METHODS OF INOCULATING CYPRESS WITH *SEIRIDIUM* SPECIES TO SCREEN FOR RESISTANCE AND PATHOGEN VARIABILITY

A thesis presented in partial fulfilment of the requirements for the degree of
Master of Applied Science in Plant health at Massey University
Palmerston North, New Zealand.

Massey University

HELEN TEKULA TSATSIA

2003

ACKNOWLEDGEMENTS

I would like to express my heart-felt gratitude to various organisations and individuals who have enabled me to produce this thesis.

Massey University: I thanked my chief supervisor, Dr Peter Long for his tremendous support and guidance given throughout the duration of this study. I am very thankful for the assistance given by Associate Professor Hossein Behboudian in terms of finding the appropriate supervisor when I first arrived at Massey University. Thanks to Mr Hugh Neilson, Lorraine Davis and Chris Rawlingson for making sure supplies essential for work were within reach. Sincere appreciation is also extended to the Seed Technology Department for allowing me to use some space under the shade cloth for the cypress work. I would also like to thank the IT staff, especially Ai lih Tan and Adam Mackres for their assistance with the use of computers. I thanked the department secretary, Pamela Howell for her assistance.

Forest Research (Rotorua): Thanks to Forest Research for providing the funds for the project. I extended my sincere thanks to staff members who were involved in the cypress work, Dr. Luigi Gea, Dr. Ian Hood (Co-supervisor and collaborator), Dr. Kathy Horgan, Judith Gardener and Trevor Faulds for their advice and assistance with plant and fungal material for the research project.

Ministry of Trade and Foreign affairs NZ Government: A word of thanks to NZ government for awarding me the NZODA scholarship. Thanks to all the Massey University staff members from the International Student's Office who were directly involved with the scholarship, Sylvia Hooker, Dianne Reilly, Charles Chua (former NZODA scholarship officer), Sue Flynn (current NZODA scholarship officer) and Jo Lee.

Public Service Division –SI Government: I would like to thank Ms Caroline Taisau for assisting me with study leave application and other procedures required by the Public Service Commission.

Friends and Relatives: My humble thanks to student colleagues: Pyone Pyone and Duangrat Thongphak for their kindness and sense of humour. I thanked Lusina and Ian Lata for their support and encouragement. I would also like to thank my relatives for their understanding and moral support (My mother. Doreen Teatu. my brothers J. Teilo. J. Levela (Jr). P. Vaia and my sister, Mary Ponave.

And lastly but not the least, I would like to thank my dear ones. Francis. Cathy and Fiona for their prayers. understanding. and patience.

I thanked God for All His Blessings and May His Holy Name Be Gloried.

To my father, the late Johnson. K. Levela who was called to rest on 25th August 2001.

ABSTRACT

The cypress species are grown for their timber value, ornamental beauty and shelter. Their existence is threatened by the presence of cypress canker disease caused by fungal pathogens of the genus *Seiridium*.

The long term solution for controlling this disease is to breed for cypress clones that are resistant to cypress canker. Screening for resistance is conducted by artificially inoculating cypress plants with the pathogen's inoculum.

This study aimed at developing reliable methods of artificial inoculation that are suitable for New Zealand's climatic conditions.

Infection of cypress plants in nature is caused by conidia but mycelial inocula are more commonly used in artificial inoculation. Several methods of inducing sporulation of *Seiridium* species were investigated. Addition of plant substrates was shown to increase sporulation of cultures of *Seiridium* isolates. Studies comparing the two types of inocula (mycelial plugs and conidial suspensions) showed that mycelium inocula caused a higher percentage of canker lesions than spore inocula. Conidial inocula offer a more consistent pathogenicity. Experiments to determine the effective spore load revealed that the percentage of canker increased with the increase of inoculum load. Pathogenicity varied between species and individual colonies of *Seiridium* isolates.

Infection of cypress in nature is thought to occur through wounds and in this work, wounding was required for infection under both glasshouse and outdoor conditions. Inoculation of the main stem and side branches showed disease symptoms develop more rapidly on side branches than on the main stem. Investigations on in vitro inoculation of tissue cultured plants and excised side shoots showed the possibility of screening cypress ramets under different environmental conditions. Temperature and percentage relative humidity were found to influence the percentage of successful inoculations on cypress plants.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF PLATES

LIST OF TABLES

LIST OF APPENDICES

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Cypress canker is a serious fungal disease of trees belonging to the *Cupressus* family. The disease causes lesions on the bark and cankers on stems and branches. Infection of the branches and crowns of highly susceptible plants can eventually lead to the death of the whole plant. Cypress canker has caused the death of cypress trees in some parts of the Northern Hemisphere during the past years. There is taxonomic evidence that two of the three *Seiridium* species causing cypress canker are present in New Zealand. The disease was first recorded in this country by Birch in 1933. He identified *Seiridium cardinale* (Wagener) Sutton & Gibson= *Coryneum cardinale* Wagener.), as the pathogen responsible for the damages. Beresford & Mulholland (1982) found *S. cardinale* to be predominant in trial plantations. In the later years, it was found that most of the damage was in fact caused by *Seiridium unicorne* (Cooke & Ellis) Sutton= *Monochaetia unicornis* (Cooke& Ellis) Saccardo (Van der Werff 1988; Self 1994).

New Zealand forestry industry supplies 1.1% of the world's total product (Anonymous 2001). At present 95 % of the soft wood timber production is from *Pinus radiata* (Aimers-Halliday et al. 1994). There is an encouraging prospect to develop cypresses as a substitute for the western red cedar and red wood currently being imported for weatherboards and exterior joinery (Miller & Knowles 1996). The *Cupressus* species have advantages over other soft wood tree species such as fast growth, wind resistance, durable heartwood, good machining properties and high quality timber (Franklin 1994).

The cypresses were introduced in to New Zealand in the 1860s from California, Kenya and Guatemala (Miller & Knowles 1996) and were grown mainly as shelterbelts. The four most important cypress species grown in New Zealand include *Cupressus macrocarpa* Hartweg, *Cupressus lusitanica* Miller, *Chamaecyparis lawsoniana* (A. Murray) and the hybrid *Cupressocyparis leylandii* (Jackson and Dallimore). Growers prefer C. *macrocarpa* because of its high quality timber and availability of market. The area planted with the important cypress species as at 1986 is listed in Table1.1. A recent

survey carried out reported an increase in the planting of *C. macrocarpa* and C. *lusitanica* by private growers during the past 10-15 years (Hood et al. 2001). The survey revealed an increase of small to medium sized woodlots, shelterbelts and hedge growers on rural land.

The survey also showed an increase of cypress canker, from South to North through out the country. The disease was more commonly found in young stands.

Cypress canker is identified as one of the major constraints in growing *Cupressus* species especially *C. macrocarpa* (Franklin 1994; Self 2000). Control methods such as the use of fungicides are not practical in forest plantations. The best alternative method for controlling cypress canker is to breed and screen for cypress clones resistant to the disease.

Forest Research in New Zealand requires a reliable method of screening isolates of *Seiridium* species for variations in pathogenicity and for screening cypress clones for resistance to cypress canker.

Table 1.1 Areas of cypress (ha) planted in North and South Island as at 1986

Cypress species	North Island	South Island
Cupressus macrocarpa	439	13000
Cupressus lusitanica	674	92
Chamaecyparis lawsoniana	329	617
Total	1442	2009

Source: Miller & Knowles (1996)

1.2 LITERATURE REVIEW

1.2.1 IMPORTANCE AND DISTRIBUTION OF CYPRESS CANKER DISEASE

The disease is caused by fungal pathogens in the genus *Seiridium*. The genus is classified under the subdivision Deutromycotina and the class Coelomycetes, of the order Blastromatineae (Sutton 1980). Susceptible cypress species become infected through wounds. The pathogens have been found to produce toxins and Graniti (1998) suggested that these could be responsible for the appearance of symptoms on damaged host tissues.

Cypress canker has spread far and wide over the last several decades; California, USA, Africa, New Zealand, Italy and other parts of Europe, and the Mediterranean region. Depending on the type of climate where *Seiridium* species are present, these pathogens can cause major losses. *S. cardinale* was reported as the major cause of serious disease in the Mediterranean and other parts of the world (Xenopoulos 1991). In New Zealand, *S. cardinale* is also reported as attacking *Cupressus macrocarpa* (Beresford & Mulholland 1982; Boesewinkel 1983). Graniti (1998) reported that *S. cardinale* affects several species of *Cupressus*, *Chamaecyparis*, *Cryptomeria*, *Cupressocyparis*, *Juniperus*, *Thuja* and other related genera of *Cupressaceae*.

Seiridium cupressi (Guba) (telemorph: *Lepteutypa cupressi*) has caused a major problem in Europe on *Cupressus* (Graniti 1998). It was also reported in New Zealand (Boesewinkel 1983), but the existence of this species is still being debated. The host range is restricted to the *Cupressacae* family.

S. unicorne on the other hand, attacks *Cupressaceae* and other botanical families (Barnes et al 2001). Boesewinkel (1983), claimed to have isolated *S. unicorne* from *Cryptomeria*. It is of minor importance in the Mediterranean region (Xenopoulous 1991). Graniti (1998) stated that the recent reports restricted the host range of population of *S. unicorne* living on cypress to *Cupressaceae* in countries such as Japan (Tobata et al. 1991) and New Zealand (Beresford & Mulholland 1982; Boesewinkel 1983; Van der Werff 1988). In North America, *S. unicorne* has been found to attack

several *Cupressaceae* and a study carried out revealed no evidence of host specificity (Tisserat et al 1991).

During 1981-1982 periods, *S. unicorne* has spread throughout New Zealand except the coast of the South Island (Van der Werff 1988). Cypress canker disease was reported as widely distributed in younger shelterbelts and woodlots. The disease is also present in older rural stands and forest plantations (Gea & Low 1997; Self 2000; Hood et al. 2001; Hood & Gardner 2002).

1.2.2 TAXONOMY

The disagreement among taxonomists regarding the number of *Seiridium* species causing cypress canker has been a long controversy. There is a history of discussions on the taxonomic species of *Seiridium* (Swart 1973; Boesewinkel 1983; Chou 1989; Viljoen et al. 1993; Graniti 1998; Morrica et al. 1999 & 2000; Barnes et al. 2000, 2001). Conidial morphology; host ranges; cultural characteristics and geographical distributions description were used in distinguishing between the three species.

Swart (1973) suggested one species with variable morphology, while Chou (1989) supported the existence of two species, *S. unicorne* and *S. cardinale*. Boesewinkel (1983) identified 3 distinct species, *S. unicorne*, *S. cupressi* Boes.combi nov.*Cryptostictis cupressi* Guba (teleomorph = *L.cupressi*) and *S. cardinale*. He used the absence of an appendage to distinguish between *S. cardinale* and the other two species and the appendage at an angle of 45° to distinguish *S. cupressi* from *S. unicorne*. Although many workers consider that there are only two species (*S. unicorne* and *S. cardinale*), modern molecular work is providing support for the three species concept. Barnes et al. (2001) found three distinct species on analysis of both β-tubulin and histone sequences.

The results of current studies have reaffirmed the morphological investigations of the three fungi causing cypress canker in New Zealand (Boesewinkel 1983) and biochemical investigations by Graniti (1998) based on appendage angle and toxin production. There is a strong support for the presence of three distinct *Seiridium* species present in different parts of the world. However, as Graniti (1998) pointed out, the existence of races or ecotypes especially with regards to *S. unicorne* cannot be ruled out.

In New Zealand, the general view of cypress canker workers is that only two species exist in the country, *S. cardinale* and *S. unicorne* .The third species identified is thought to be a variation of *S. unicorne*.

1.2.3 SYMPTOMS AND DISSEMINATION OF CYPRESS CANKER DISEASE

The *Seiridium* species that cause canker disease on barks of cypress trees are wound parasites. However, under favourable conditions, the pathogens can enter through the epidermis (Raddi & Panconesi 1981). The canker name itself describes the symptom of the disease. Upon entering through the wounds on the barks of cypress plants, lesions form around the wound as the pathogen progresses though the bark. Resinous canker develops on the bark and in young plants the stems are girdled (Plate 1.1).

Canker formation is found on the tree trunk as well as on the branches. Symptoms of disease include yellowing, browning and wilting of foliage of cankered branches. Infection can result in the loss of foliage and eventually death of susceptible trees. Cankered trunks of mature trees become deformed and reduce the timber quality.

Factors such as cold, wind, insects that cause wounds on bark of cypress plants spread the disease (Raddi & Panconesi 1981). The conidiomata of the *Seiridium* species open wide on canker surfaces under moist conditions (Graniti, 1998). Slimy conidial masses exposed under moist conditions when dry are dispersed by wind. Rain water also disperses conidia from acervuli over short distances. Insects or air borne ascospores could be responsible for spreading the disease on branches high up the trunk of tall mature cypress trees.

Plate 1.1 (a): Wilted side branches (indicated by a white arrow) next to a canker on the main stem of *C. macrocarpa*. (b): Resinous canker on the stem

1.2.4 FACTORS AFFECTING CYPRESS CANKER DISEASE

Cypress canker is believed to spread from one continent to another through the distribution of diseased planting material. However, the establishment of the disease within a locality depends on fungal pathogenicity; environmental conditions such as relative humidity and temperature; and the defence mechanism of the host plant.

1.2.4.1 Fungal pathogencity

Research carried out in Europe revealed that the pathogenicity and host ranges differ between the *Seiridium* species (Barnes et al. 2001). For instance, *S. cupressi* has been found be an aggressive pathogen in Europe on *Cupressus* species. Xenopoulos (1991) reported pathogenicity variation in his study. *S. cardinale* and *S. cupressi* caused bigger and severe cankers than all strains of *S. unicorne*. Initially cankers caused by *S. cardinale* were bigger than those of *S. cupressi* but two years after inoculation, the cankers of *S. cupressi* become larger and more severe than cankers of *S. cardinale* (Xenopoulos 1991). Work done in New Zealand also showed overall higher pathogenicity and less variability among *S. cardinale* isolates than those of *S. unicorne* isolates (Chou 1990). The study also revealed that isolates of *S. unicorne* displayed vast differences in pathogenicity and therefore caused difficulty in determining pathogenicity. Despite the variation in pathogenicity, Chou (1989) reported that apart from the difference in the presence or absence of conidial appendages, *S. unicorne* and *S. cardinale* are similar in many biological characteristics.

1.2.4.2 Host resistance

The susceptibility of the host plant to cypress canker is one of the important factors that influence the rate of spread within a locality. The literature showed that there are considerable differences in susceptibility among cypress species and among individuals within the same species (Raddi & Panconesi 1981; Beresford & Mulholland 1982; Raddi & Panconesi 1984; Xenopoulos 1990). *Cupressus macrocarpa* has been found to be susceptible to cypress canker compared to *C. sempervirens*, *C. arizonica*, *C. lusitanica* and *C. torulsa*. Resistant cypress species are useful for incorporating resistance into breeding programmes.

The resistance mechanism of cypress to attack by *S. cardinale* has been reported to be based on the ability of cypress trees to compartmentalize wounds (Xenopoulos 1990). It also suggested that the resistant mechanism was under apparent polygenic control. This view was also supported by Spanos and co-workers (Spanos et al. 1999). Spanos and co-workers expressed the view that anatomical responses to wounding and infection considered to be stable polygenic process might cause difficulty in manipulation in breeding programmes.

Apart from genetic resistance of certain cypress species, plant maturity appeared to play a role in host resistance. Van der Werff (1988) reported that canker infection on *C. macrcarpa* decreases as the tree age increases. Tree age however, was not correlated with disease incidence on *C. lusitanica* and *Ch. lawsoniana*. A study on the effects of infection and tree age on the progress of *S. unicorne* by Yamada et al (1994) supported the observation by Van der Werff. Yamada & Ito (1995) also found a similar result when inoculations of *S. unicorne* were carried out at heights of 1, 2, 3 metres. The study showed non-wounding inoculations induced infection at height of 3 m and not at height 1 and 2 m. It was concluded that the preformed outer bark was the most responsible factor for fewer infections. However, observation on younger *Cupressus* plants showed a contradicting result. Chou (1990) reported that young seedlings (3-6 months old) of *C. macrocarpa* and *C. lusitanica* were found to be highly resistant but infection occurred when one and half year old plants were used. The study also revealed that the basal part of the stems were infected but not on the inoculations made on the upper green stems of the same plant. A study on *Chamaecyparis obtuse* ("hinoki") showed that infection caused by *S. unicorne* in 3 year old trees spread more rapidly than in younger trees (Kato 1996). It was also noted in Israel that natural infection by *S. cardinale* was observed more frequently on adult trees than on young plants (Solel et.al. 1983).

An artificial study also showed that under greenhouse conditions, the bark maturity prompted infection. These observations were similar to what Chou (1990) observed with cypress plants used in his study. However, the study in Israel also revealed that with artificial inoculation in field conditions, canker development did not differ either between young thin branches and older ones or between inoculations at the base and at the tip of the branches.

1.2.4.3 Environmental factors

Different observations on bark resistance mentioned in the previous section could be related to environmental factors and due to the fact that bark canker resistant trait is not stable as reported by some workers (Casini & Santini 1995; Santini et al 1997).

Geographic-climatic barriers may also responsible for low disease rate among cypresses with no genetic resistance to cypress canker (Santini & Lonardo 2000). The most important environmental conditions critical include relative humidity, water and

temperature. Grantiti (1998) reported that for *S. cardinale*, the conidia failed to germinate when the relative humidity approaches 80 %. On the other hand, Solel et al. (1983) stated that high relative humidity enhanced artificial infection of nursery seedlings.

Water plays the most important role in spreading conidia of cypress canker pathogen (Raddi & Panconesi 1981). Xenopoulos (2000) suggested that drought stress seems to be the main factor for the infection of susceptible host plants.

The optimum temperatures for pathogenicity of each *Seiridium* species differ. The optimum temperature for growth and sporulation of *S. cardinale* and *S. cupressi* is 25°C (Graniti, 1998; Sasaki & Kobayashi 1976). Graniti (1998) reported that *S. cardinale*, is the most thermophilic of the three *Seiridium* species and its conidia can germinate, and colonies can grow up to 35°C. It was also observed that the growth of *S. cardinale* in host tissues is slowed or stopped during the hottest months of the year, resuming again in autumn. The pathogen spreads in the host tissues during winter. For *S. unicorne* the optimum temperature is 20°C (Graniti 1998). Low temperatures during winter or frost can cause damage on the bark of trees and provides an easy entry of the pathogens (Moricca et al. 2000). In New Zealand Van der Werff (1988) reported that infection increased with the increase in temperature across the country.

1.2.5 CYPRESS CANKER DISEASE CONTROL

1.2.5.1 Cultural management

Good management practises can prevent the spread of cypress canker in young plants and small scale plantings such as those used for shelter belts and ornamental cypresses. Selection of clean planting material is always the best option to prevent plant disease spreading within a locality. In the nursery the disease can be controlled by removal of diseased seedlings and obtaining planting material from healthy stock plants. Removal of diseased branches could be easily done with plants grown for ornamental and wind breaks purposes. This practice becomes difficult as the plants grow tall and cannot be applied to forest plantation situations.

1.2.5.2 Chemical control

Studies in Italy have shown that application of benomyl or benomyl and captafol were partly effective in controlling cypress canker in the first stage of infection (Panconesi & Raddi 1986). Work done in New Zealand also showed that *S. cardinale* can be controlled by chemicals such as benomyl and chlorothalonil (McCain 1984). Parrini & Panconesi (1991) stated that chemical trials carried out on *C. sempervirens* in the nursery showed systemic products such as benomyl and thiophanate-methyl proved to be more effective than conventional contact fungicides. However, chemical control is considered as expensive and impractical in the plantation situations.

1.2.5.3 Breeding for resistance to cypress canker

Some cypress species have some level of resistance to cypress canker disease. Some of species include *C. lusitanica*, *C. arizonica* and *C. torulosa*. Breeding for resistant to cypress canker appears to be best alternative of controlling the spread of cypress canker disease. A number of countries in Europe have breeding programmes to screen for resistant clones. It is difficult to develop universally resistance clone due to the fact that the canker resistances has be found to be unstable (Casini & Santini 1995).

1.2.6 ARTIFICIAL INOCULATION METHODS

1.2.6.1 Introduction

Screening for cypress clones resistant to canker disease caused by *Seiridium* species involves artificial inoculation. Inoculations are normally done on young plants in the field and in glasshouse conditions. In vitro inoculation of cypress with *S. cardinale* had been done in Europe and the result has a potential for use in cypress canker screening programmes (Spanos et al. 1997 a). Mycelium is the common type of inoculum used in screening programmes in several countries (Strouts 1973; Raddi & Panconesi 1984; Chou 1990; Xenopoulos 1990; Tisserat et al. 1991; Santini et al. 1997). However, spore suspension was also used in New Zealand by Beresford and Mulholland (1982) and by Ponchete and Andeoli (1989) and Strouts (1973) in England.

1.2.6.2 Wounding technique

The most widely used inoculation technique is to create wounds or insertion on stems or branches of cypress plants. The inoculum, which can be either conidial suspension or agar plugs containing mycelium are placed on the wounds. Wounds are made using cork borers or scalpels. This technique is now commonly used in Europe (Raddi & Panconesi 1984). In New Zealand, the convenient inoculation technique used is reported in Chou (1989, 1990) and Self (1994). Wounds are created by making a V-shape cut on the stem and placing mycelial plugs in the wound. The same technique is used in other, overseas countries (Strouts 1973; Spanos et al. 1999).

1.2.6.3 Fungal inoculum

Infection of cypress plants in nature is by conidia (Raddi & Panconesi 1981). The use of mycelium could mask the type of reaction of the same plant in the natural habitat when it is exposed to conidial inocula. Use of high inoculum could result in early elimination of clones of desirable characters that could be used in the breeding programmes. Raddi & Panconesi (1984) suggested that the variation in screening results could be due to the fact that inoculations might have been carried out with mycelium taken from a surface mutant sector of fungal colony with either higher or lower pathogenicity. Hood & Gardner (2002) expressed a similar concern, that with *S. unicorne* cultures, the ability of cultures to degenerate and loose virulence poses an additional complication as reported by Chou (1989) and Self (1994).

1.2.6.4 Methods of inoculum production for artificial inoculation

1.2.6.4.1 Methods of culturing *Seiridium* species

Isolation is normally done by obtaining small sections of bark or other diseased tissues and culturing on artificial media after surface sterilization using ethanol or sodium hypochlorite. Cultures of *Seiridium* species can be maintained on 1.5- 2.0% potato dextrose agar (Tisserat et al.1991; Graniti et al.1992; Barnes et al. 2001). Other media

being used included malt agar (Viljoen et al 1993; Spanos et al.1997a). Single spores have also been isolated from conidiomata embedded in the bark (Tisserat et al. 1991).

1.2.6.4.2 Methods of inducing sporulation of *Seiridium* species

The use of conidial suspension for artificial inoculation requires adequate supply of conidia. *Seiridium* species in some cases sporulates easily on artificial media and natural substrates such as bark of cypress. However, it has been reported that after series of subcultures or long storage, *Seiridium* isolates often loose their sporulating capability (Tisserat, et al. 1991).

Different methods of spore production have been reported in (Strouts, 1973; Initini & Panconesi, 1974; Sasaki & Kobayashi, 1976; Solel et al. 1983; Chou 1989; Tobata et al. 1991; Sanchez & Gibbs 1995). The length of incubation reported in these references for the appearance of conidia ranged from one week to several weeks. Inoculation of sterilized cypress twigs and exposure of *Seiridium* isolates to near UV light seemed the most common method of inducing spore production. Plant material added to agar has been found to increase sporulation of some fungi (Fisher et al. 1982; Wang et al.. 1985; ; Hu & Wu, 1997; Wyss et al. 2001). .Variability of *Seiridium* isolates was also observed in conidia production.

1.2.6.4.3 Conidial inoculum load

Artificial inoculation with conidia is considered more natural than the use of mycelia. Conidial inocula were used in the past for artificial inoculation. Methods of conidial inoculum applications have been described in (Strouts, 1973; Beresford & Mulholland, 1982; Solel et al., 1983; Ponchet & Andreoli, 1984; Ponchet & Andreoli, 1989; Panconesi & Raddi, 1991). The concentration of conidial suspension varied and in most cases the number of conidia used per wound was not reported. A study by Ponchet & Andreoli (1984) revealed that for *S. cardinale* the minimal effective dose was 50 conidia per wound and the optimum was 500, but it is not known whether there is a difference between *Seiridium* species or isolates of the same species relating to the minimal effective dose.

1.2.7 ASSESSMENT OF CANKER INFECTION IN ARTIFICIAL INOCULATION

From the literature method of assessment carried out in most studies involved measurement of the canker sizes, visual observation of plant growth, disease symptoms and presence of fungal fruiting bodies. The measurements are done from few weeks to few years after the artificial inoculation of cypress plants. For cypress canker disease resistant screening purposes, the method of assessment used is normally based on a descriptive scale. The different methods of assessment are described in various research areas, some of which include (Beresford & Mulholland, 1982; Solel et al. 1983; Xenopoulos 1990; Santini & Lonardo 2000).

1.3 CONCLUSION AND RESEARCH AIMS

Having reviewed work done overseas and New Zealand, it is obvious that screening for cypress canker resistant plants in this country is still at its early stage. A complication is that two of the three pathogenic *Seiridium* species have been confirmed in New Zealand but there is a possibility of existence of a third species. It is important that the number of species is confirmed in order to develop a reliable screening programme. Contradictory results are likely to be related to misidentification of the *Seiridium* isolate used in studies because they may behave differently under the same environmental conditions. Variability in resistance has also been observed between ramets of the same clone growing at the same site (Raddi & Panconesi, 1984). Most of research work on cypress canker in the past decade has been done on *S. cardinale* and *S. unicorne* under overseas climatic condition and the results may not be directly applicable to New Zealand.

Forest Research requires reliable method of artificial inoculation of cypress with *Seiridium* species to screen for canker resistance under New Zealand climatic condition.

The overall goal of the study was to develop reliable methods of artificial inoculation of cypress with *Seiridium* spp. in order to detect any clonal differences in resistance to these pathogens.

The aims were to:

1. Consistently obtain abundant spore production in culture since large numbers of conidia would be required as inoculum.
2. Compare main stem and side branch inoculations.
3. Compare agar plug and conidial suspension inocula.
4. Determine the effective dose for conidial inoculum.
5. Assess inoculation of ramets in vitro and in vivo.
6. Determine whether wounds are required for infection.
7. Assess the pathogenic variability of isolates.
8. Assess the effect of environment on infection.