled Petals and Infusion

Petals (60 g/500 ml) were boiled in distilled water until the colour was lost (~15 min). The petals were strained from the liquid and added to Difco PDA prior to autoclaving (15 min at 121°C). The liquid infusion was used to make up Difco PDA.

Homogenised Petals

Petals (60 g/500 ml) were homogenised in a Waring Blender (~3 min). The homogenate was topped up to 800 ml with ROW (autoclaved 15 min at 121°C).

Propylene Oxide Petals

Ten petals were placed in 400 ml Schott bottles with cotton wool soaked in propylene

oxide. The bottles were sealed and left for 108 h. The lids were loosened and the propylene oxide allowed to evapourate over 48 h. Petals were placed onto freshly poured plates of Difco PDA.

Homemade PDA

200 g potato (old potatoes best)

20 g D-glucose

20 g agar

1 Litre SDW

The unpeeled potatoes were scrubbed clean, chopped into cubes and 200 g weighed and rinsed in distilled water, wrapped in cheesecloth and added to 1 Litre of boiling ROW (~20 min). The liquid was squeezed out of the cheesecloth and the potatoes discarded. Twenty g agar and 20 g glucose were added to the liquid which was then made up to 1 Litre with ROW and autoclaved (15 min at 121°C).

Adapted from the CMI (Commonwealth Mycological Institute) Plant Pathology Handbook, 1968.

Leonian's Agar

0.625 g peptone

6.25 g maltose

6.25 g malt extract

1.25 g KH_2PO_4

0.625 g $MgSO_4.7H_20$

20 g agar

1 Litre distilled water

From Tuite (1969).

Nutrient Agar (NA)/Nutrient Broth (NB)

8 g nutrient broth

15 g agar (NA only)

1 Litre distilled water

Autoclave (15 min at 121°C).

Nutrient Yeast Dextrose Agar (NYDA)/Nutrient Yeast Dextrose Broth (NYDB)

8 g nutrient broth

5 g yeast extract

10 g D-glucose

15 g agar (NYDA only)

1 Litre distilled water

Autoclave (15 min at 121°C).

APPENDIX II

EXAMPLE OF SAS CODE

Example of SAS code for a three factor ANOVA.

Non-optional SAS code is represented in capital letters, optional code (name of data set and variables) is underlined. Code for transformation and Tukey tests are marked with * and may be omitted where appropriate.

```
DATA NAME;
INPUT ISOLATE MEDIA TEMP AVERAGE;
*LAVERAGE=LOG(AVERAGE + 1);*
CARDS;
(insert data set)
;
RUN;

PROC PRINT;
VAR ISOLATE MEDIA TEMP AVERAGE *LAVERAGE*;
RUN;

PROC GLM DATA=NAME;
CLASS ISOLATE MEDIA TEMP;
MODEL AVERAGE (*LAVERAGE*)=ISOLATE|MEDIA|TEMP/SS1;
MEANS AVERAGE (*LAVERAGE*)=ISOLATE|MEDIA|TEMP;
*MEANS ISOLATE MEDIA TEMP/TUKEY;*
OUTPUT OUT=RDIS P=PREDICT R=RESIDUAL;
RUN;
```

```
PROC PLOT DATA=RDIS;
PLOT RESIDUAL*PREDICT;
RUN;
PROC CHART DATA=RDIS;
VBAR RESIDUAL/TYPE=FREQ;
RUN;
PROC UNIVARIATE DATA=RDIS PLOT;
VAR RESIDUAL;
RUN;
```

APPENDIX III

SURVEY SITES 1997 AND 1998

Sites are listed by year of survey and by North or South Island. Addresses are listed under the town, or nearest town in which they were found. Towns and cities are listed in alphabetical order for easy reference. Plant centres, churches, parks, etc. are listed first in alphabetical order. Street addresses are listed in alphabetical order, and where more than one site in one street was surveyed, these are listed in numerical order. Where the name of the main road is not known (which was frequently the case in small towns) I have listed the site as (e.g.)'4 SH6' meaning 'letterbox number 4 on main road through town X'. In some cases it was not possible to give a street address, so I have given the name on the letterbox, or directions (e.g. 'east of town') or other directions. Most of these sites can be easily located during the camellia flowering season.

1997 NORTH ISLAND

Address	No. Plants (total)	No. with Diseased Flowers
Ashurst		
cnr Bamford St	4	0
216 Cambridge Ave	1	1
224 Cambridge Ave	1	0
Craven Cres	3	0
143 Stanford St	1	0
57 Worchester St	1	0
Aokoutere		
Portch family	43	3
Bell Block (near New Plymouth)		
Hulke Cres	1	0
981 Main Rd	1	0
east of town	11	0
east of town	8	0

Bulls		
Bulls Tea Shop	3	0
Police Station	1	0
81 Holland Rd	2	0
73 main rd	1	0
Martin Rd	4	0
Bunnythorpe		
28 main rd	2	0
Carterton		
Carrington	>30	1
Memorial Square	6	0
Cheltenham		
north of town	5	1
north of town	2	1
Ekatahuna		
road verge north of town	20	0
garden opp. these	1	0
Eltham		
264 SH3	4	2
Featherston		
house near Fire Station	23	0
public gardens	6	1
Feilding		
north of town	2	0
Norfolk Gardens	5	0
Norfolk Gardens	>30	27
BP Station, Aorangi Rd	3	0
Kowhai Gardens	50	24
Foxton		
pool	1	1
school	3	1
Hunterville		
2km north	4	0
next to Post Office	1	0
St Andrews Presbytarian Church	5	0
cnr Feltham St	3	0
main st	13	1

Inglewood		
milk no. 1337	4	4
31 Rimu St	2	2
Library	2	2
Hawera		
185 SH3	1	1
187 SH3	2	1
233 SH3	1	1
Kaitoke (near Upper Hutt)		
Kaitoke Country Gardens	>20	3
Kimbolton		
Crosshills	11	1
Rhododendron Association Garden	35	2
'Marama'	51	12
Plunket	7	0
south of town	1	0
Levin		
Rose Gardens	9	4
Manaia		
Golf Club	8	8
near Tangatora Strm	3	0
Mangaweka		
school	6	1
cnr Te Kapua St	1	0
Utiku Church	2	0
Martinborough		
near main square	1	0
Presbytarian Church, Weld St	5	1
second house entering west of town	5	0
Marsterton		
Queen Elizabeth Gardens	87	5
Solway Park Motel	15	0
cnr Chapel & Michael Sts	7	0
174 Chapel St	3	1
cnr High & Derby Sts	7	0
137 High St	1	0
141 High St	1	0
Marton		
110 Wanganui Rd	4	2

lower Beaven Rd	8	0
Midhurst		
opp. Bare Bistro	1	1
New Plymouth		
Brooklands Zoo	8	3
Pukerua Park	1	0
335 Devon St	6	3
419 Devon St	2	1
420 Devon St	2	2
Redcoat Lane	12	7
Ngatui		
farm	>20	0
Normanby		
opp. Atkinson St	2	0
Okato		
Fire Station	8	0
beside Home Kills	8	0
18 SH45	1	0
opp. 18 SH45	4	0
Opiki		
school	15	0
Opunake		
sports ground	3	0
Allison St	2	0
62 Fox St	6	0
cnr Gisborne Tce & Havelock St	3	0
43 Gisborne Tce	8	2
Otaki		
Mill Rd	3	2
Pahiatua		
first house on right after bridge	3	1
main st	11	0
Palmerston North		
Esplanade	23	1
Massey University Arboretum	37	3
18 Hughes St	18	1
Summerhill Dr	45	3
Victoria St	6	2

Paraparaumu		
in three gardens	14	10
Airport	2	2
Patea		
rear of council offices	3	0
191 SH3	3	3
195 SH3	1	0
Pukeiti		
Pukeiti Gardens	25	0
Pungarehu		
St Martins Catholic Church	2	0
Rahotu		
school grounds & opp.	2	0
Rata		
The Ridges Garden & Nursery	1	0
Raumati South & Beach		
cnr Ngaio & Matua Rd	1	1
Poplar Ave	6	4
Rosetta Rd	1	1
Sanson		
Caltex	1	0
26 main rd	1	0
Stratford		
opp. 33 Broadway	6	2
Taihape		
Gumboot Manner Tearoom	1	0
St Davids Presbyterian Church	1	0
Kokako St	4	0
Upper Hutt		
Harcourt Park	45	34
151 Colston Park Rd	4	4
opp 151 Colston Park Rd	1	1
149 Colston Park Rd	1	0
Waitara		
AFFCO Dommet St	26	0
149 Raleigh St	5	1

Pukekohe Domain	18	0
3 Melern St	15	0
Wanganui		
Glen Logie Rose Gardens	12	7
Waverley		
5 SH3	5	0
Community Park	14	11
Winiata		
driveway at junction	7	1

1998 NORTH ISLAND

Town	No. Plants (total)	No. with Diseased Flowers
Acacia Bay		
Acacia Bay Lodge	2	0
Auckland		
Albert Park	2	0
Auckland College of Education	13	1
Auckland Regional Botanic Gardens	175	0
Bible College, Henderson	5	0
Centre Point Nurseries, Albany	3	0
Eden Gardens	>200	12
Featherstones Garden Centre	18	0
Forest Hill Nurseries	5	0
Ivy Garden Centre, Pakuranga	22	0
Orinda Mitre 10, Howick	7	0
Pakuranga College	2	0
Palmers, Browns Bay	4	0
Palmers, Glen Eden	16	0
Palmers, Hobsonville	8	0
Palmers, Newmarket	2	0
Plant Barn, Remuera	12	0
Remuera Gardens	6	0
Selwyn Village, Point Chevalier	11	0
Southern Cross Hospital, Mt Eden	1	0
Sunhill, St Johns	21	0
University of Auckland	5	0
Wholesale Plants, Takanini	38	0
222 Campbell Rd, Onehunga	2	0

111 Cook St, Howick	1	0
6 Epsom Ave, Epsom	1	0
16 Epsome Ave, Epsom	1	0
72 Forest Hill, Henderson	1	0
130 Glenmore Rd, Sunnyhills	1	0
12 Gollan Rd, Mt Wellington	2	0
14 Gollan Rd, Mt Wellington	8	0
45 King George Ave, Epsom	1	0
74 King George Ave, Epsom	1	0
132 Manukau Rd, Epsom	1	0
106 Marua Rd, Ellerslie	1	0
Minerva Tce, Howick	2	0
145 Mountain Rd, Epsom	1	0
16 Poronui St, Mt Eden	6	0
60 Prince Regent Dr, Half Moon Bay	1	0
45 Ranfurly Rd, Epsom	1	0
29 St Andrews Rd, Epsom	1	0
7A Stokes Rd, Mt Eden	1	0
23 Windmill Rd, Mt Eden	1	0
Cambridge		
St Andrews Anglican Church	1	0
Town Hall	3	0
Tudor Garden Centre	9	0
Alpha St, verge	2	0
Clive		
18 Mill Rd	2	0
20 Mill Rd	2	0
32 Mill Rd	3	0
Dannevirke		
Dannevirke Park	8	0
Hamilton		
Ascot Lodge Motel	1	0
Church of Nazarath	4	0
Greens	22	0
Hamilton Gardens	82	0
Jesmond Park	6	0
Lake Rotoroa	29	0
Palmers, Lincoln St	16	0
Parana Park	22	0
50A Alison St	1	0
96 Ellicott Rd	1	0
114 Ellicott Rd	2	0
27 Grey St	1	0
41 Grey Lynne Ave	2	0

50 Grey Lynne Ave	1	0
42 Hibiscus Dr	2	0
246 Hukanui Rd	1	0
26 Kahikatea Dr	1	0
cnr Lincoln & Massey Sts	2	0
1 Newcastle Rd	1	0
8 Newcastle Rd	1	0
391 Peachgrove Rd	3	0
55 Tawa St	1	0
116 Tawa St	1	0
263 Te Rapa Rd	1	0
cnr Ulster & Mill Sts	1	0
Hastings		
Cornwall Park	36	0
Hastings Womens Country Club	1	0
300 King St	1	0
406 Tomoana Rd	1	0
800 Tomoana Rd	1	0
Hatepe		
Hatepe General Store	3	3
Havelock North		
Havelock North Garden Centre	1	0
Mitre 10	1	0
26 Duart Rd	1	0
Huntly		
railway tracks, SH1	2	0
Trinity Church	1	0
Kihikihi		
Frasers Plant Centre	12	0
2833 SH3	3	0
Manunui		
Caltex Service Station	1	0
Napier		
Botanic Gardens	9	0
Ngaruwahia		
4 Gallileo St	1	0
Ohakune		
Ohakune Country Motel	1	0
opp. Fire Station	1	0

Okato		
Gossling St Hall	1	0
beside Home Kills, SH45	3	0
Fire Station	6	4
2 Gossling St	1	0
Opunake		
New Life Garden Nurseries	5	0
Opunake Motel & Backpackers	6	1
Opunake Sports Ground	2	1
St Pauls Methodist Presbyterian	1	0
70 Ihaia Rd	1	0
78 Ihaia Rd	1	0
8 Ponderosa Pl	1	0
Otorohanga		
opp. sports ground	2	0
Otorohanga Greenworld	16	0
Police Station	1	0
Windsor Park	5	0
5 Sangro Cres	3	0
56 Turongo St	2	0
Paeroa		
park in Willoughby St	8	0
St Pauls Anglican Church	5	0
70 Arney Rd	4	0
Piopio		
The Village Green	1	0
60 SH3	1	0
21 Tui St	2	0
Piriaka		
house by store	2	0
Pukeiti		
Pukeiti Gardens	19	0
Pukekohe		
St Josephs School	5	0
29 Churchill St	1	0
42 Kitchener Rd	1	0
3 Wesley St	2	0
Pungarehu		
on left before petrol station	1	0

St Martins Catholic Church	1	0
Putaruru Arms		
Putaruru Arms	5	0
verge north of town	25	0
Raetihi		
Motorcamp, SH4	4	0
17 Pitt St	1	0
cnr Ward & Pitt Sts	3	0
Ward St	1	0
SH4 farm 553	1	3
Rahotu		
opp school	4	0
Rotorua		
District Court House	1	0
Neil Hunt Park	7	0
Princes Hotel	4	0
Rotorua Airport	2	0
Rotorua Gardens	90	0
524 Te Ngae Rd	2	0
Taihape		
Gumboot Manor Tearooms	1	0
St Davids Presbyterian Church	1	0
cnr Kokako & Huia Sts	1	0
11 Kokako St	2	0
19A Kokako St	1	1
Taumaranui		
Farmlands	5	0
Mansons Gardens	4	0
Mitre 10	3	0
Seventh Day Adventist Church	1	0
Womens Club	1	0
187 Hakiana St	2	0
Taupo Rd	1	0
Taupo		
Botanic Gardens	241	4
Cedar Lodge	1	0
Country Mews Hotel	1	1
Oasis Motel	1	0
Taupo Library and surrounds	13	0
8 Chesham Ave	8	5
Horomatangi St, opp Whitcoulls	2	0

74 Shepard Rd	2	0
Tauranga		
Elizabeth Heights	1	0
Holy Trinity Anglican Church	10	0
Otumoetai Primary School	2	0
148 Bellvue Rd	1	0
68 Kings Ave	3	0
14 Levers Rd	1	0
86 Levers Rd	1	0
218 Levers Rd	1	0
266 Ngati Rd	1	0
264 Otumoetai Rd	2	0
Smiths Rd, verge	6	0
350 Waihi Rd	1	0
7 Woods Ave	1	0
Te Awamutu		
Interlact Nutrition Ltd	1	0
Pioneer Walk	38	0
Sherwin Park	2	0
Te Awamutu Primary School	1	0
Te Awamutu Rugby Sports Club	2	0
cnr Mutu & Mahoe Sts	3	0
27 Princess St	1	0
96 Raikes Ave	1	0
204 Raikes Ave	1	0
382 Teasdale St	1	0
452 Teasdale St	1	0
Te Kuiti		
main st by railway tracks	2	0
Muffins Coffee Lounge	2	0
St Lukes Anglican Church	18	0
Te Kuiti Baptist Church	1	0
3 Gladstone Rd	2	0
Te Puke		
McLoughlin Park	10	0
400 SH2	1	0
Thames		
Aeroview Greenworld	14	0
Thames Emergancy Xray Unit	2	0
801 Rolleston St	1	0
Tokaroa		
between Warehouse & playground	1	0

Super Liquor	4	0
verge south of town, petrol station	6	0
verge south of town, CHH	6	0
Bonaly St, toilets	4	0
Turangi		
Turangi shopping centre	3	0
5 Hingaia St	1	0
20 Hingaia St	2	0
55 Hingaia St	1	0
48 Tautahanga St	1	0
50 Tautahanga St	1	0
54 Tautahanga St	1	0
Urenui		
cnr SH3 & Ngapapa Rd	1	0
Waihi		
on road verge, SH2	6	0
Waihi South School	1	0
Moresby St, opp. school	2	0
Tauranga Rd, telephone box	4	0
Waipawa		
clock tower, SH2	1	0
Waipukarau		
Plunket	1	0
St Marys Anglican Church	5	0
1 Kitchener St	1	0
12 Kitchener St	1	0
15 Kitchener St	1	0
Waitakaruru		
Plunket	2	0
Woodville		
Woodville School	4	0
3 SH3	1	1
19 SH3	1	0

1998 SOUTH ISLAND

Address	No. Plants (total)	No. with Diseased Flowers
Ahaura		
Napoleon St, next to church	1	0
Akaroa		
Chez la Mer	1	0
Memorial Ground	6	0
St Peters Anglican Church	3	0
Woodstock Garden Centre	10	0
11 Rue Balguerie	4	0
21 Rue Balguerie	1	0
38 Rue Balguerie	1	0
67 Rue Balguerie	1	0
70 Rue Jolie	4	0
117 Rue Jolie	1	0
3A Rue Lavaud	3	0
89 Rue Lavaud	13	0
opp. 1 Watson St	1	0
Amberley		
Amberley House Nurseries	43	0
Holy Innocents Anglican Church	1	0
Hurunui District Offices	8	0
9 Douglas Rd	2	0
Ashburton		
Ashburton Domain	33	0
Ashburton Hotel	2	0
Clocktower	32	0
Garden Place	148	0
Jordans	>1000	0
Millichamps Garden Centre	110	0
Trotts Gardens	127	0
along East St at shops	81	0
29A Grove Belt St	1	0
53 Grove Belt St	2	0
69 Grove Belt St	1	0
103 Grove Belt St	2	0
98 Park St	6	0
124 Park St	1	0
cnr West & Havelock Sts	2	0
7 West St	3	0
72 Wills St	3	0

Balclutha		
Balclutha Backpackers	1	0
Plunket	5	0
Shand House	4	0
7B Argyle St	1	0
13 Argyle St	1	0
214 Clyde St	1	0
Belfast		
EW Nichol Furniture Manufacturer	6	0
opp. New World, main rd	3	0
St Philips Presbyterian Church	2	0
Sheldon Park	1	0
737 Main North Rd	1	0
753 Main North Rd	1	0
820A Main North Rd	1	0
Blenheim		
Adams Garden Centre	31	0
Blenheim School	18	0
Grove Park Hotel	19	0
Maungatea Nursery	333	0
Pollard Park	53	14
Roselands Greenworld	24	0
Seymour Square	5	0
75A Alfred St	15	0
12 Andrew St	3	0
91 Battys Rd	84	46
33 Coleman Rd	14	0
10 Fyffe St	250	0
14 Lakings Rd	58	0
63 Leefield St	3	0
7 Martin Tce	62	2
69 McLauchlan St	1	0
85 McLauchlan St	3	0
26 Muller Rd	23	7
52 Percy St	11	0
57 Parker St	1	0
19 Perkis St	70	0
Bluff		
cnr Gore & Boyne Rds	1	0
17 Henderson St	1	0
Brightwater		
33 Lord Rutherford St	1	0
36 Lord Rutherford St	1	0

58 Lord Rutherford St	2	0
68 Lord Rutherford St	4	0
178 River Terrace Rd	2	0
48 Waimea West Rd	1	0

Christchurch

Athol McCulley, Wairakei Rd	161	1
Christchurch Botanic Gardens	74	0
Mona Vale Gardens	7	2
Smiths City car park, Riccarton	30	0
Sumner School	12	0
8 Allister Ave, Merivale	11	3
14 Allister Ave, Merivale	1	0
89A Aorangi Rd, Bryndwyr	18	16
91 Aorangi Rd, Bryndwyr	1	0
93A Aorangi Rd, Bryndwyr	1	0
122 Aorangi Rd, Bryndwyr	1	0
124 Aorangi Rd, Bryndwyr	3	0
308 Aorangi Rd, Bryndwyr	1	0
310 Aorangi Rd, Bryndwyr	1	0
21 Arnold St, Sumner	1	0
28 Arnold St, Sumner	2	0
4 Bristol Pl, St Albans	1	0
14 Bristol Pl, St Albans	1	0
58 Brookside Tce, Bryndwyr	1	0
70 Brookside Tce, Bryndwyr	2	0
6 Clark St, Sumner	3	0
7 Clark St, Sumner	3	0
2 Colwyn St, Bryndwyr	1	0
4 Colwyn St, Bryndwyr	1	1
7 Colwyn St, Bryndwyr	3	2
11 Colwyn St, Bryndwyr	3	0
8A Corfe St, Upper Riccarton	1	0
133 Fendalton Rd, Fendalton	1	0
36 Flavell St, Heathcote Valley	4	3
12 Glandovey Rd, Fendalton	1	0
23 Glandovey Rd, Fendalton	3	0
39 Gordon Ave, St Albans	1	0
17 Innes Rd, St Albans	1	0
74A Innes Rd, St Albans	2	0
80 Innes Rd, St Albans	1	1
151 Leinster Rd, Merivale	9	2
142 Main Rd, Redcliffs	1	0
127 Memorial Ave, Fendalton	1	0
207 Memorial Ave, Fendalton	1	0
6 Murdoch St, Bryndwyr	1	0
50 Nayland St, Sumner	5	3
116 Nayland St, Sumner	1	0

52 Onslow St, St Albans	7	1
42 Rugby St, Merivale	4	4
52 Rugby St, Merivale	2	1
90 Rugby St, Merivale	1	1
31 Sayers Cres, Avonhead	36	3
78 Straven Rd, Riccarton	1	0
6 Torquay Pl, Bryndwyr	5	0
218 Wairakei Rd, Bryndwyr	1	0
232 Wairakei Rd, Bryndwyr	2	0
239 Wairakei Rd, Bryndwyr	1	0
24 Wairarapa Tce, Fendalton	2	1
52 Whiteleigh Ave, Addington	1	0
88 Wiggins St, Sumner	1	0
132 Yaldhurst Rd, Riccarton	3	0
406 Yaldhurst Rd, Riccarton	1	1
Clinton		
4 SH1	1	0
Collingwood		
Haven Rd	1	0
Vicarage	2	0
Cromwell		
Mall, car park	1	0
Dunedin		
Dunedin Botanic Gardens	162	0
Fairfield Tavern	8	0
Fairfield Tavern Reserve	9	0
Jac's Mitre 10	16	0
Matheson & Roberts	62	0
St Mathews Anglican Church	3	0
42 Argyle St	2	0
27 Baldwin St	1	0
39 Baldwin St	1	0
30 Belgrave Cres	2	0
88 Cavell St	1	0
34 Fairfield Rd	2	0
40 Hawthorne Ave	1	0
162 Kenmure Rd	2	0
8 Lindie St	1	0
72 Middleton Rd	112	0
11 Nile St	28	0
10B Queens Dr	18	0
1 West Ave	1	0
2 Whitby St	1	0
23 Whitby St	1	0

28 Whitby St	1	0
East Takaka		
B Manson	16	1
Gore		
Deer Park	10	0
Gore A&P Association	4	0
St Andrews Presbyterian Church	1	0
Salesyards	8	0
41 Ardwick St	1	0
44 Broughton St	2	0
57 Broughton St	1	0
12 Crombie St	2	0
Govenors Bay		
Queen Charlotte Dr	1	0
Greymouth		
Albert Mall	4	0
Dixon Park	10	0
Garden Place	90	0
Greymouth Court House	4	0
Holy Trinity Anglican Church	3	0
Mitre 10	4	0
War Memorial Baths	1	0
185 High St	1	0
11 Tainui St	1	0
12 Turumaha St	1	0
67 Turumaha St	1	0
The Grove		
Queen Charlotte Dr, TR Buckland	1	0
Halswell		
2 Sabys Rd	1	0
70 Sabys Rd	6	0
74 Westlake Dr	16	0
Hanmer Springs		
Cheltenham House	1	0
Glenalvon Lodge	4	0
St Roch's Catholic Church	4	0
Hari Hari		
bridge, north end of town	2	0

Havelock		
Havelock Garden Centre	24	0
Pelorus Motel	1	0
Police Station	1	0
War Memorial, main rd	1	0
48 SH6	12	0
72 SH6	2	0
Hokitika		
Hokitika Hall	1	0
St Marys Catholic Church	1	0
Westland District Council	1	0
113 Hampden St	1	0
127 Hampden St	1	0
173 Hampden St	1	0
121 Julie St	2	0
154 Stafford St	1	0
159 Stafford St	3	0
Hope		
532 SH6	2	0
549 SH6	5	0
SH6	12	0
Hornby		
Gardenways	8	0
Mitre 10	26	0
Warehouse	18	0
271A Main South Rd	1	0
Ikamatua		
Ikamatua Hotel	1	0
north of Big Grey River bridge	1	0
172 SH7	2	0
Inangahua		
orange house, visual only	1	0
Invercargill		
Mitre 10	7	0
Queens Park	127	0
Nichols Greenworld, North Rd	112	0
Nichols Greenworld, Tay St	54	0
Wachner Pl	5	0
Waihopai School	1	0
Waikiwi Garden Centre	44	0
Warehouse	28	0
70 Carlyle St	1	0

225 Gala St	2	0
231 Gala St	1	0
85 Herbert St	7	0
152 Herbert St	2	0
173 Inglewood Rd	1	0
74 Mary St	3	0
105 Mary St	1	0
145 Mary St	2	0
9 Morton Rd	1	0
66 Morton Rd	1	0
67 Queens Dr	1	0
193 Queens Dr	1	0
537 Tay St	1	0
614 Tay St	1	0
30 Thomson St	3	0
512 Tweed St	1	0
Kaiapoi		
Kaiapoi shopping carpark	3	0
St Bartholomews Anglican Church	2	1
51 Cass St	2	0
97 Cass St	3	0
161 Ohoka Rd	1	0
173 Ohoka Rd	1	0
44 Sewell Rd	2	0
256 Williams St	1	0
312 Williams St	1	0
Kaikoura		
Mitre 10, West End	3	0
Mountain View Garden Centre	28	0
20 Churchill St	2	0
67 Torquay St	2	0
Lincoln		
Lincoln University	181	0
36 Edward St	3	0
Little River		
Little River Hotel	2	0
River Gardens	>400	0
Lower Moutere		
47 Moutere Highway	4	0
51 Moutere Highway	2	0
69 Moutere Highway	1	0

Lumsden		
54 Diana St	1	0
Lyttelton and Bays		
Ortan Bradley Park, Charteris Bay	27	0
Simeon Quay, Lyttelton	1	0
Taunton Gardens	35	0
126 Diamond Harbour Rd	2	0
422 Diamond Harbour Rd	1	0
Mapua		
111 Higgs Rd	1	0
Iwa St	2	0
27 Iwa St	4	0
Toru St	5	0
Mataura		
at bridge south end of town	8	0
22 SH1	2	0
Milton		
Tokomairiro High School	4	0
65 Johnson St	1	0
68 Cowper St	2	0
Momorangi Bay		
Queen Charlotte Dr	1	0
Mosgiel		
Hotel Motel Taieri	1	0
Mosgiel Garden Place	51	0
1 Inglis St	3	0
6 Inglis St	1	0
15 Inglis St	1	0
15 Irvine St	3	0
15 Tay St	1	0
24 Tay St	3	0
29 Tyne St	1	0
Motueka		
Grain & Grape, car park	1	0
Gothic Gourmet	1	0
Jacaranda Park	27	0
Woodlands Nursery	44	1
Greenlane	20	1
343 High St	1	0
361 High St	1	0
425 High St	1	0

433 High St	1	0
435 High St	1	0
472A High St	4	0
585 High St	1	0
588 High St	2	0
Queen Victoria St	2	0
28 Whakarewa St	2	0
32 Whakarewa St	2	0
45 Whakarewa St	3	0
132 Whakarewa St	1	0
2 Woodlands Ave	3	0
24 Woodlands Ave	1	0
30 Woodlands Ave	10	0
41 Woodlands Ave	2	0
Motupipi		
447 main rd	4	0
Murchison		
Murchison Area School	2	0
Brunner St	11	0
20 Hampden St	1	0
50 SH6	2	0
52 SH6	1	0
Nelson		
Anzac Park	23	16
Fairfield Park	9	4
Nelson Cathedral	35	27
Ocean Lodge Motel	3	1
Queens Gardens	27	21
Baigent Rd	4	0
56 Collingwood St	1	1
Tory St	1	1
Oamaru		
Breens Nursery	12	0
Oamaru Public Gardens	54	0
5 Caledonian St	1	0
20 Caledonian St	1	0
23 College St	7	0
29 Tamar St	1	0
Teschmakers Rd	12	0
324 Thames Highway	1	0
326 Thames Highway	2	0
392 Thames Highway	1	0
434 Thames Highway	1	0
436 Thames Highway	1	0

6 Witham St	1	0
20 Witham St	1	0
Okaramio		
SH6	1	0
Patons Rock		
Holmwood	198	0
Picton		
Beachcomber Inn, Waikawa Rd	1	0
Blue Anchor Holiday Park	2	0
Ferry Terminal	1	0
Mariners Mall car park	1	0
Picton Foreshore Reserve	9	0
Shelly Park Marina	1	0
7 Canterbury St	1	0
12 Canterbury St	1	0
17 Canterbury St	1	0
44 Devon St	1	1
48 Devon St	1	0
70 Devon St	2	0
52 Hampden St	1	0
60 Kent St	2	0
25 Milton Tce	1	0
25A Milton Tce	2	0
53 Milton Tce	1	0
61 Milton Tce	1	0
cnr Newgate & Waikawa	5	1
10 Newgate St	6	0
23 Oxford St	2	0
25 Oxford St	1	0
2 Seaview Cres	2	0
5 Sussex St	7	1
90 Waikawa Rd	1	1
92 Waikawa Rd	1	0
128 Waikawa Rd	1	0
53 York St	1	0
Pohara		
main rd	3	0
Queenstown		
Copthorne Resort	12	0
Garden Court Motel	1	0
Gardens Park Royal	2	0
Millenium	8	0
Queenstown Gardens	7	0

St Peters Anglican Church	3	0
Shotover Garden Centre	76	0
cnr Earl & Camps Sts	2	0
cnr Earl & Marine Pde, car park	2	0
cnr Earl & Marine Pde, toilets	2	0
cnr Frankton & Brisbane St	2	0
10-1 Frankton Rd	1	0
10-2 Frankton Rd	2	0
6 Hallenstein St	1	0
27 Melbourne St	1	0
21 Sydney St	1	0
Rai Valley		
school	2	0
Rangiora		
Hide's House & Garden	18	0
John Knox Presbyterian Church	9	0
McAlpines Mitre 10	46	0
Seventh Day Adventist Church	1	0
112 Blackett St	6	0
52 Percival St	1	0
Reefton		
cnr Bridge & Church Sts	2	0
60 Bridge St	1	0
6 Broadway	1	0
20 Broadway	1	0
104 Broadway	1	0
6 Mace St	2	0
Smith St, opp. rotunda	3	0
Renwick		
36 Brydon St	1	0
8 High St	1	0
25 Rouse Hill St	4	0
15 Uxbridge St	6	0
Richmond		
Henley School	3	3
Catholic Church, William St	13	4
Washbourn Gardens	33	23
2 Aston Pl	1	0
12 Aston Pl	1	0
3 Edward St	1	0
5 Edward St	2	1
7 Edward St	2	0
11 Edward St	1	1

13 Edward St	1	0
19 Edward St	3	0
28 Edward St	2	0
6 Farnham Dr	1	0
18 Farnham Dr	2	0
21 Farnham Dr	1	0
30 Farnham Dr	3	0
31 Farnham Dr	1	0
35 Farnham Dr	1	0
43 Farnham Dr	3	0
cnr George & Queen Sts	2	0
2 George St	4	0
20 George St	1	0
24A George St	1	0
26A George St	1	0
32 George St	1	0
123 Queen St	1	0
129 Queen St	1	0
162 Queen St	1	1
166 Queen St	1	1
25 Surrey Rd	3	3
10 Washbourn Dr	1	0
33 William St	3	3
39 William St	4	2
Battesby Orchard, Ranzau	137	7
Bay Nurseries	124	1
Gardens of the World	16	1
Thirkettles Nursery	54	8
Riwaka		
D. Arundel	1	0
Dehra Doon Rd	4	0
cnr SH6 & Dehra Doon Rd	1	0
Rockville		
D. Grant	9	4
Ruby		
1 Tait St	3	0
Sedden		
Barewood Rd	18	0
Springs Creek		
St Lukes Anglican Church	2	0
46 Ferry Rd	1	0

Stoke		
Broadgreen	93	10
Isle Park	54	28
Marsden Valley Cemetary	58	52
Marsden Valley Substation	2	2
The Ridgeway	2	0
Taitapu		
Hawthorne Cottage	18	0
Templeton		
Bell Property, Barters Rd	243	4
Temuka		
264 King St	5	0
Timaru		
Green Leaf Plant Centre	1	0
Timaru Gardens	133	0
Timaru Library	6	0
18 Butler St	2	0
76 Otipua Rd	2	0
201 Otipua Rd	1	0
257 Otipua Rd	1	0
262 Otipua Rd	1	0
cnr Perth & Arthur Sts	9	0
38 Wai-iti Rd	5	0
66 Wai-iti Rd	1	0
135 Wai-iti Rd	2	0
168 Wai-iti Rd	1	0
200 Wai-iti Rd	2	0
Totara Flat		
Totara Flat School	1	0
Upper & Lower Takaka		
Golden Bay High School	19	17
Hamama	94	5
Jade Cottage	1	0
opp. school corner	0	2
St Andrews Presbyterian Church	0	2
Upper Takaka	160	0
War Memorial	4	0
Commercial St	2	0
47 Commercial St	1	0
95 Commercial St	3	1
Reilly St	1	0

Upper Moutere		
1362 milk no. highway	2	0
Wainui Inlet		
J & M Robinson	18	18
Wakefield		
Wakefield School	2	0
56 Arrow St	1	0
69 Pitfure Rd	3	0
83 Whitby Rd	2	0
111 Whitby Rd	1	0
113 Whitby Rd	2	0
199 SH6	1	0
204 SH6	2	0
Wanaka		
71 Upton St	1	0
50 Youghal St	5	0
Westport		
Buller River bridge, north end	4	0
Municipal Chambers	2	0
Victoria Park	5	0
Westport Motor Hotel	3	0
26 Bright St	1	0
43 Bright St	1	0
cnr Brougham & Peel Sts	2	0
69 Brougham St	4	0
55 Dommett St	1	0
32 Henley St	1	0
north end Lyndhurst St	2	0
13A Lyndhurst St	1	0
54 Peel St	1	0
58 Peel St	1	0
64 Peel St	1	0
76 Queen St	1	0
90 Queen St	1	0
Whataroa		
Whataroa Court House	2	0
Winton		
Holy Trinity Anglican Church	1	0
park opp. BNZ	3	0
Westpac	3	0
17 Albert St	1	0
20 Albert St	4	0

42 Park St	3	0
Woodend		
Woodend Nurseries	57	0
Woodend School	1	0
2 Main North Rd	2	0
9 Woodend Rd	1	0
4 Woodend Rd	1	0

APPENDIX IV

SURVEY SUMMARIES 1997 AND 1998

Summaries are listed by year of survey and as North or South Island. Summaries are grouped into small blocks for quick reference. Where there are several main highways in a region I have listed these first, but where regions can be considered units, e.g. Buller and Westland, I have not included State Highway numbers.

1997 NORTH ISLAND

Town	No. Sites	No. Plants (total)	No. with Diseased Flowers
SH1 + 3 Wellington-Wanganui			
Raumati South	3	8	6
Paraparaumu	2	16	12
Otaki			
Levin	1	9	4
Foxton	3	4	2
Opiki	1	15	0
Sanson	2	2	0
Bulls	3	5	0
Martin	3	16	2
Wanganui	1	12	7
SH 3 + 45Wanganui-New Plymouth			
Waverley	2	19	11
Patea	3	7	3
Hawera	4	8	7
Manaia	2	11	8
Opunake	5	22	2
Rahotu	1	2	0
Pungarehu	1	2	0
Okato	4	21	0
Pukeiti Gardens	1	25	0
Normanby	1	2	0
Eltham	1	4	2
Stratford	2	9	5
Midhurst	1	1	1
Inglewood	3	8	8

Waitara	5	66	1
New Plymouth	5	31	16
SH3 New Plymouth-Hamilton			
Bell Block	3	10	0
Urenui	4	14	1
Wai-iti	1	8	0
Uruti	1	14	0
Awakino	1	3	0
Heo Reo Farm	1	2	0
Herangi Station	1	1	0
Piopio	2	4	0
Te Kuiti	3	7	0
Otorohanga	3	11	0
Te Awamutu	3	22	0
Ohaupo	1	8	0
Hamilton	2	51	0
SH1 Palmerston North-Taihape			
Palmerston North	5	55	11
Rata	1	1	0
Hunterville	5	26	1
Mangaweka	3	10	1
Utiku	1	2	0
Winiata	2	9	1
Taihape	4	7	0
Kimbolton	5	105	1
Cheltenham	2	7	2
Feilding	6	92	51
Bunnythorpe	1	2	0
Ashurst	7	21	1
Aokautere	2	88	6
SH4 Wanganui-Taumaranui			
Upokongaro	1	4	3
Kakatahi	1	4	2
Raetihi	1	10	0
Ohakune	2	9	0
Owhango	1	7	0
Piriaka	1	3	0
Taumaranui	1	8	0
SH1 + 5 Turangi-Rotorua-Hamilton			
Turangi	3	13	1
Te Rangi-ita	1	2	0
Hatepe	1	5	3
Waitehanui	1	9	0
Taupo	2	10	0

Rotorua	2	53	0
Tokoroa	2	9	0
Putaruru	4	14	0
Cambridge	4	9	0
Wairarapa			
Pahiatua	2	14	1
Ekatahuna	2	21	0
Ngatui	1	20	0
Carterton	9	158	7
Martinborough	3	11	1
Featherston	3	52	4
Kaitoke	1	23	20
Upper Hutt	4	56	40
Hawkes Bay			
Woodville	1	3	0
Dannevirke	1	6	0
Norsewood	1	3	0
Waipukurau			
Waipawa	1	2	0
Havelock North	1	2	0
Hastings	1	10	0

1998 NORTH ISLAND

Town	No. Sites	No. Plants (total)	No. Diseased
Greater Auckland	40	672	13
Pukekohe	4	9	0
Huntly	2	3	0
Ngaruwahia	1	1	0
Hamilton	26	216	0
Cambridge	4	15	0
Putaruru	2	30	0
Tokaroa	5	21	0
Acacia Bay	1	2	0
Taupo	8	270	10
Turangi	7	10	0
Taihape	5	6	1
SH3 + 45 Te Awamutu-Opunake			
Te Awamutu	11	52	0
Kihikihi	2	15	0
Otorohanga	7	29	0

Te Kuiti	5	25	0
Piopio	3	4	0
Urenui	1	1	0
Pukeiti	1	19	0
Okato	4	7	4
Pungerehu	2	2	0
Rahotu	1	4	0
Opunake	7	17	2
SH4 Taumaranui-Ohakune-Raetihi			
Taumaranui	7	17	0
Manunui	1	1	0
Piriaka	1	2	0
Ohakune	2	2	0
Raetihi	4	9	0
SH2 Thames-Tauranga-Rotorua			
Waitakaruru	1	2	0
Thames	3	17	0
Paeroa	3	17	0
Waihi	4	13	0
Tauranga	13	31	0
Te Puke	2	11	0
Rotorua	6	106	0
SH2 Napier-Woodville			
Napier	1	9	0
Clive	3	7	0
Hastings	5	40	0
Havelock North	3	3	0
Waipawa	1	1	0
Waipukarau	5	9	0
Dannevirke	1	8	0
Woodville	3	6	1

1998 SOUTH ISLAND

Town	No. Sites	No. Plants (total)	No. Diseased
SH6 + 60 Picton-Nelson-Waimea			
Picton	28	59	4
Governors Bay	1	1	0
Momorangi Bay	1	1	0
The Grove	1	1	0
Havelock	6	41	0

Nelson	8	103	71
Stoke	5	209	92
Richmond	33	100	42
Waimea	4	331	17
Mapua	4	12	0
Ruby Bay	1	3	0
Motueka	23	131	2
Riwaka	3	6	0
Lower Moutere	3	7	0
Upper Moutere	1	2	0
Brightwater	6	11	0
Wakefield	8	14	0
Hope	3	19	0
Golden Bay			
Upper & Lower Takaka	10	294	27
Patons Rock	1	198	0
Collingwood	2	3	0
Rockville	1	13	4
Wainui Inlet	1	18	18
Pohara	1	3	0
Motupipi	1	4	0
East Takaka	1	17	1
Malborough			
Rai Valley	1	2	0
Okaramio	1	1	0
Renwick	4	12	0
Blenheim	22	1155	107
Springs Creek	2	3	0
Seddon	1	18	0
SH1 + 75 Kaikoura-Christchurch-Akaroa			
Kaikoura	4	35	0
Hanmer Springs	3	9	0
Amberley	4	54	0
Woodend	5	62	0
Belfast	7	15	0
Kaiapoi	9	16	0
Rangiora	7	81	0
Templeton	1	247	4
Halswell	3	23	0
Hornby	3	53	0
Christchurch	58	452	46
Lincoln	2	184	0
Taitapu	1	18	0
Lyttelton & Bays	5	66	0
Little River	2	>402	0

Akaroa	13	49	0
SH1 Ashburton-Dunedin-Bluff			
Ashburton	17	>1554	0
Temuka	1	5	0
Timaru	14	167	0
Oamaru	14	96	0
Dunedin	22	435	0
Mosgiel	9	63	0
Milton	3	7	0
Balclutha	6	13	0
Clinton	1	1	0
Gore	7	29	0
Mataura	2	10	0
Invercargill	25	408	0
Bluff	2	2	0
SH6 Winton-Queenstown-Wanaka			
Winton	6	15	0
Lumsden	1	1	0
Queenstown	16	121	0
Cromwell	1	1	0
Wanaka	2	6	0
Westland-Buller			
Whataroa	1	2	0
Hari Hari	1	2	0
Hokatika	9	12	0
Greymouth	13	122	0
Ahaura	1	1	0
Totara Flat	1	1	0
Ikamatua	3	4	0
Reefton	7	12	0
Inangahua	1	1	0
Murchison	5	17	0
Westport	17	30	0

APPENDIX V

UNKNOWN CAMELLIA VARIETIES

Camellia are listed by letter, with a brief description of their colour and shape or variety with which they look most similar. Missing letters indicate the flower was identified.

B	light pink, formal double, looks like 'E. G. Waterhouse'
C	white, minature semi-double, looks like 'Cinnamon Cindy'
D	white, semi-double, trumpet-shaped
E	pink with white fimbrulated edges, looks like 'Elegans Splendar'
F	rose-pink, looks like *C. grandthamiana*, furry buds, unusual foliage
H	deep red, black veins, semi-double, stamens pink and two petaloids
I	pink, large semi-double, bright stamens
J	light pink, large semi-double, bright stamens, looks like 'Donation'
K	white, apricot sport, many petaloids
L	red with white virus marbling
M	red, tiered pointed petals
N	red, looks like 'William Hertrich'
O	pink, ruffles, no stamens
P	red, single, bell-shaped flowers
Q	pink, curly petals, no stamens
R	white, large semi-double, stamens
Z	white, crinkly petals, yellow stamens

APPENDIX VI

INFECTION STUDY DATA

Experiment 7

Number of Petals Infected by *C. camelliae* Ascospores in Each Stage of Bud Burst

Camellia	Stage of Bud Burst											
	Nonwounded/agar + Cc3 (/5)						Wounded/agar + Cc3 (/5)					
	1	2	3	4	5	6	1	2	3	4	5	6
Tree 3	0	0	0	0	0	0	0	0	0	1	3	3
Tree 10	0	0	0	0	1	0	1	0	0	2	2	5
Tree 15	0	0	0	1	2	0	0	1	0	3	4	3
'Kanjiro'	0	0	0	0	0	3	0	0	2	0	5	4
'Kanjiro'	0	0	0	0	0	0	5	1	5	2	5	5
'Moonlight'	0	0	0	0	0	0	1	0	2	3	2	4
'Yuletide'	0	0	0	0	0	0	1	1	2	2	2	5
'Yuletide'	0	0	0	0	0	0	0	0	1	2	1	4
'Buddha'	0	0	0	0	0	3	4	5	4	5	5	5
'Cinnamon Cindy'	1	2	0	0	0	0	1	2	1	4	3	5
'Debutante'	0	0	0	0	0	0	4	4	5	5	5	5
'Debutante'	1	0	0	0	0	0	5	5	5	5	5	5
'Dolly Dyer'	1	0	0	0	1	1	3	5	5	5	5	5
'Jubilation'	0	0	2	0	1	1	4	5	3	1	4	5
'Lady Clare'	0	0	0	0	0	4	4	2	3	4	5	5
'Lady Loch'	0	0	0	0	0	0	3	5	5	5	5	5
'Lady Loch'	0	0	0	0	0	0	5	5	5	5	5	5
'Lady Loch'	0	0	1	0	0	3	5	5	5	5	5	5

Experiment 7 (cont.)

Number of Petals Infected by *C. camelliae* Ascospores in Each Stage of Bud Burst

'Madame Hahn'	0	0	0	0	0	0	3	4	5	5	5	5
'Sunnybank'	0	0	0	0	0	0	3	5	5	5	5	5
Tree B	0	0	0	0	0	2	3	5	4	5	5	5
Tree C	0	0	0	0	0	0	4	2	2	4	3	5
Tree D	0	0	0	0	0	0	1	1	3	2	3	5
Tree E	0	0	0	0	0	0	4	5	5	5	5	5
Tree F	0	0	0	0	0	0	4	5	4	4	5	5
Tree G	0	0	0	0	0	0	3	5	5	5	5	5
Tree H	0	0	0	0	3	2	2	2	5	5	5	5
Tree H	0	0	1	1	0	3	0	1	5	5	5	5
Tree H	0	0	0	1	0	0	0	0	0	2	0	3
Tree I	0	1	0	0	1	3	0	4	5	4	4	5
Tree J	0	0	0	0	0	1	4	4	5	5	5	5
Tree K	0	0	0	1	0	0	3	5	5	0	0	0
Tree K	0	0	0	0	0	0	0	1	0	0	0	5
Tree L	0	0	1	0	0	0	4	5	5	5	5	5
Tree L	0	0	0	0	0	1	3	5	5	5	5	5
Tree M	0	1	0	0	0	0	5	3	5	5	5	4
Tree N	0	0	0	0	0	0	0	0	1	0	5	5
Tree N	0	0	0	0	1	1	2	5	5	5	5	4
Tree O	0	0	0	0	0	1	5	5	5	5	5	5
Tree P	1	0	0	1	0	1	2	5	5	5	5	5
Tree Q	0	0	0	0	0	0	4	5	5	5	5	5
Tree R	0	0	0	1	1	3	3	5	5	5	5	5
Tree S	0	0	0	0	0	0	2	3	4	5	5	5
C. chekioangolosa	0	0	0	0	0	1	4	4	5	5	5	5
C. yunnanensis	0	0	0	0	0	3	1	4	5	4	5	5

Experiment 8

Infection of Spring-Flowering Camellia Bud Burst Stages by Ascospores.

Camellia	Stage of Bud Burst											
	Uninoculated Control (/5)						Ascospore Inoculation (/5)					
	1	2	3	4	5	6	1	2	3	4	5	6
'Debutante'	0	0	0	0	0	0	0	0	0	3	0	5
'Desire'	0	0	0	0	1	5	0	0	0	0	0	3
'Easter Morn'	0	0	0	0	0	0	0	0	0	0	3	2
'Hulyn Smith'	0	0	0	0	0	0	0	3	5	5	5	5
'Lady Loch'	0	0	0	0	2	0	0	0	3	2	5	5
'Mark Alan'	0	0	0	0	0	1	1	2	4	5	5	5
'Midnight'	0	0	0	0	0	0	0	0	0	0	0	5
'Pilida'	0	0	0	0	0	0	0	2	4	5	5	5
'Roger Hall'	0	0	0	0	0	0	0	0	0	0	0	5
'Rose Bouquet'	0	0	0	0	0	0	0	0	0	5	4	5
'Sprite'	0	0	0	0	0	0	0	0	0	2	1	4
'E. G. Waterhouse'	0	0	0	0	0	0	0	0	0	0	1	2
Tree M	0	0	0	0	0	0	0	2	0	5	5	4
Tree N	0	0	0	0	0	1	0	0	3	4	5	5
Tree Q	0	0	0	0	0	0	2	0	3	5	5	5

Tests repeated with similar results are listed separately, but where the first test resulted in no infections and the second test did, only the second test is cited as this indicated that infection by ascospores did not take place during the first inoculation.

Experiment 10

Infection of Camellia Species and Varieties by Ascospore Inoculation Chamber.

Camellia	Petal Treatment	
	Uninoculated Control (/10)†	Ascospore Inoculated (/20)†
C. japonica		
'Aquarius'	0	20
'Ballerina'	9	20
'Desire'	9	19
'Dixie Knight'	0	10
'Dona Herzilla de Freitas Magalhaes'	2	15
'Easter Morn'	0	14
'Elegans Champayne'	1	20
'Grand Prix'	4	3
'Hagoromo'	0	20
'Kramer's Supreme'	0	20
'Lady Loch'	8	8
'Lady Loch'	0	20
'Laurie Bray'	3	20
'Leonora Novick'	4	20
'Madam Hahn'	9	11
'Margaret Davis'	0	20
'Mark Alan'	0	20
'Maroon & Gold'	0	20
'Mary Agnes Patin'	0	20
'Midnight'	1	20
'Nuccio's Gem'	4	18
'Preston Rose'	0	20
'Roger Hall'	0	14
'Roger Hall'	0	20
'San Dimas'	0	20
'Songbird'	0	15
'Tiffany'	2	17
'Wilamina'	0	20
'Zambo'	6	17

Experiment 10 (cont.)
Infection of Camellia Species and Varieties by Ascospore Inoculation Chamber.

C. reticulata		
'Brian'	0	20
'Hulyn Smith'	2	20
'Lovely Lady'	0	10/10
'Miss Rebecca'	0	20
'Simpatica'	10	17
'Terrell Weaver'	3	20
'Tom Durrant'	0	20
'Warwick Berg'	0	20
'Zhangjia Cha'	0	20
C. saluenensis		
'Sunnybank'	1	15/15
Hybrids		
'Adorable'	1	20
'Annette Carol'	10	20
'Dreamboat'	0	19
'Dresden China'	0	20
'Edith Mazzei'	1	20
'E. G. Waterhouse'	0	20
'E. G. Waterhouse'	2	20
'Fairy Wand'	0	20
'Mirage'	0	19
'Mona Jury'	0	18
'Pilida'	0	20
'Nicky Crisp'	0	20
'Night Rider'	0	20
'Night Rider'	0	20
'Nonie Haydon'	0	20
'Pink Dahlia'	0	20
'Rendezvous'	0	20
'Snippet'	0	20
'Spring Festival'	3	17
'Spring Mist'	0	0

Experiment 10 (cont.)
Infection of Camellia Species and Varieties by Ascospore Inoculation Chamber.

'Spring Mist'	0	0
'Sprite'	0	20
Unknown		
Tree C	0	20
Tree N	0	20
Tree O	2	20
Tree Z	0	20
Species		
C. chekiangoleosa	0	12
C. cuspidata	0/11	0/11
C. euphlebia	0	20
C. forrestii	0	3
C. forrestii	0	9
C. fraterna	1	4
C. gigantocarpa	6/8	13/13
C. grijsii	0	0
C. grijsii	0	2
C. longicarpa	0	0
C. longicarpa	0	0
C. lutchuensis	0	0
C. lutchuensis	0	0
C. lutchuensis	0	1
C. pitardii var. *pitardii*	2	19
C. polydonta	0	20
C. rosabelle	0	19
C. rosiflora	0	8
C. transarisanensis	0	1
C. transnokoensis	1	0
C. wabasuki	0	20
C. yuhsienensis	0/4	0/10
C. yunnanensis	0	1
C. yunnanensis	0	2
C. yunnanensis	0	4

† numbers cited out of 10 or 20 petals unless otherwise indicated