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Biocontrol of Blackberry (*Rubus fruticosus* L. agg.)

by *Phragmidium violaceum* (Schultz) Winter

A dissertation

submitted in partial fulfilment

of the requirements for the Degree

of

Masters of Applied Science

at

Lincoln University

by

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Lincoln University

November 1996

Abstract of a dissertation submitted in partial fulfilment of the requirements for the
Degree of Master of Applied Science in Plant Protection

Abstract

**Biocontrol of blackberry (*Rubus fruticosus* L. agg.) by
Phragmidium violaceum (Schultz) Winter**

by Judith Pay

Blackberry (*Rubus fruticosus* L. agg.) is a serious weed in Australia and New Zealand. Control is both difficult and costly. Spores of the rust pathogen *Phragmidium violaceum* (Schultz) Winter were released in Victoria, Australia in 1984 as a biological control agent specific to this weed. *P. violaceum* was first reported on blackberry in Canterbury, New Zealand in February 1990. It has subsequently been reported as being widespread over both main islands and as far east as the Chatham Islands. Nothing is known about either the races of the rust that have become established in New Zealand or the host/pathogen interactions.

The life-cycle of *P. violaceum* on blackberry in New Zealand was similar to that described in Chile and Australia and all spore stages were produced. Susceptibility of four hosts differed depending on species and age of leaf. *R. echinatus* was the most common species in Canterbury and was the most infected species in the field. It was only moderately infected in detached leaf studies. In contrast, *R. laciniatus* was the

least common species and only moderately infected in the field. However, it was the most infected species in detached leaf studies. *R. ulmifolius* was never infected in the field but was susceptible to two isolates of urediospores, collected from Farewell Spit and Seddonville, in detached leaf studies.

Of the three ages of leaves tested, the youngest, fully expanded leaves were the most susceptible to infection ($P < 0.001$) compared to leaves from the middle of the cane ($P < 0.05$) and the oldest leaves ($P < 0.001$).

Eight putative physiological races of *P. violaceum* were identified provisionally from sixteen samples tested. Amongst these some isolates infected none of the host species tested and others infected all host species.

Urediospores germinated at 10-25°C with maximum germination occurring within 7-10h. Germination was most rapid, with maximum achieved within 3h, at 20°C. There was some evidence for adaptation to temperature in one sample. Teliospores germinated after 45h at temperatures between 5 and 25°C after cold water treatment (5°C) for two weeks.

The significance of host specialisation and conditions suitable for host infection are discussed in relation to biocontrol of blackberry with *P. violaceum*.

Keywords: Blackberry; *Rubus fruticosus* L. agg.; *Phragmidium violaceum*; biocontrol agent; host specialisation; physiological races.

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1. GENERAL INTRODUCTION

Early settlers who came to Australia and New Zealand from Europe brought with them many plants from their homelands both for sentimental reasons and as potentially useful plants. Some of these introduced plants failed to grow but some others, finding the soils and climate to their liking grew far more vigorously than in their place of origin aided by the fact that often pathogens and predators that had kept them under control in their place of origin were not transported to the new environment with their hosts. One plant introduced by the early settlers which has become a serious weed in both Australia and New Zealand is European blackberry (*Rubus fruticosus* L.agg.).

Blackberry in Australia and New Zealand, after its introduction, spread quickly and became a weed taking over valuable land that had potential for crop production. It displaces pasture and is unpalatable to stock because of its prickly nature, it smothers native vegetation and reduces plant diversity as well as restricting access to rivers for recreational purposes and the watering of stock (Amor and Harris 1979). This has led to extensive research into methods of control. However, all techniques tried to date have been either ineffective, costly or both. Unfortunately the fungus *Phragmidium violaceum* (Schultz) Winter, that parasitizes blackberry in Europe, was not introduced when blackberry was first brought to Australia and New Zealand. The presence of *P. violaceum* in New Zealand was first recorded in 1990 (Noonan, Pay and Close 1990). This observation led to investigations into the possible use of this parasite as a biological control agent for blackberry. The results of some of the investigations are the subject of this dissertation.

Blackberry taxonomy

Plants commonly known as European blackberry, or bramble, belong to a closely related group within the genus *Rubus* (Rosaceae). The relationships between species are still debated by taxonomists with no general agreement globally. Webb and Given (1988) stated that " the subgenus *Rubus* is very problematic and hundreds of segregates are recognised in Europe alone-most belonging to the aggregate *R. fruticosus* L., the common blackberry." This is probably the most useful designation available until further taxonomic work has clarified the limits of species, cultivars etc. In this dissertation members of the subgenus *Rubus* have been keyed to and described under *R. fruticosus* agg. This allows the aggamospecies to be recognised individually and they will be referred to as "species".

Distribution of blackberry

Members of the *R. fruticosus* L. aggregate are found in Europe, the Middle East and north-west Africa, North America, Chile, South Africa, Australia and New Zealand (Laundon and Rainbow 1969). Blackberry grows mainly in areas where the annual rainfall exceeds 760mm and between latitudes 30⁰ to 65⁰ N and 28⁰ to 45⁰ S. In drier areas (eg. Canterbury, NZ) it is often found growing near creeks and irrigation channels close to areas of early European settlement. Blackberry can grow in the open or the shade, often growing at the edge of forests and plantations where the light intensity may be as little as 10% of full sunlight (Amor and Richardson 1980). Blackberry was introduced to Australia and New Zealand by early European settlers as they valued the berries for jam and pie making and the plants were used extensively as hedgerows (Amor and Richardson 1980). In New Zealand, species belonging to the *Rubus fruticosus* aggregate have discrete regional distributions

reflecting patterns of settlement and are represented by 19 species and one common hybrid (Webb and Given 1988).

Growth and development of blackberry

Blackberry is a perennial shrub often forming large clumps (0.3m to 7m high). The canes bear prickles and form thickets impenetrable to people and grazing animals. New canes (primocanes), formed in the spring from buds on the crown or the roots, elongate rapidly and form single compound leaves but do not bear flowers or fruit. These canes grow to the top of the canopy and some of them eventually arch towards the ground where, in autumn, they take root. New plants are formed the following spring from buds and roots at the apices of these rooted primocanes (Fig.1) thus contributing to the survival and spread of the thicket. In their second year canes (floricanes) bear flowering branches and fruit. The root system is perennial and canes live for at least 2 or 3 years, sometimes longer. Blackberry plants lose most of their leaves in the winter and are dormant until cane growth commences in the spring, but in mild climates, such as the West Coast of the South Island of New Zealand, leaves may remain on the canes all year.

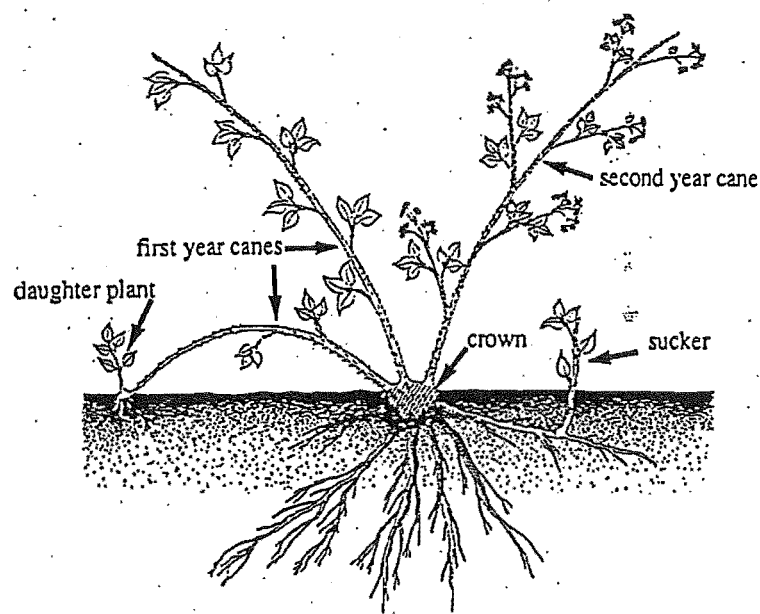


Fig 1. Growth pattern of blackberry

Blackberry can spread vegetatively, from root segments, cane root-tipping and adventitious shoots (suckers) that form occasionally on roots. Seed, produced by pseudogamy, contributes to the spread of the weed when the berries are eaten by birds and the seeds are dispersed in their droppings (Amor and Richardson 1980).

Apomixis in *R. fruticosus* agg. is facultative, and new apomictic taxa arise occasionally by hybridisation between sexual and pseudogamous species. Plants derived by these means give rise to a whole spectrum of individuals each differing from its neighbours by only a small number of characteristics and making identification difficult.

Blackberry as a weed

Blackberry is regarded as a serious weed in Western USA, Chile, South Australia and New Zealand (Gilkey 1957, Bridge 1963, Parsons 1958, Hilgendorf 1952). In 1975 the amount of land infested with blackberry in Victoria, Australia, was estimated to be 663 000 ha, of which 58% was in native forest, 28% in agricultural land, 10% in sown forest, 2% in road sides and 2% was other use (Amor and Richardson 1980)

Control is costly and often difficult because blackberry forms impenetrable thickets and the type of terrain it invades often has difficult access. Limited control can be achieved by grubbing, mowing, burning or ploughing, and heavy grazing with sheep and cattle will reduce the number of plants formed at the cane apices. Goats have been used at high stocking rates to suppress blackberry in both New Zealand (Von Pein 1961) and Australia (Amor and Richardson 1980) but these animals have themselves become pests. Complete suppression of blackberry can be achieved only by a combination of cultural and chemical treatments. Herbicides such as 2,4,5-T were most commonly used until the late 70's when they became unpopular for environmental and health reasons. More recently sprays containing metsulfuron, picloram, glyphosate and thiazafluron have been found to give

reasonable control (Amor and Richardson 1980). Many of the large areas of blackberry that exist do so because presently available control measures are not economic. These areas form a reservoir of material from which new infestations occur. Significant amounts of money are spent on the control of this weed. In 1979 the Department of Crown Lands and Survey of Victoria, Australia spent over \$1M on blackberry control (Amor and Richardson 1980) and less costly and more efficient means of control are constantly being sought. In New Zealand there do not appear to be any national records kept of the amount of land infested with this weed or of the cost of control. However, a postal survey of farmers in the South Island (Bascand 1979) estimated that approximately 32% of farmable land was infested, 5-25% of that unusable because of this weed. Any alternative method of control of this weed less costly than the ones now employed would be of significant benefit to Australian and New Zealand agriculture.

Biological control of weeds

Traditional weed control was either by hand, which was very labour intensive, or mechanical. Chemical weed control technology has been developed over the last 50 years and increased yields of many crops can be attributed partly to the control of weeds with herbicides. However, the escalating costs of research and development, the increasing public concern regarding the persistence of residues in soil and water, injury to non-target crops by spray drift and problems associated with herbicide resistance are limitations to continued chemical herbicide use. In a period of increasing environmental awareness there is a move towards biological control strategies as a component of weed control systems.

One method of biological control utilises natural enemies of the target plants for their control. Entomologists have used both parasites and predators to manage insect

populations and in some cases insects have been used to control weeds (Sailer 1972, Hill *et al* 1991). Bacterial, fungal and viral plant pathogens have the potential to cause severe damage to their host plants especially if the equilibrium is altered in favour of the pathogen and so are potentially good agents for weed control. The subsequent introduction of pathogens that did not arrive with their hosts when they were brought to a new country may redress the situation where the host has become a weed. In certain cases this may produce long and durable control that is highly specific and does not cause significant environmental damage. The introduction of the pathogen may be by one of two strategies, the inundative tactic or the classical tactic

The inundative approach is to use a pathogen as a mycoherbicide by applying single or multiple, severe or lethal dose(s) to the weed as might be done in the case of chemical herbicides. This approach is more suitable for annual weed control where control is desirable within one growing season than for situations where complete control is not necessary. The pathogen may be introduced or may already be present on the host but in low numbers which are not damaging and because of poor dissemination or failure to over-winter does not multiply sufficiently to produce lethal effects. To date only three products in this category have been commercialised in any part of the world. The first was *Phytophthora palmivora* for control of strangler vine (milkweed) in the citrus groves of Florida. The potential of this pathogen was discovered in 1972 (Ridings *et al* 1975) and marketed in 1981 as DeVINE, a liquid spore suspension. It is applied with a boom and nozzle sprayer system to the soil surface under tree canopies and can reduce the strangler vine population by 90% within 1-2 years. The second very successful example of a pathogen used in this way is the control of northern jointvetch *Aeschynomene virginica* (L.) B.S.P. in rice fields in Arkansas USA with *Colletotrichum gloeosporioides* (Penz.) Sacc.

The potential of this organism was discovered by Daniel *et al* (1973) and marketed in 1982 as the mycoherbicide COLLEGO . It is applied aerially once a year and kills plants within 4-6 weeks. However, Greaves and Macqueen (1990) have suggested that from a commercial standpoint, DeVINE and COLLEGO have not been very successful. DeVINE persists in the soil giving several season's control from one application thereby reducing sales and COLLEGO has only achieved about 20% market penetration. The third mycoherbicide, BIOMAL, was registered in 1992. BIOMAL is a preparation of *Colletotrichum gloeosporioides* f.sp. *malvae* and is used to control round-leaved mallow. This biocide was discovered and developed in the USA by Mortensen (1988).

The classical biocontrol approach is the introduction and release of a pathogen derived from the hosts original, or another, environment. Control is dependent on the pathogen reproducing and dispersing naturally in the new environment. This approach is best suited for perennial weeds in less intensively managed areas such as roadways, railways, waterways and edges of forests where area-wide applications of herbicides are not economically justified or desirable and complete control is not necessary. The most cited classical biocontrol success story is the introduction of Chondrilla rust, *Puccinia chondrillina* Bubak and Syd., which is indigenous in the Mediterranean area, into Australia to control rush skeletonweed, *Chondrilla juncea* L. (Templeton *et al* 1979). Another success has been the control of pamakani weed (*Ageratina riparia*) in Hawaii with the white smut *Cercospora ageratinae* (Te Beest 1991). Rusts and smuts lend themselves to this type of exploitation because they produce many generations of infective propagules in one season which are disseminated by the wind and spread naturally, compared to some other fungal pathogens which may produce few generations per season or are poorly dispersed.

Rusts as pathogens

Members of the Uredinales are the causal agents of rust diseases. They are among the most important parasitic fungi, causing great losses to a wide variety of plants including cultivated crops such as cereals, coffee, beans, asparagus, and scores of others (Alexopoulos and Mims 1979). The life cycles of some rusts are among the most complex of any fungi. Up to five types of spores, both sexual and asexual, may be produced by some rusts (macrocytic). Other species may have a reduced cycle (leptocyclic) and some may produce as few as two spore types (brachycyclic). All of the spore types may occur on one host (autoecious) or, in some species, (heteroecious) two separate hosts are involved (Alexopoulos and Mims 1979).

Urediospores are the repeating stage of most rusts since many generations of these spores may be produced in one growing season. These spores may be transported long distances by wind and occasionally enough spores remain viable long enough to begin a new infection cycle on a susceptible host. The spread of urediospores by wind has been well documented, e.g. in the USA clouds of urediospores of *Puccinia graminis* f.sp. *tritici*, the causal agent of stripe rust on wheat, were tracked for distances of up to 2,000 miles (Webster 1970).

Pathogens and insects associated with blackberry-Australia

The growing public concern of the side effects of 2,4,5-T used for the control of blackberry, along with the reported success of the biological control of blackberry in Chile with the rust *Phragmidium violaceum* (Schultz) Winter (Oehrens and Gonzales 1974, 1977), resulted in the commencement of a biocontrol research programme in Australia. Bruzzese (1980) surveyed *Rubus* spp in Victoria and recorded forty-two species of phytophagous insects and mites associated with *R. fruticosus* agg. but found none of them caused more than minor

damage to the plant. The cane-boring sawfly *Hartigia albomaculatus* was the only insect species with any potential as a biological agent. A survey in Victoria in 1976-77 found eight fungal pathogens on *R. fruticosus* agg. (Field and Bruzzese 1985). The most commonly found diseases were blackberry orange rust (*Kuehneola uredinis*), leaf spot (*Septoria rubi*) and anthracnose (*Elsinoe veneta*). There were large differences within the aggregate host species in response to *K. uredinis*, from premature defoliation and cane dieback on the most susceptible species to little or no effect on other species (Amor and Richardson 1980). None of these insects or fungi were eventually found to be suitable as biocontrol agents (Amor and Richardson 1980).

Pathogens and insects associated with blackberry -New Zealand

In New Zealand fourteen insect pests have been recorded on *R. fruticosus* agg (Spiller and Wise 1982) and eleven pathogens (all fungal) (Pennycook 1989) Only *K. uredinis* appears to cause any damage of any consequence to blackberry and while it is commonly observed it is usually only severe in regions with high rainfall or in wet seasons with prolonged humid conditions (Laundon and Rainbow ,1969). In 1989, when Pennycook published his list of plant diseases found in New Zealand, *P. violaceum* had not been recorded in New Zealand..

***Phragmidium violaceum* as a biological control agent of blackberry**

Blackberry leaf rust, *Phragmidium violaceum* (C.F Schultz) Winter is an autoecious, macrocyclic rust. It is a pathogen specific to members of *R. fruticosus* agg., and is found throughout Europe ,North Africa and the Middle East (Laundon and Rainbow, 1969). The host specialisation of the rust has been studied by Vleugel (1908), Klebahn (1912) and Jorstad (1953)(all cited in Wilson and Henderson, 1966) who concluded that the rust attacks species within some subsections of *R. fruticosus*.

P. violaceum was first considered as a potential biological control agent of European blackberry in the Southern Hemisphere as early as the nineteen twenties when Cunningham (1927) imported spores from Europe to N.Z. However, the spores failed to germinate in quarantine. In 1973 a strain of *P. violaceum* from Europe was introduced to Chile to control two species of European blackberry (*R. constrictus* and *R. ulmifolius*) introduced by German immigrants (Oehrens 1974). Urediospores were released in several localities in the vicinity of the city of Valdivia and within 18 months the rust was present in a area with a radius of 70km from its point of release. After 3 years it was concluded that the rust inhibited the spread of *R. constrictus* but had little effect on *R. ulmifolius* (Oehrens & Gonzalez, 1974, 1977).

In Australia the evaluation of *P. violaceum* for the biological control of European blackberry naturalised in that country began in 1978 at Montpellier, France, where specificity tests were carried out with a number of rust isolates (Bruzzese & Hasan 1986). The pathogenicity of *P. violaceum* on members of the Rosaceae growing in Australia and New Zealand was found to be confined to a limited number of all members of the genus *Rubus*. All the cultivars of European blackberry naturalised in Australia, some of which are also present in New Zealand, were found to be either moderately or highly susceptible to the rust. However valuable commercial brambleberries such as raspberry, boysenberry and loganberry were not affected. The five species of *Rubus* endemic to New Zealand were found to be moderately susceptible to the rust.

Susceptibility of plants from *R. fruticosus* aggregate to the rust was found to vary between members of the aggregate when they were inoculated with a mixed pool of isolates, and Bruzzese and Hasan (1986) suggested that it would require more than one isolate to give

good control of blackberry naturalised in Victoria, Australia. In February 1984, before permission had been obtained to introduce a suitable virulent strain of the rust into Australia, *P. violaceum* was recorded at several sites in Victoria (Marks *et al.* 1984) where it had apparently been deliberately, and illegally, introduced. This rust strain spread very quickly in south-eastern Australia but appeared to be less damaging to the predominant blackberry species (*R. procerus* P.J. Muell.) than the strains selected in France (Bruzzese and Field 1984). Once the host range of this illegally introduced strain was tested and shown to be limited to weed species of blackberries, it was released in Western Australia in 1984-85. Although this strain is now found wherever blackberry grows in this State it is of low virulence and has not caused significant damage to its host (Dodd and Lloyd, 1992). In 1990 permission was finally given to import a more virulent strain (F15) into Australia from Europe. Urediospores of this isolate were released in Victoria in the summers of 1990/91, 91/92 and 92/93. They were released early in the spring, when the illegally introduced strain was at the pycnial and aecial stages, in the hope that the new introduction would out-compete the first strain and become the dominant one (Bruzzese 1992). F15 was also released in Western Australia in late October 1991 in an effort to control *R. discolor*, the predominant *Rubus* species in that state (Dodd and Lloyd 1992). Apart from visual assessments of the severity of attack on the most common species of blackberry in Australia, (*R. discolor*=*R. procerus*) there was no test available at that time to determine which strain had become established. The evaluation of the effect of both strains on blackberry in Victoria and Western Australia is continuing. (Bruzzese, pers. comm).

Effects of *P. violaceum* on blackberry

P. violaceum infects the leaves of blackberry and under some circumstances the calyces. Heavy infections of rust on susceptible members of *R. fruticosus* L. agg. causes premature leaf fall up to five months earlier than normal (Bruzzese pers. comm). As a result stems do not lignify, they may be invaded by secondary pathogens and affected by frosts, resulting in death of the terminal 30cm of the stems. In severe infections the numbers of viable seed may be reduced by up to 45% (Oehrens 1977). Defoliation allows light to penetrate the thickets, allowing the establishment of other vegetation, especially in autumn and winter. Although rust epidemics may look spectacular with most of the young leaves completely covered in uredia, blackberry is a vigorous, persistent plant and repeated infection over several years may be necessary to cause the root system to die back (Bruzzese pers. comm.)

Arrival of *P. violaceum* in New Zealand

P. violaceum was first reported in New Zealand in February 1990 (Noonan, Pay and Close 1990). Blackberry thickets of *R. echinatus* heavily infected with rust were discovered at Rakaia, Canterbury, and the rust was identified by the characteristic teliospores as being *Phragmidium violaceum* (Schultz) Winter. If it is assumed that *P. violaceum* came into New Zealand as airborne spores from Australia, this would be the “illegal” strain introduced to Australia in 1984 because the F15 strain was not released until the summer of 1990/91. Subsequent to the identification of the rust as *P. violaceum* in February 1990 the authors found that the pathogen was wide spread on the east coast of the South Island. The following year, 1991, there were many reports of the rust in the North Island and as far east as the Chatham Islands. (P. Johnston pers. comm.)

New Zealand lies 2000 km across the Tasman Sea, southeast of Australia and in the path of prevailing westerly winds. Anticyclones and low pressure troughs move across the Tasman Sea from Australia to New Zealand. The main mountain ranges of New Zealand provide a partial barrier to the prevailing westerly winds and form a natural trap for windborne material (Close *et al*, 1978) Depending on the weather conditions the winds may travel either around the northern or southern tips of the country and up or down the east coast (Fig 2). The aerial transfer of biological material, eg. insects, pollen and spores, has been well documented over both short and long distances (Gregory, 1973; Ayler, 1990). Five likely cases of trans Tasman dispersal involving rust species were identified by Close *et al* (1978): stem rust of wheat (*Puccinia graminis* Pers. f.sp. *tritici* Erikss. and E. Henn.); antirrhinum rust (*Puccinia antirrhini* Diet. and Holw.); *Euphorbia* rust (*Melampsora euphorbiae* (Schub.) Cast.); sunflower rust (*Puccinia helianthi* Schw.); and two poplar rusts (*Melampsora medusae* Theumen and *Melampsora larici-populina* Klebahn). A more recent arrival has been stripe rust of wheat (Close 1982). This fungus was first detected in Victoria, Australia, in October 1979 and was expected to cross the Tasman Sea and infect New Zealand wheat. This did occur in the spring of 1980 when stripe rust was detected in November in crops of the highly susceptible cultivar Tiritea, in an area near Gore in Southland. During the 1980-81 season the disease spread throughout Southland and in 1981 appeared in Canterbury and then Marlborough and up to Manawatu in the North Island.

While *P. violaceum* is now found on blackberry throughout most of New Zealand nothing is known of the strains of the rust present or of the host/pathogen characteristics as they occur in New Zealand.

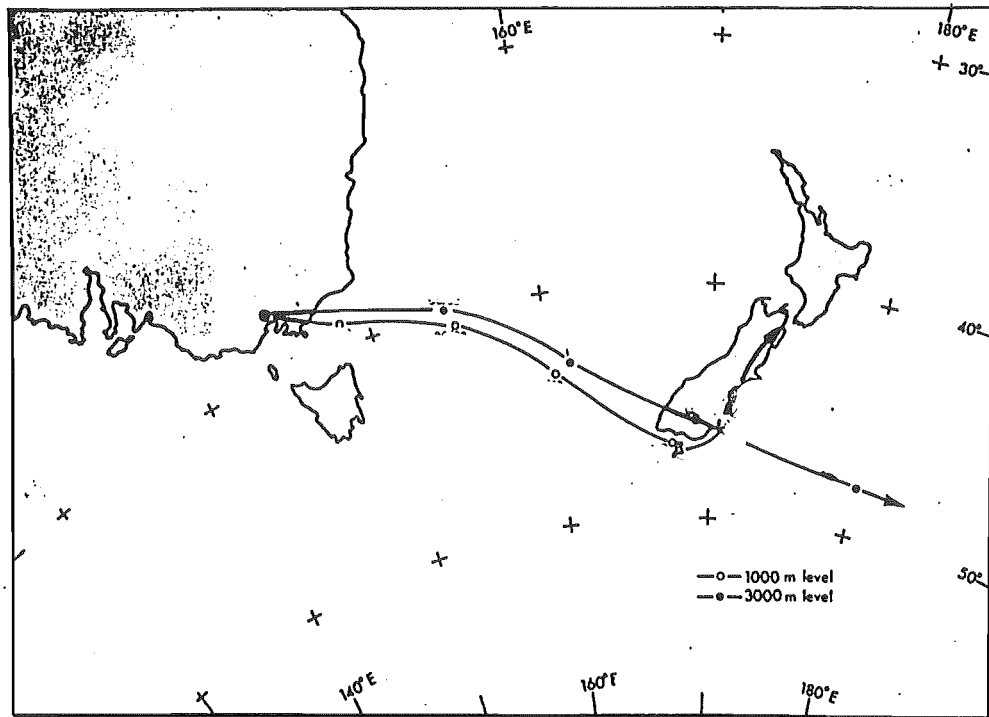


Fig 2. Trans-Tasman trajectories at 1,000 and 3,000 m levels, originating from Melbourne, providing suitable conditions for transfer of fungal spores to New Zealand. (Redrawn from Close *et al* 1978)

OBJECTIVES OF THIS RESEARCH

After *P. violaceum* had been identified in New Zealand and the literature concerning the biological control of blackberry had been reviewed it became clear that further research was required to ascertain whether the fungus could be managed as a potentially valuable agent for the control of blackberry in New Zealand.

To this end the following aspects of the fungus and its relationships to the host were investigated.

- Observation and identification of the life-cycle stages of *P. violaceum* as it occurred on *R. fruticosus* L. agg in Canterbury, New Zealand.
- Testing of the hypothesis that host specialisation occurs in species of blackberry growing in Canterbury.
- Verification of the suggestion by Bruzzese and Field (1984) that age of leaf affects the susceptibility of the host
- Determining optimum conditions for the germination of uredospores and teliospores *in vitro*.

2. OBSERVATIONS OF THE LIFE-CYCLE OF *PHRAGMIDIUM* *VIOLACEUM* IN THE FIELD

Identification of *P. violaceum* in New Zealand

P. violaceum was first described by C.F. Schultz in 1806 under the name *Puccinia violacea*. Subsequently Winter (1880) changed the name to *Phragmidium violaceum*. The most recent formal description is that of Loundon and Rainbow (1969), taken from CMI Description 209 (Fig 3). (Commonwealth Mycological Institute)

Phragmidium violaceum (C. F. Schultz) Winter, *Hedwigia*, 19: 54, 1880.
≡ *Puccinia violacea* C. F. Schultz, *Prodr. Fl. Starg.*, p. 459, 1806.

Autoecious; macrocyclic. *Pycnia* epiphyllous, crowded together in the centre of the spots, indefinite, becoming fused, subcuticular, 30–40 μ high. *Aecia* mostly hypophyllous on large purplish blotches usually with a yellowish or brownish centre, caeomoid, circular or annulate up to c. 1 mm diam., orange yellow, paraphysate. *Aeciospores* globose or ellipsoid, 19–35 μ diam; wall hyaline, echinulate, the spines 2.5–5 μ apart \times 1–1.5 μ high, 3–3.5 μ thick; pores scattered, obscure. *Uredia* hypophyllous on small or large blotches like those bearing the aecia, 0.2–1 mm diam. orange yellow, paraphysate. *Urediospores* broadly ellipsoid to obovoid, 22–32 \times 19–24 μ ; wall hyaline, echinulate, the spines 3–5 μ apart \times 0.75–1 μ high, 2.5–3.5 μ thick; pores scattered, obscure. Paraphyses clavate-capitate, incurved, 50–70 \times 15–23 μ . *Telia* like the uredia but black and up to 2 mm diam. *Teliospores* cylindrical, not or slightly constricted at the septa, rounded at the apex and usually surmounted by a rounded papilla 1–9 μ long \times 6–9 μ wide, 3–5 celled, dimensions excluding the apiculus: 3 celled spores 58–75 \times 30–40 (mean 63–70 \times 32–38) μ , 4 celled spores 79–100 \times 32–40 (mean 82–90 \times 33–38) μ , 5 celled spores 101–115 \times 30–40 (mean 103–111 \times 31–38) μ ; wall sienna to dark umber brown, sometimes markedly layered, coarsely warted, 5–7.5 μ thick; pores 2–4 in each cell; pedicels 90–150 μ long \times 7–10 μ wide at the neck, swollen below to 12–19 μ .

Fig. 3 C.M.I. Description of *Phragmidium violaceum*

This description was used by Bruzzese and Tenni (1992) to identify the fungus when it was introduced into Australia and is used in this dissertation.

In New Zealand blackberry showing severe rust infection was observed at Rakaia, Canterbury by Noonan, Pay and Close (1990). The infected material was examined in the laboratory and the shape and size of the spores noted. The black teliospores were 1-4 celled, (2-celled range 38-55 x 28-31 μ m, 3-celled range 54-77 x 28-31 μ m, 4-celled 75-90 x 28-31 μ m), papilla 4-5 μ m long, persistent hyaline pedicel. These results were in agreement with the description of Loundon and Rainbow (1969) and, together with symptom expression, confirmed that this infection was caused by *P. violaceum*. The orange uredospores were less easily identified but were strongly aculeate-verrucose and distinguished from *K. uredinis* by the spines being further apart. This rust, which is also present in New Zealand on blackberry, has also been described by Loundon and Rainbow (1969) in CMI Descriptions 202 (Fig 4).

Kuehneola uredinis (Link) Arthur, *Int. bot. Congr.*, Vienna, 1905, p. 342, 1906.
≡ *Oidium uredinis* Link in Linn., *Sp. Pl.* ed. 4, 6(1): 123, 1824.
= *K. albida* (Kuhn) Magn., 1899.

Autoecious; brachycyclic. *Pycnia* mostly epiphyllous or caulicolous, one to several in small groups, large and prominent, pustular, subcuticular, conical-lenticular, indeterminate and becoming fused, 50-60 μ high. Spermata ellipsoid-obovoid, 4-5.5 x 2.5-3.5 μ . *Aecial uredia* mostly epiphyllous or caulicolous, surrounding the pycnia in an annulate ring about 1 mm diam. or along the veins and stems in large ellipsoid-annulate groups up to 10-20 mm long. Spores like the true urediospores. *Uredia* caulicolous and forming elongated slits 1-10 mm long or hypophyllous on purplish spots or on minute yellowish spots sometimes surrounded by a purplish halo, scattered, often crowded, 0.1-0.2 mm diam., deep orange yellow when fresh, nonparaphysate. *Urediospores* broadly obovoid, 21-30 x 16-23 μ ; wall hyaline, closely echinulate, the spines 1.5-2.5 μ apart x 0.5-0.75 μ high, about 1.5 μ thick but often appearing thinner (0.5-0.75) because the outer layer is conspicuous whilst the inner layer is almost invisible; pores very obscure, said to be 3-4, equatorial. *Telia* like the uredia but floccose and pale yellowish buff or almost white. *Teliospores* in chains of mostly 4-7 cells, chains rather easily fragmenting, trapezoid-cylindric, 15-40 x 14-24 μ ; wall hyaline, smooth but often bearing coronate projections or bumps at the top (in apical spores) or upper rim (in intercalary spores), about 0.5 μ thick at the sides, thickened to 2-4 μ at the apex; pores apical in short projections; pedicels short, hyaline and fragile.

The uredia are readily distinguished from those of *Phragmidium* species by not possessing the distinctive paraphyses.

Fig. 4 C.M.I. Description of *Kuehneola uredinis*

Selection and description of sites

Twenty-two sites were chosen in Mid-Canterbury. Nine sites had been monitored annually from 1990 when *P. violaceum* was first reported, the remaining thirteen sites were selected in October 1994. The location, aspect, host species, and site characteristics were recorded for each site (Table 1). All sites were easily accessible from the road and geographically separated; eighteen sites consisted of mixed hedgerows of blackberry and gorse along minor country roads, three were isolated thickets in pasture and one was a thicket at the edge of a public scenic reserve. The sites were visited every four weeks from October 1994 until March 1995. A selection of diseased leaves were collected from each site and brought to the laboratory for examination.

The blackberry plants at each site were identified using the key of Webb and Given (1988). At two sites the plants showed intermediate characters between recognised species; at site 11 plants were closest to *R. echinatus* and at site 16 they were most similar to *R. ulmifolius*.

Species of blackberry present in Canterbury

Using the criteria of Webb and Given (1988) plants collected in Canterbury during the course of this investigation were identified as follows.

- *R. cissburiensis* W.C. Barton et Riddelsd., large green-backed leaves,
 - *R. laciniatus* Willd., dissected or cut-leaflets,
 - *R. echinatus* Lindley, fierce stem armature,
 - *R. procerus* Muller, white-backed, large leaflets,
- R. ulmifolius* Schott., waxy stem and pink flowers.

Table 1 Location and description of monitored sites of blackberry infected with *Phragmidium violaceum* in Canterbury during the summer of 1994/95

Site	Location	Grid ref.	Aspect	<i>Rubus spp.</i>	Description of location	Comments on location	Infection and infestation levels
1	Springston. Leeston Hyw	S43°40 E172°25	East	echinatus	Farm fence	Gateway to old farm shed subject to spraying	Much dieback and dead canes
2	Robinsons Rd Lincoln	S43°37 E172°30	North	echinatus	Farm fence	Opp. railway house subject to cutting	Very little infection at any stage
3	Ellesmere Rd Halswell	S43°35 E172°32	North	echinatus	Farm fence	Shaded by large trees, irrigation ditch	Infection increasing annually
4	Trices Rd Halswell	S43°35 E172°35	East	cissburiensis	Farm fence	Sheltered opp. #3 site cut back	Susceptible at pycnial stage.
5	Groynes Rec. Centre	S43°27 E172°37	Nor/West	cissburiensis	Public reserve	Picnic area #2 inside gate to Kirihihi	Little infection at any time
6	Gardners Rd Chch	S43°28 E172°37	West	ulmifolius	Private hedge	High hedge occasionally trimmed	Nil infection at any time
7	Springs Rd Lincoln	S43°39 E172°28	Nor/West	cissburiensis	Waste land	University dairy farm edge of stream	Severe insect infestation
8	Springs Rd Lincoln	S43°39 E172°28	Nor/East	cissburiensis	Farm fence	University farm fence line	Low levels all times
9	Rakaia Huts Rakaia Nth	S43°53 E127°14	East	echinatus	Waste land River bank	Edge of stream original site of rust	Severe infection 5th consecutive year
10	Rakaia Huts Rakaia Nth	S43°53 E127°14	West	procerus	Farm fence	Fence close to holiday huts	Infection levels slowly increasing
11	Bealey Rd Mid-Cant	S43°32 E172°16	South	unknown (like echinatus)	Farm fence	3km west turn off West Coast rd	Low infection
12	Bealey Rd Mid-Cant	S43°32 E172°17	South	echinatus	Farm fence	Opp. farm letter box "Aldridge"	Increased levels over season
13	Charing Cross	S43°33 E172°09	North	echinatus	Hedge	Derelict church opp #14	Little rust large insect infestation
14	Charing Cross	S43°33 E172°09	South	echinatus	Waste land	Corner opp. #13 tree clearing	Die-back severe
15	Darfield Nth	S43°29 E172°06	East	echinatus	Farm fence	Opp. pine plantation road to Kimbereley	<i>K. uredinis</i> severe early and late in season
16	Old West Coast Rd	S43°25 E172°06	North	unknown (like ulmifolius.)	Farm fence Mixed spp.	Opp. group large black poplars	Spots on top of leaves but no sporulation
17	S.W. Oxford	S43° 19 E172°09	East	laciniatus	Farm fence Mixed weeds	9km. from Waimak. river bridge	<i>K. uredinis</i> severe early in season
18	S.W. Oxford	S43° 19 E172°09	East	echinatus	Farm fence Mixed weeds	Opp. 2 concrete water tanks. close to #17	Little infection
19	Nth Oxford	S43° 16 E172°12	S/East	cissburiensis	Hedge	Opp. subdivision "Somerset Downs" sprayed in Jan	Susceptible at early stages..
20	Nth Oxford	S43° 15 E172°12	West	echinatus	Farm fence	Opp. hydroponics farm	Moderate infection
21	Tram Rd	S43° 20 E172°20	South	echinatus	Farm fence	Near Poyntz Road intersection	Many dead canes
22	Tram Rd	S43° 23 E172°34	South	echinatus	Farm fence	Beside AA "cow" sign site mown	Many dead canes

Footnote: Site 23 and 24, Harts creek, Lake Ellesmere, was not monitored but chosen later for samples for host specialisation experiments

METHOD OF MONITORING THE PROGRESS OF THE DISEASE

At the first visit to all sites in October 1994 old leaves from the previous season were examined for the presence of telia, and new-season leaves were inspected for pycnia, aecia and uredia. The life-cycle stages were assessed as either nil (-), slight(+), moderate (++) or severe (+++). The occurrence of blackberry orange rust (*K. uredinis*) and insect infestation was also recorded. On subsequent visits the development of *P. violaceum* over the season, the timing of each stage, and the severity of the epidemic was recorded (Table 2). Samples of leaves showing stages of the life cycles of *P. violaceum* and *K. uredinis* were brought back to the laboratory and were examined microscopically, sectioned on a freezing microtome, and photographed.

SPORE TYPES OF *P. VIOLACEUM* OBSERVED IN THE FIELD

The life-cycle of *P. violaceum* on blackberry in Canterbury was found to be similar to that in Chile described by Oehrens (1974) and in Victoria, Australia (Bruzzese and Field 1984).

At the first visit in October telia of *P. violaceum*, that had overwintered on leaves remaining on the canes, were found at all sites except Gardeners Road, (#6, Table 2), the only site where *R. ulmifolius* occurred. Although this site was observed periodically for three years previously it had never been found to be infected with either *P. violaceum* or *K. uredinis* (Pay, unpublished). The plants remain uninfected up to November 1996.

Table 2 Date of first appearance of life-cycle stages of *Phragmidium violaceum* recorded at monthly observations. Severity of epidemic by December 1995 and presence of *Kuehneola uredinis*.

Site	Location	<i>Rubus spp.</i>	<i>Phragmidium violaceum</i>					Severity late Dec	<i>K. uredinis</i>
			Old Telia	Pycnia	Aecia	Uredia	New Telia		
1	Springston.	echinatus	E. Nov ++	E. Nov +	L. Nov	Nov ++	Jan +	4	-
2	Robinsons Rd	echinatus	E. Nov +	E. Nov +	Dec Sprayed	Jan +	Jan +	1	-
3	Ellesmere Rd	echinatus	E. Nov +	E. Nov +	E. Dec +	M. Dec ++	Jan ++	5	-
4	Trices Rd	cissburiensis	E. Nov ++	E. Nov ++	E. Dec +	M. Dec ++Mown	Jan +	3	-
5	Groynes	cissburiensis	E. Nov +	E. Nov +	E. Dec +	E. Dec +	Jan +	2	-
6	Gardners Rd	ulmifolius	-	-	-	-	-	0	-
7	Springs Rd	cissburiensis	L. Nov +	L. Nov +	L. Nov +	Jan +	Jan +	2	-
8	Springs Rd	cissburiensis	L. Nov +	L. Nov +	L. Nov +	Jan +	Jan +	2	-
9	Rakaia Huts	echinatus	E. Nov ++	E. Nov +	E. Nov +	M. Dec ++	Jan ++	5	-
10	Rakaia Huts	procerus	E. Nov +	-	-	M. Dec +	Jan +	2	-
11	Bealey Rd	unknown (like echin.)	E. Nov +	E. Nov +	-	Jan +	Feb +	2	Feb +
12	Bealey Rd"	echinatus	E. Nov +	E. Nov +	E. Nov +	E. Dec ++	M. Dec +	3-4	Feb +
13	Charing Cross	echinatus	E. Nov +	E. Nov +	E. Nov +	E. Dec +	M. Jan +	2	Feb +
14	Charing Cross	echinatus	E. Nov +	E. Nov +	E. Nov +	E. dec ++	M. Jan ++	5	Dec +
15	Darfield Nth	echinatus	E. Nov +	E. Dec +	-	E. dec +	M. Dec +	3	Nov-Mar ++
16	Old West Coast Rd	unknown (like ulm)	E. Nov +	E. Nov +	E. Nov +	E. Dec +	Feb +	2	Feb +
17	S.W. Oxford	laciniatus	E. Nov +	E. Nov +	E. Dec +	M.Dec +	Feb +	3	Nov-Mar ++
18	S.W. Oxford	echinatus	E. Nov +	E. Dec +	E. Dec +	M. Dec +	Feb +	3	Mar +
19	Nth Oxford	cissburiensis	E. Nov +	E. Nov ++	E. Nov +	M. Dec ++Sprayed	Feb +	3	-
20	Nth Oxford	echinatus	E. Nov +	E. Nov ++	E. Nov +	M. Dec +	Feb +	2	Feb +
21	Tram Rd	echinatus	E. Nov +	E. Nov +	E. Nov +	E. Dec +	Jan +	5	-
22	Tram Rd	echinatus	E. Nov +	E. Nov +	E. Nov +	M. Dec ++	Jan +	4	-

Severity scale: 0 = nil infection ,5 = severe infection

Key: E = Early, M = Mid, L = Late

+ = few, ++ = Moderate, +++ = Severe, - = not observed

Pycnia of *P. violaceum* were present at all the other sites on 9 November, as small, raised, orange blisters on the adaxial surface of new leaves in the lower canopy (Plate 1), often beneath a leaf occupied by telia (Plate 2) which could indicate basidiospores were transferred from above. Pycnia were often clustered and formed rings as a result of radial growth within the tissue. Pycnia were subcuticular and were often fused (Plate 3). Plants at sites 4, 12 and 19 had the most severe infection. Pycnia were present in small numbers at sites 4, 5, 11, 12, 14-17, 19-22 as late as 9 December.

Aecia (caemoid-with paraphyses but without peridium), were also found at many of the sites on 9 November, in conjunction with pycnia either on the abaxial surface below the pycnia (Plate 4) or in the centre of pycnial rings on the adaxial surface. Aecial rings were conspicuous but not raised or shiny as were the pycnia (Plate 5). Aecia were formed beneath the epidermis initially but broke through on maturity. Aeciospores were pale yellow, globose, echinulate, formed in chains with intercalary cells and there were slightly curved hyaline paraphyses (Plate 6). Only a few aeciospores were present on 9 December at most sites but they were plentiful at site 19, which also had a late production of pycnia.

Uredia were observed as early as 23 November at sites 1 and 9 : both these sites had severe disease levels the previous year. Uredia were produced on the abaxial surface of young leaves of primocanes and floracanes, and in severe infections uredia occurred also on sepals (Plate 7). Plants at sites 2, 6, 7 and 11 had no uredia by 6 December and these sites had very low disease severity during the late summer (Table 2). By mid December 9 sites(1, 3, 4, 9, 12, 14, 19, 21,



Plate 1. Pycnia of *Phragmidium violaceum* on blackberry leaves



Plate 2. Blackberry leaves from previous season, with characteristic red spots of *P. violaceum* infection, above new season's leaf with pycnia

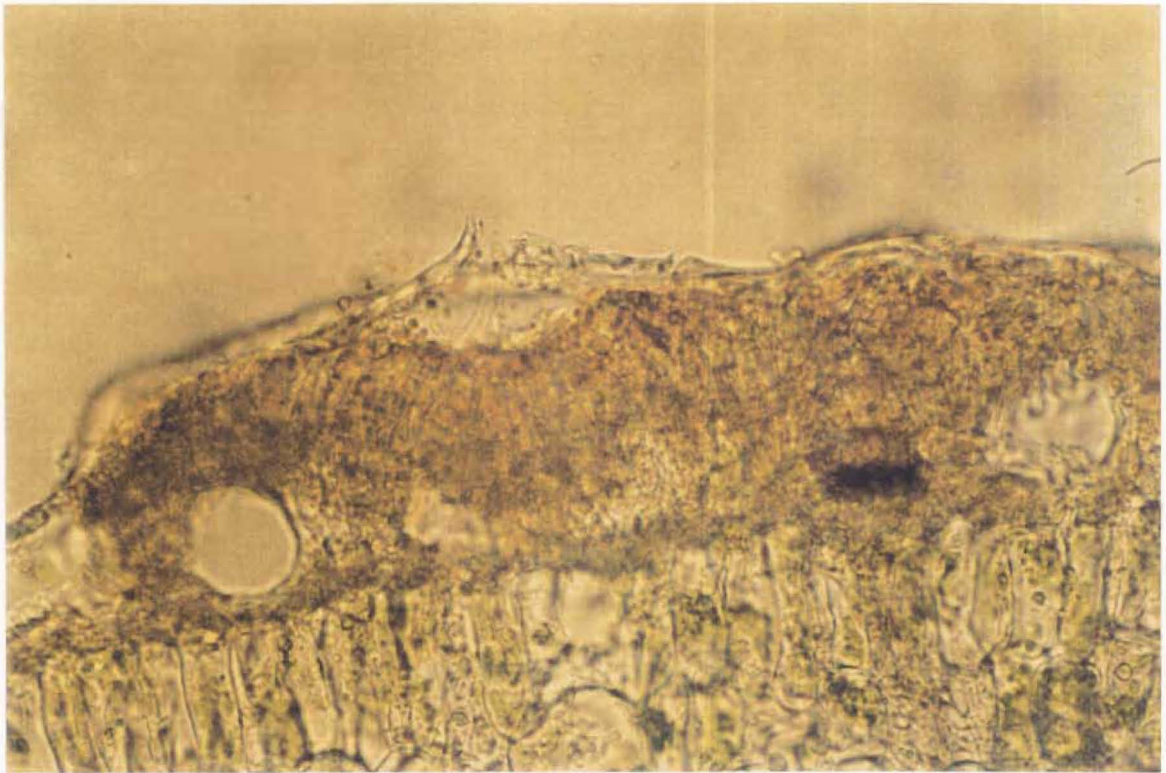


Plate 3. Subcuticular pycnium with broken epidermis (x 400)

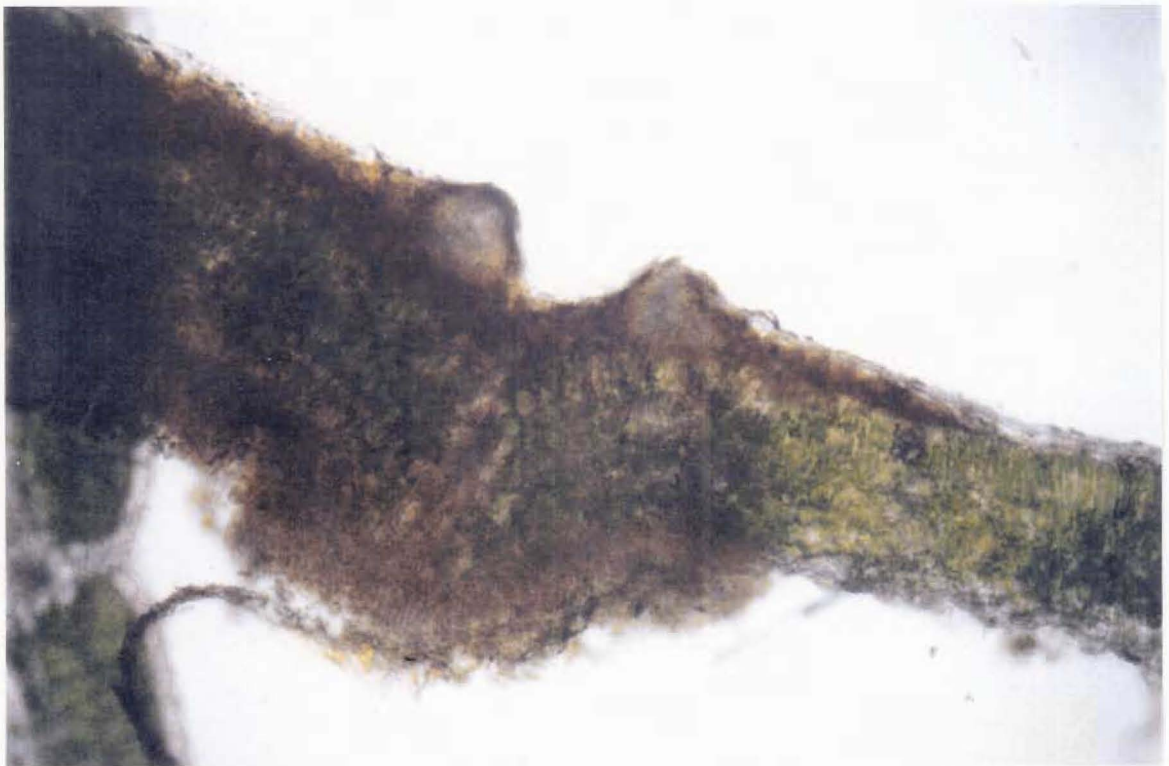


Plate 4 A pycnium on the top surface of the leaf with an aecium below.(x100)



Plate 5. Aecial pustules raised above shiny pycnia on top surface of leaf(x 40)

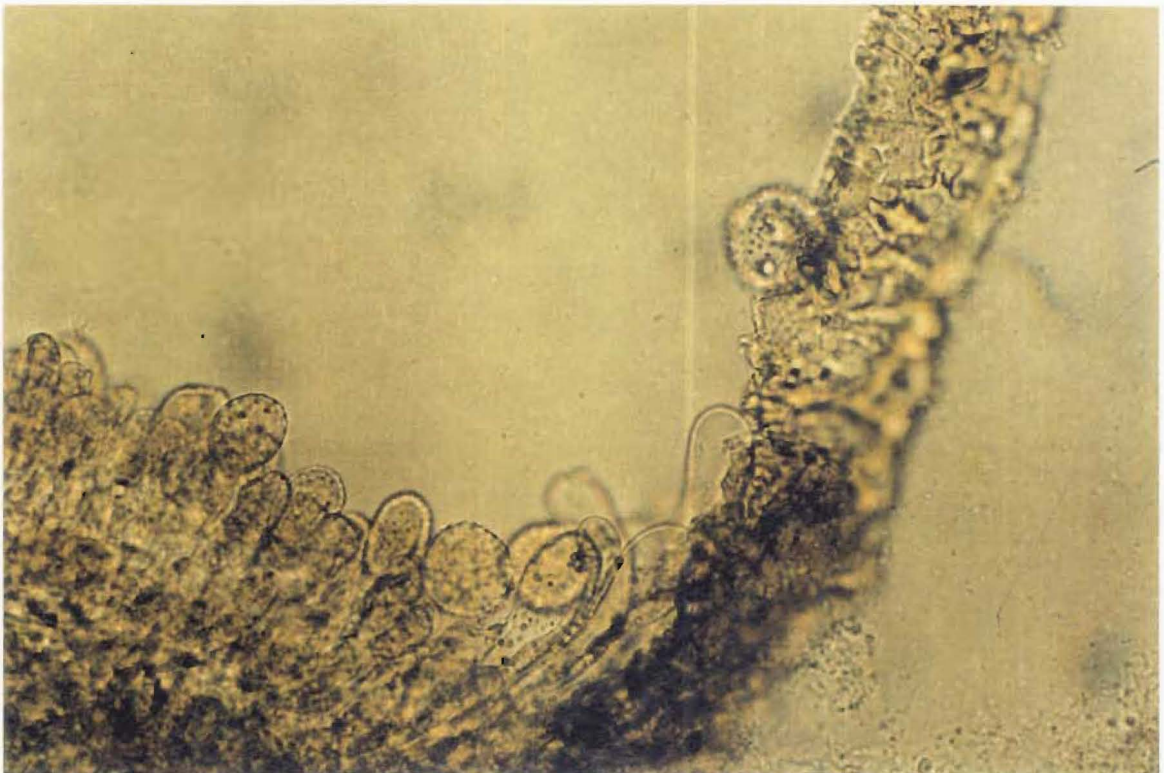


Plate 6. Aecial chains with paraphyses, with torn epidermis on right side (x 640)

22) had moderate (approx. 50% of the youngest leaves infected) to severe infection (ie. almost 100%), with many curled leaves which became necrotic and abscised prematurely. The tips of canes also died back at these sites (Plate 8).

Teliospores were produced amongst the urediospores 2-4 weeks after the first observation of uredia, often beneath or within the yellow pustules. Ultimately the pustules became black and sooty (Plate 9), as telia developed rapidly throughout the summer. By mid-January telia were forming at most sites. It was common to see telia only on the older leaves, mixed pustules of urediospores and teliospores on the middle canopy and uredia only on the upper canopy with young leaves (Plate 10). By the 25 March most plants were producing teliospores only, except at sites 1, 3, 9, 14, 21, and 22 where plants were severely affected by the rust and all young leaves were infected with urediospores. *R. echinatus* was the species most severely infected in any of the sites.

Plants at site 4 were cut back in December and site 19 was sprayed with a herbicide in February so disease development, which in November and December had been severe in both cases, was curtailed.

K. uredinis was present at 15 of the 22 sites by the 9 November and was severe at sites 15, 17 & 18. At all but these sites, the rust was at low incidence over the summer months but it increased in severity in February and March. Telia of *K. uredinis* were never observed at any of the monitored sites.



Plate 7. Severe infection of blackberry with *Phragmidium violaceum*. Leaves curled with yellow urediospore infection and characteristic red spots on tops of leaves



Plate 8. Die-back of blackberry canes following severe infection with *Phragmidium violaceum* the previous season.

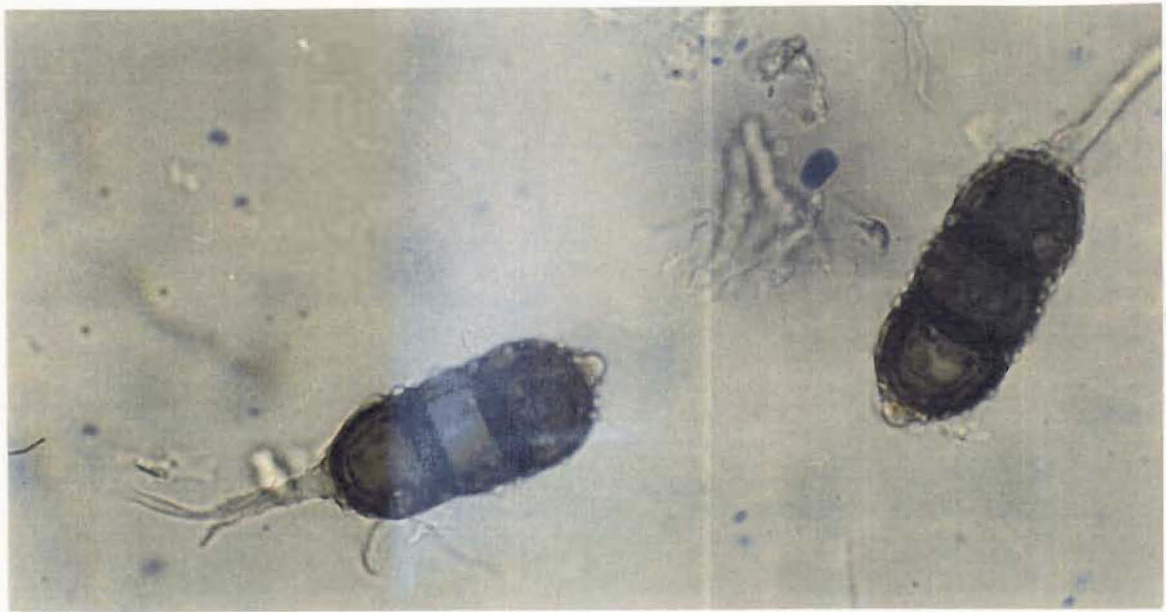


Plate 9. Teliospores of *Phragmidium violaceum* (x400)

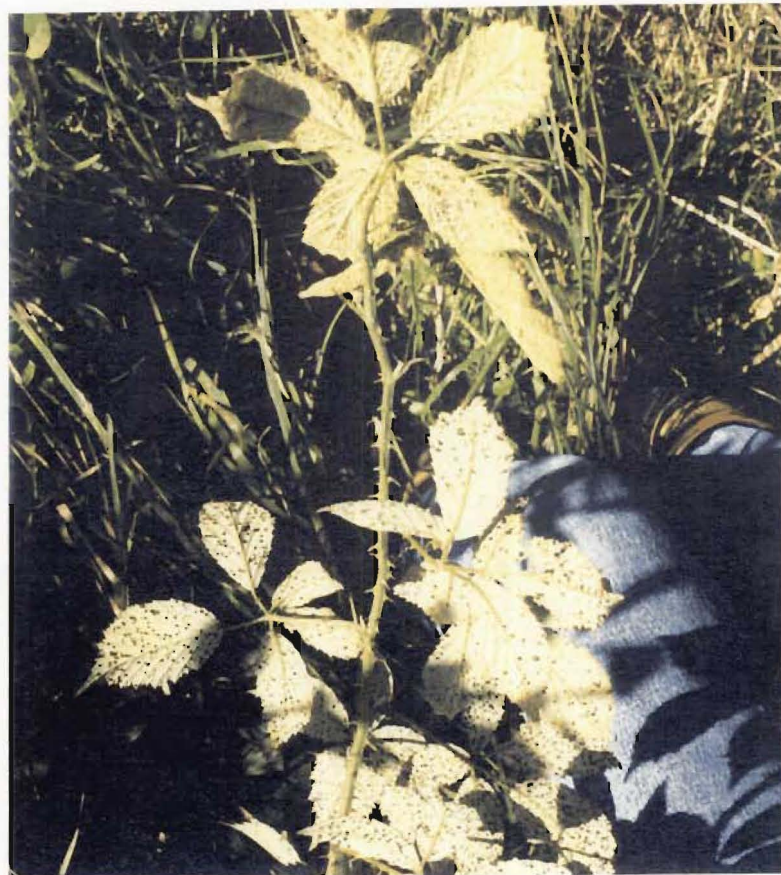


Plate 10. Teliospores on lower, older leaves and urediospores on the top, younger leaves.

Infestation of blackberry plants by unidentified insects caused some moderate damage to leaves at sites 6, 7, 11 and 20 and was severe only at site 13. Insect infestation appeared to be related to aspect of the site and was not host specific.

DISCUSSION

Teliospores of *P. violaceum*, that had been produced the previous season, were observed to overwinter in telia on leaves that remained on the canes. These spores may germinate in the following spring releasing basidiospores which began a new cycle of disease and they effectively formed a "bridge" between the seasons. The initial survey was not made until late October by which time many new leaves had appeared on the canes and these were already infected with both pycnia and aecia. To identify the onset of infection and the duration of the sexual stage of the life cycle of *P. violaceum* as it occurs in Canterbury it will be necessary to begin observations earlier in the spring.

Generally the plants growing in north-facing sites exposed to the sun and the hot, dry north-west winds, did not develop severe infections of *P. violaceum* but were more likely to be subject to insect infestations (Table 1). Plants in sites facing south or east often had more leaves which were subject to severe rust infections but little or no insect damage. For example, sites 13 and 14 were on opposite sides of the road, 20 meters apart and consisted of the same species (*R. echinatus*) but showed different amounts of infection by *P. violaceum*. Site 13 was facing north and was exposed to the sun and wind of a Canterbury summer while site 14 faced

south and was shaded and sheltered by large pine trees providing environmental conditions such as extended leaf wetness which has been shown to favour infection by some rusts (Stakman and Harrar 1957). Of the 13 sites that faced either south or east or were shaded by large trees, 7 were severely infected by the rust. Premature defoliation of infected leaves on which urediospores were being produced was common in those sites where the disease was moderate to severe and cane death of severely diseased plants occurred as early as 11 February. None of the hosts in the 9 north-facing sites developed severe epidemics, regardless of host.

The summer of 1994/95 was extremely dry and windy in Canterbury . Weather data recorded at the Lincoln Climate Station shows that there was lower than average rainfall and above average total wind between September 1994 and April 1995 (Appendix 1). These conditions may have played a part in preventing spore germination and therefore infection. The conditions necessary for germination of both urediospores and teliospores were subsequently examined in laboratory studies.

3. AN INVESTIGATION OF THE SUSCEPTIBILITY OF NEW ZEALAND SPECIES OF BLACKBERRY TO *P. VIOLACEUM*

3.1 INTRODUCTION

Bruzzese and Hasan (1986) tested the host specificity of all members of the sub-family Rosoideae of the Rosaceae that they considered to be of importance in Australasia with *P. violaceum*. Possible hosts tested included species of *R. fruticosus* naturalised in Australia, cultivated *Rubus* varieties, native Australasian *Rosaceae* never before exposed to *P. violaceum* (including five species of *Rubus*, subgenus *Lampobatus*, native to New Zealand), and many ornamental plants in the Rosoideae. Only five of the twenty members of *R. fruticosus* agg. that grow in New Zealand were included in those tests. *P. violaceum* had a limited host range on the genus *Rubus* and within the taxa that were infected by the rust, the response varied from immune to highly susceptible. However, these results cannot be transposed to the same species growing in New Zealand because, as discussed previously, the so-called species may be different genetically in each country (Bruzzese, pers. comm).

Therefore it was important to test the susceptibility of the species of blackberry growing in New Zealand to the strain of *P. violaceum* found in New Zealand. The first series of experiments was designed to determine the susceptibility of the species of blackberry growing in New Zealand to strains of *P. violaceum* collected in New Zealand. Two aspects of susceptibility were tested: host specialisation and leaf age.

Another factor that can affect the spread of a plant pathogen is the presence of races of the pathogen that can parasitise the host plants present in a particular area. Species of plant pathogenic fungi may be at least as variable in their genetic composition as the species of plants which they parasitise. Within a species of fungus there may be races that differ in their ability to parasitise a particular strain of the host. Isolates of a pathogenic fungus may be grouped into races on the basis of their ability to parasitise known strains of their host. This phenomenon has been particularly well illustrated with the wheat cultivar Ceres to *Puccinia graminis* f. sp. *tritici* (Stakman and Levine 1922), and for resistance of wheat cultivars in New Zealand to *Puccinia striiformis* f. sp. *tritici* (Cromie, 1990).

In this investigation races of *P. violaceum* were provisionally identified using the method based on Stakman and Harrar (1957).

While the species of host is known to be of importance in susceptibility of plants to disease, other factors also play a part. For example, it is known that plant pathogens may differ considerably in their ability to infect different organs or tissues of their hosts and in some cases the age of the plant part is the deciding factor (Stakman and Harrar 1957). For understanding and controlling plant diseases it is essential to understand these interactions. In experiments 1 and 2, leaves of different ages were used to test the claim made by Washington in 1987 that the resistance of blackberry leaves to *P. violaceum* increases with age.

3.2 METHOD

Leaves infected with *P. violaceum* were collected in November 1994 from eight sites around the South Island of New Zealand (Fig 5). They were stored in a refrigerator at 5°C until required. In the laboratory the percentage germination of urediospores from each collection site was determined using the following method. Spores were removed from the abaxial surface of the infected leaf with a wire loop, suspended in sterile distilled water and a 30 µl drop of spore suspension diluted to 10⁴ spores/ml was placed on 1cm² blocks of 1.5% water agar on glass slides. The slides were placed in 90mm Petri dishes lined with damp filter paper and incubated for 18h at 20°C in the dark (in earlier experiments germination was found to be inhibited by light). Only samples having a minimum of 30% germination were selected for further study. This percentage was mid-way between the 10% considered satisfactory by Bruzzese and Hasan (1984) and the 50% used by Washington (1987).

Increase of inoculum

Because only a limited number of spores were present on the leaf samples it was necessary to increase the quantity of inoculum. Young leaves of *R. laciniatus* were used throughout as earlier experiments had shown this host to be highly susceptible to *P. violaceum*.. Newly expanded leaves of *R. laciniatus* were collected from plants grown in the glasshouse and immediately placed in 90mm Petri dishes lined with damp filter paper abaxial side up, because stomata occur only on the abaxial side of blackberry leaves. Spore suspensions were prepared as described above and each leaf was inoculated with a single 30 µl drop of the spore suspension and incubated at 20⁰ C in the dark for 18hr to ensure germination. Then they were

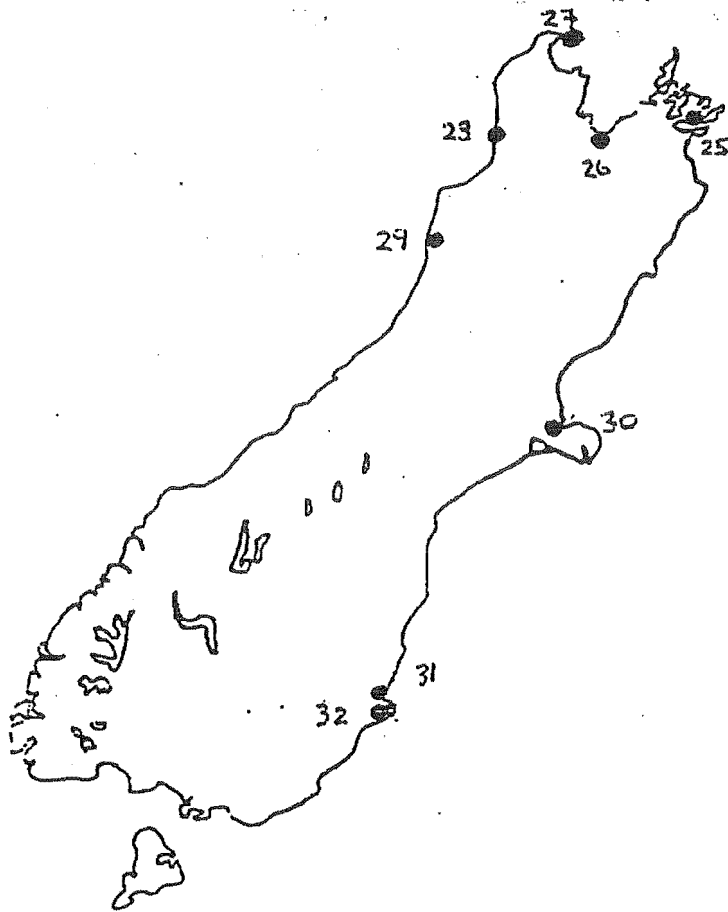


Fig. 5 Blackberry collection sites around the South Island, New Zealand.

- | | |
|---|---------------------------------|
| 25: Shakespeare Bay, Marlborough | 29: Cobden, West Land |
| 26: Havelock, Nelson District | 30: Halswell, Canterbury |
| 27: Farewell Spit, Golden Bay | 31: Waitati, Otago |
| 28: Seddonville, West Land | 32: Dunedin, Otago |

incubated for 28 days at the same temperature with a 12h photoperiod. Light levels between 220-260 $\mu\text{E m}^{-2}\text{s}^{-1}$ were supplied by daylight incandescent and fluorescent lights. Leaves were misted with water every second day to avoid desiccation. After 28 days uredia appeared at each of the inoculation sites. The urediospores were harvested and used as inoculum.

Propagation of plants for detached leaf studies

Plants which had previously been propagated from rooted stem apices of *R. cissburiensis*, *R. laciniatus*, *R. echinatus*, and *R. ulmifolius* were grown in 14cm diameter pots containing potting mix (80% composted bark, 20% washed crusher dust-particles up to 5mm diameter, 4kg dolomite/m³ and 8-9 month Osmocote Plus pellets [16:3.5:10 N:P:K @5kg/m³]). The plants were grown in a glasshouse under natural light and temperature range 15-30°C. The canes were cut back periodically to soil level when longer than 30-40cm and allowed to re-grow to produce new, young leaves for detached leaf inoculations experiments. Three leaf ages were used in inoculation tests: the youngest fully expanded leaf (1), the oldest leaves at the base of the cane (3), and leaves between these extremes (2). Detached leaves were used because, according to Bruzzese and Hasan (1986), they gave similar results to leaves on entire potted plants kept under the same conditions. Furthermore detached leaves were more convenient because they allowed greater replication and cross contamination between tests could be avoided more easily.

The methods used for inoculation and incubation were based on the work of Bruzzese and Hasan (1984) and the method of assessment was based on the suggestion by Washington (1987) who reported that “the percentage of leaflets with sporulating lesions appears to be a

useful parameter.....it is easily observed and recorded, and is apparently well correlated with susceptibility to infection.”

Using the results from experiments 1 and 2 combined, the method based on Stakman and Harrar (1957) was used to determine if physiological races of *P. violaceum* could be identified. In this method leaves of host plants which had been inoculated with urediospores of different races of the fungus are designated as susceptible (+) or resistant (-) depending on whether or not sporulation occurs. Variants of the pathogen are then assigned a number according to which hosts they infect.

Detached leaf studies

Experiment 1

Samples of the urediospores from each of 8 sites were tested for germination and all showed more than 30% germination. A single 30 µl drop of spore suspension containing 10^4 spores/ml was inoculated onto leaves of 3 ages from each of the 4 hosts. Each Petri dish contained two leaves of the same age taken from one of the hosts. Leaves were placed abaxial side up on damp filter paper in a 90mm Petri dish and lightly misted with water. Each leaf was inoculated as illustrated in Figure 6a. Each treatment was replicated four times. The Petri dishes were incubated in a controlled environment room at 18°C day: 10°C night (+/-2°C). They were kept in the dark for the first 18h to enhance spore germination, and subsequently with a 16h photoperiod. The leaves were turned adaxial side up after two days (to prevent condensation in the dishes dropping on the inoculated surfaces) and misted with water every second day to avoid desiccation. After 31 days the presence of uredia on each leaf was recorded.

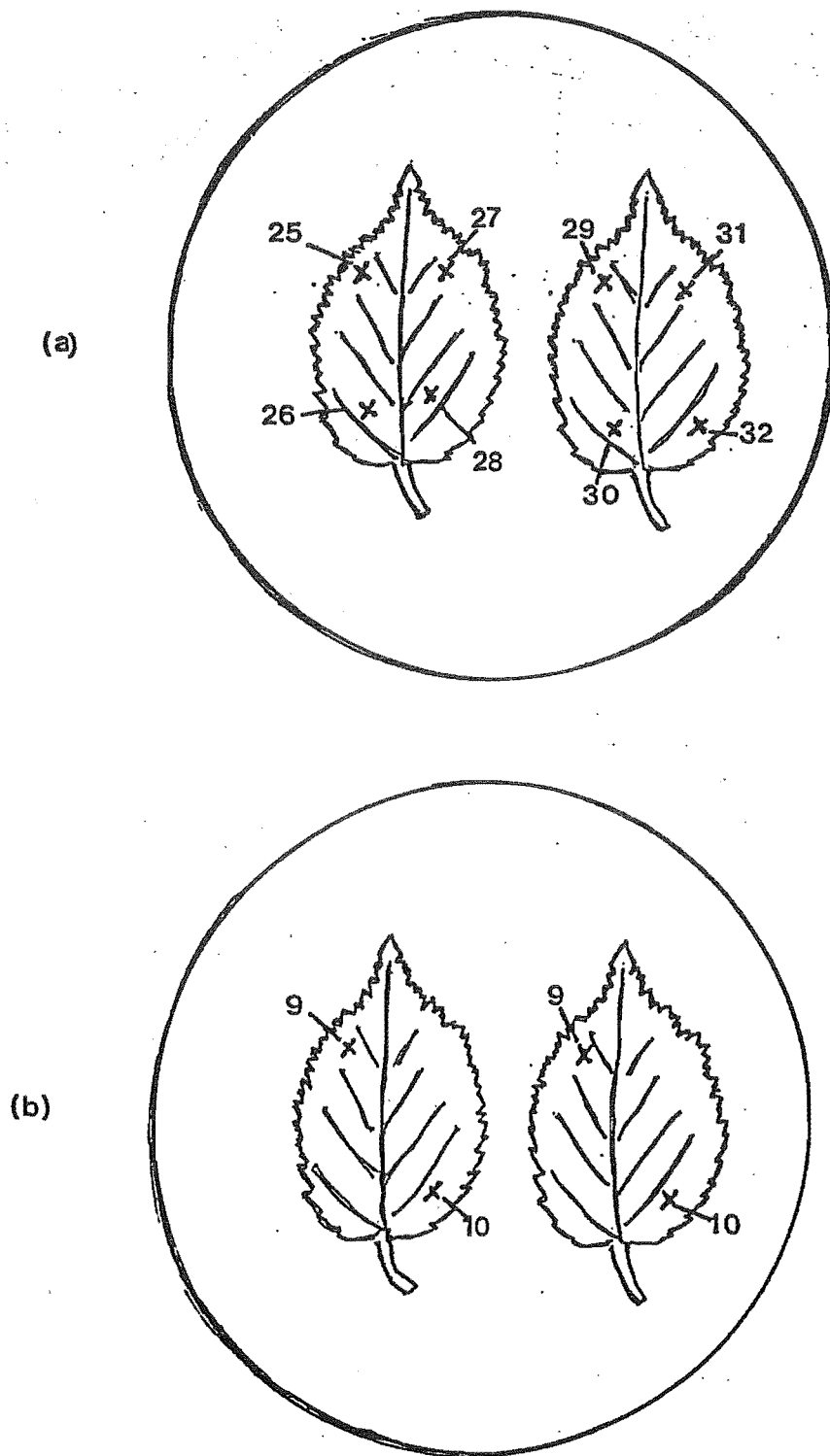


Fig. 6 Examples of leaf inoculation methods: (a) samples 25-32 from South Island collections; (b) samples from Canterbury sites.

Experiment 2

Early in January 1995 uredospore samples of *P. violaceum* were collected from infected plants at eight sites in Canterbury: sites #3, 4, 5, 9, 10, 17, 23, 24 (Table 1).

Spores were removed from infected leaves with a wire loop, suspended in distilled water and diluted to give a spore suspension of 10^4 spores/ml. Germination was checked as in experiment 1 before inoculation. Each host and leaf age was prepared and inoculated as in experiment 1 but in this experiment only two samples per leaf were used as illustrated in Fig 6b. Replication and incubation conditions were the same as in experiment 1. The presence of uredia on each leaf was recorded after 31 days.

3.3 RESULTS

Experiment 1

In this experiment leaves from the four hosts showed different responses to urediospores of *P. violaceum* collected from the eight South Island sources (Table 3). No infection occurred on *R. ulmifolius* from any sites other than 27 and 28 (Farewell Spit, at the north of the South Island, and Seddonville, West Land, respectively).. Three out of four young leaves of *R. ulmifolius* were infected by samples which had been collected from unidentified hosts. These samples infected all species. *R. laciniatus* was the most susceptible host

Table 3 Percentage of infected detached leaves,3 different ages, of 4 host species of *Rubus fruticosus* 31 days after inoculation with samples of *Phragmidium violaceum* collected from South Island sites (n=4).

Test Host:		<i>R.cissburiensis</i>			<i>R.laciniatus</i>			<i>R.echinatus</i>			<i>R.ulmifolius</i>			
Sites	Inoculum sources	Leaf Age ^a			Leaf Age			Leaf Age			Leaf Age			
		1	2	3	1	2	3	1	2	3	1	2	3	
25	Shakespeare	Unkn	0	0	0	0	0	0	0	0	0	0	0	0
26	Havelock	Unkn	0	0	0	0	0	0	0	0	0	0	0	0
27	Farewell Spit	Unkn	75	0	0	50	25	25	0	50	0	75	0	0
28	Seddonville	Unkn	0	100	25	50	75	50	25	0	0	75	0	0
29	Cobden	Unkn	0	0	0	0	25	0	0	0	0	0	0	0
30	Halswell	<i>Cissb</i>	0	0	0	25	0	0	0	0	0	0	0	0
31	Waitati	Unkn	0	0	25	25	0	0	25	0	0	0	0	0
32	Dunedin	Unkn	0	0	25	0	0	0	0	0	0	0	0	0

^a 1=youngest leaf at tip of cane 2=middle leaf 3=oldest leaf on cane

Table 4 Percentage of infected detached leaves,3 different ages, of 4 hosts of *Rubus fruticosus* 31 days after inoculation with samples of *Phragmidium violaceum* collected from local Canterbury sites (n=4)

Test Host:		<i>R.cissburiensis</i>			<i>R.laciniatus</i>			<i>R.echinatus</i>			<i>R.ulmifolius</i>			
# Sites	Inoculum source	Leaf Age ^a			Leaf Age			Leaf Age			Leaf Age			
		1	2	3	1	2	3	1	2	3	1	2	3	
9	Groynes	<i>Ciss</i>	25	0	0	25	25	0	25	0	0	0	0	0
10	Ellesmere	<i>Echin</i>	0	0	0	25	25	0	50	0	0	0	0	0
11	Trices Rd	<i>Ciss</i>	75	0	0	50	0	0	0	0	0	0	0	0
12	Rakaia	<i>Proc</i>	50	0	0	0	0	0	0	0	0	0	0	0
13	Harts Ck	<i>Proc</i>	50	0	0	25	0	0	75	0	0	0	0	0
14	Harts Ck	Unkn	0	0	0	0	0	0	25	0	0	0	0	0
15	Rakaia	<i>Proc</i>	25	0	0	100	25	25	50	25	0	0	0	0
16	Oxford	<i>Lacin</i>	0	25	25	100	25	50	0	25	0	0	0	0

^a Leaf Age 1=youngest leaf at tip of cane, 2=middle leaf, 3=oldest leaf on cane

Experiment 2

In this experiment leaves from the four hosts also reacted differently to urediospores collected from known hosts from the eight Canterbury sites (Table 4). As in experiment 1 *R. laciniatus* was the most susceptible host, 6 out of 8 of the samples causing infection on this host, sample 9 (ex *R. echinatus*) and sample 17 (ex *R. laciniatus*) infected all leaf ages. *R. echinatus* and *R. cissburiensis* had similar susceptibility to the pathogen and were less infected than *R. laciniatus*.

The total number of leaves infected was low in both experiments with many isolates failing to cause infection on some hosts. Statistical analysis of differences between host was therefore not possible and only general observations could be made. Eight physiological races were provisionally identified from the sixteen samples of *P. violaceum* tested (Table 5)

Effect of leaf age on susceptibility

Data from experiments 1 and 2 were combined and subjected to chi-square analysis for determination of the effect of leaf age on susceptibility. Records were omitted for those samples where no infection occurred on any of the three ages of leaves of the 4 replicates.

A leaf age category was recorded as infected if any of the four inoculations were successful.

There was a significant effect of age ($p < 0.001$) on the amount of infection. A chi-squared test for each pair-wise comparison of three ages indicate significant differences ($p < 0.05-0.001$).

Table 5. Provisional identification of physiological races of *P. violaceum* by combining the results of host specialisation experiments

Site	Location	Rubus spp.	TEST HOST SPECIES				Races ^a
			Cissb	Lacin	Echin	Ulmif	
3	Ellesmere	<i>R.echinatus</i>	-	+	+	-	6
4	Trices Road	<i>R.cissburiensis</i>	+	+	-	-	5
5	Groynes	<i>R.cissburiensis</i>	+	+	+	-	7
9	Rakaia	<i>R.echinatus</i>	+	+	+	-	7
10	Rakaia	<i>R.procerus</i>	+	-	-	-	2
17	Oxford	<i>R.laciniatus</i>	+	+	+	-	7
23	Harts Creek	<i>R.procerus</i>	+	+	+	-	7
24	Harts Creek	Unknown	-	-	+	-	4
25	Shakespeare Bay	Unknown	-	-	-	-	1
26	Havelock	Unknown	-	-	-	-	1
27	Farewell Spit	Unknown	+	+	+	+	8
28	Seddonville	Unknown	+	+	+	+	8
29	Cobden	Unknown	-	+	-	-	3
30	Halswell	<i>R.cissburiensis</i>	-	+	-	-	3
31	Waitati	Unknown	+	+	+	-	7
32	Dunedin	Unknown	+	-	-	-	2

Key: + uredia present
 - uredia absent

^a putative races only

Race 1=nil infections all hosts

Race 2=*R. cissburiensis* only

Race 3=*R. laciniatus* only

Race 4=*R. echinatus* only

Race 5=*R. cissburiensis* & *R. laciniatus*

Race 6=*R. laciniatus* & *R. echinatus*

Race 7=*R. laciniatus*, *R. echinatus* & *R. cissburiensis*

Race 8=all hosts infected

3.4 DISCUSSION

Effect of species on disease severity

The species of blackberry host had a marked effect on disease severity. *R. echinatus* was only moderately infected in detached leaf studies but was the species found to be most severely infected in the field (Table 2), especially at the uredial and telial stages. *R. echinatus* was the most common species observed in Canterbury and large numbers of teliospores overwintered on the canes forming a large inoculum pool for the next season.

Although *R. laciniatus* was the most susceptible in detached leaf inoculation experiments it was only moderately infected in the field. The reason for this may be that this species is rarely found in Canterbury and therefore there could be a lack of inoculum adapted to this host. In the field *R. cissburiensis* appeared to be very susceptible at the early pycnial stage at many sites monitored but only moderate levels of disease developed at the later uredial stage compared to *R. echinatus* (Table 2). This contrasted with the results of detached leaf studies where young leaves of *R. cissburiensis* were very susceptible to uredospore inoculation (Table 5). A differential response by a blackberry species to infection by different spore stages of *P. violaceum* in the field has also been noted in Chile (Oehrens 1977). Other workers have also reported this differential response in macrocyclic and autoecious species of rust fungi, eg. *Phragmidium rubi-idaei* DC. on *Rubus idaeus* L. (Anthony and Shattock 1985), *Puccinia asparagi* DC. on *Asparagus officinalis* L. (Blanchette *et al.*, 1982), and *Melampsora lini* (Ehrenb.) Lev. on *Linum usitatissimum* L. (Flor. 1959; Statler & Gold, 1980). These results highlight the need for disease assessments to be made throughout the growing season.

R. ulmifolius was completely resistant both in the field and in detached leaf studies to urediospores collected from Canterbury. This resistance of *R. ulmifolius* was consistent with results from Chile but at odds with those from Victoria (Oehrens 1974, Bruzzese and Hasan 1984). This apparent contradiction may be attributable to a different genotype of this host species in Australia compared to Chile and New Zealand. However, *R. ulmifolius* was susceptible to samples of the rust collected from the West Coast of the South Island which suggests that either the strain F15 has established on the Coast or another more virulent strain has evolved there which was not included in the samples collected in Canterbury.

Plants at site 16 (Old West Coast) which could not be identified but were similar to *R. ulmifolius*, had characteristic red/purple blotches indicative of *P. violaceum* infection on the adaxial surface of the leaves. However, no sporulation was observed, indicating this plant was probably *R. ulmifolius* or a hybrid of *R. ulmifolius*. *R. ulmifolius* is the only member of the aggregate known to form hybrids and at least one (*R. ulmifolius* x *R. cissburiensis*) has been recognised in the NZ Flora. *R. ulmifolius* hybrids gave mixed results in the Australian specificity tests ranging from very susceptible to resistant (Bruzzese and Hasan 1986). In Chile, Oehrens (1977) found that there were marked differences in field and glasshouse responses to uredospore inoculations of *R. ulmifolius*. He reported that there was very little disease to be found on this species unless it was growing in close proximity to the more susceptible species. He also noted that there was a change in the size of the pustules over time, possibly showing adaptation of the rust within 2-3 years of its being introduced. He suggested this adaptation was similar to "slow rusting" in cereals, which is often regarded as a form of quantitative or horizontal resistance (Van der Plank, 1975)

When the results from experiment 1 and 2 were combined to show host range, inoculum derived from a particular host always reinfected the same host, sample 30(Table 5) was the only exception. Spores originating from *R. echinatus* infected only one leaf of *R. cissburiensis* (age 1), infected most of the leaves of *R. laciniatus*, regardless of age , and only young leaves of *R. echinatus*, the original host from which the spores were taken. Conversely spores from *R. cissburiensis* infected only one leaf of *R. echinatus* (age 1), but 50% of young leaves of both *R. laciniatus* and *R. cissburiensis*

While the author can be confident about the positive inoculations confirmation is required for those inoculations that were negative, i.e. no infection in any of the leaf ages or replicates.

While differences in disease development in the field may be explained by environmental parameters the results of these detached leaf studies go some way to support the Australian results (Bruzzese and Hasan, 1984) that susceptibility varied among members of *R. fruticosus* agg. to *P. violaceum*. Provisional identification of eight races of *P. violaceum* suggests that isolate pathogenicity may vary in populations of the rust in the South Island of New Zealand.

However, for conclusive evidence of host specialisation and physiological races these experiments would need to be repeated using inoculum arising from single spore isolations.

This would eliminate any variability of inoculum sources and ages of populations which were some of the limiting factors in the above experiments.

4. EFFECT OF TEMPERATURE AND SAMPLE ON UREDIOSPORE GERMINATION

4.1 INTRODUCTION

The germination of fungal spores is affected by a number of physical factors. These include light, temperature and moisture, all of which can inhibit or stimulate germination. Some fungi require free water to be present before germination can take place while others, like powdery mildews, are inhibited. Temperature is also a determining factor in the abundance, rate and sometimes type of germination (Stakman and Harrar, 1957). The minimum, maximum and optimum temperatures not only differ between species but may also in some cases differ between isolates of the same species e.g. *Puccinia graminis tritici* (Prabhu and Wallin, 1971). Those fungi, or strains of fungi, that can germinate quickly will have a biological advantage in a competitive infection situation or if spores have limited viability (Stakman and Harrar, 1957).

Although the conditions that favour the germination of urediospores of *P. violaceum* have not been fully studied, Washington (1991) has suggested that temperatures of 18-21°C and the presence of free moisture on leaves for up to 18hrs are required for maximum infection.

Similar temperatures but a shorter continuous moisture period is required for infection by *Phragmidium mucronatum* and *P. tuberculatum*, the cause of rose rust in New Zealand (Horst 1983).

A series of experiments were undertaken to determine the effect of temperature on the germination of urediospores of three samples of *P. violaceum*.

4.2 METHOD AND MATERIALS

To investigate the temperature range and time required for germination, urediospores of *P. violaceum* were collected on leaves of blackberry infected with the fungus, from sites 1, 9, 10 (Table 1) on 2nd March 1995. These sites were chosen to give a range of host species showing differing responses to the rust.

Spores were removed from the leaves with a wire loop and suspended in 5ml of distilled water with 0.01% Tween 80 [conc 1×10^3 spores/ml]. Tween 80 was added to ensure the spores were not clumped together so that they could be counted easily and did not have an inhibitory effect on each other. Preliminary germination trials, where the spore suspension was placed on glass slides, proved unsatisfactory because humidity was not controlled and at least half the samples dried out before the experiment finished. Another method tried was by placing the spore suspension in a cavity slide but the most reliable method was the following: three 1cm^2 blocks of 1.5% water agar were placed side by side on a sterilised glass microscope slide and one drop of spore suspension from each of the three sites was placed on separate agar blocks. Each treatment was replicated 4 times. The slides were placed in sterile 90mm Petri dishes which had been lined with damp filter paper and incubated in the dark at 5, 10, 15, 20, 25 and 30° C. After 3, 5, 7, 9, 11, and 24 hours the agar blocks were examined at x10 magnification and the number of spores germinated were recorded from a random selection of 50 urediospores. An urediospore was considered to have germinated when the germ tube was

longer than the width of the spore. The data were analysed by ANOVA and for each sample the differences between temperatures and periods of incubation were assessed by an LSD test.

4.3 RESULTS

There were significant main effects of temperature ($P < 0.001$), sample ($P = 0.097$) and time ($P < 0.001$) on germination of urediospores (Appendix II). There were significant interactions between sample and temperature ($P = 0.001$); between temperature and time ($P < 0.001$) and between sample and time ($P = 0.02$). All samples germinated at 10-25°C (Fig 7) with maximum germination occurring within 7-10h (Fig.8). Germination was especially rapid (3h) at 20°C (Figs.8) and in sample 2 there was a significant difference in germination at 20°C compared to 15 and 25°C.

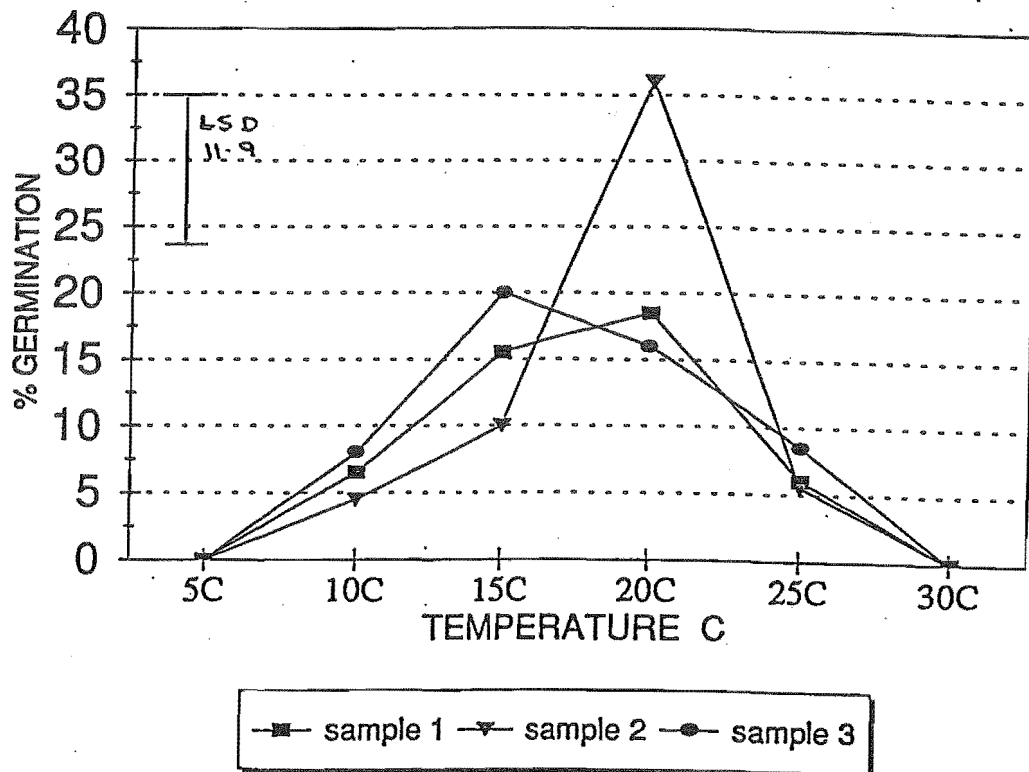


Fig.7 Percentage germination of three samples of urediospores after 24 hours incubation at 6 temperatures.

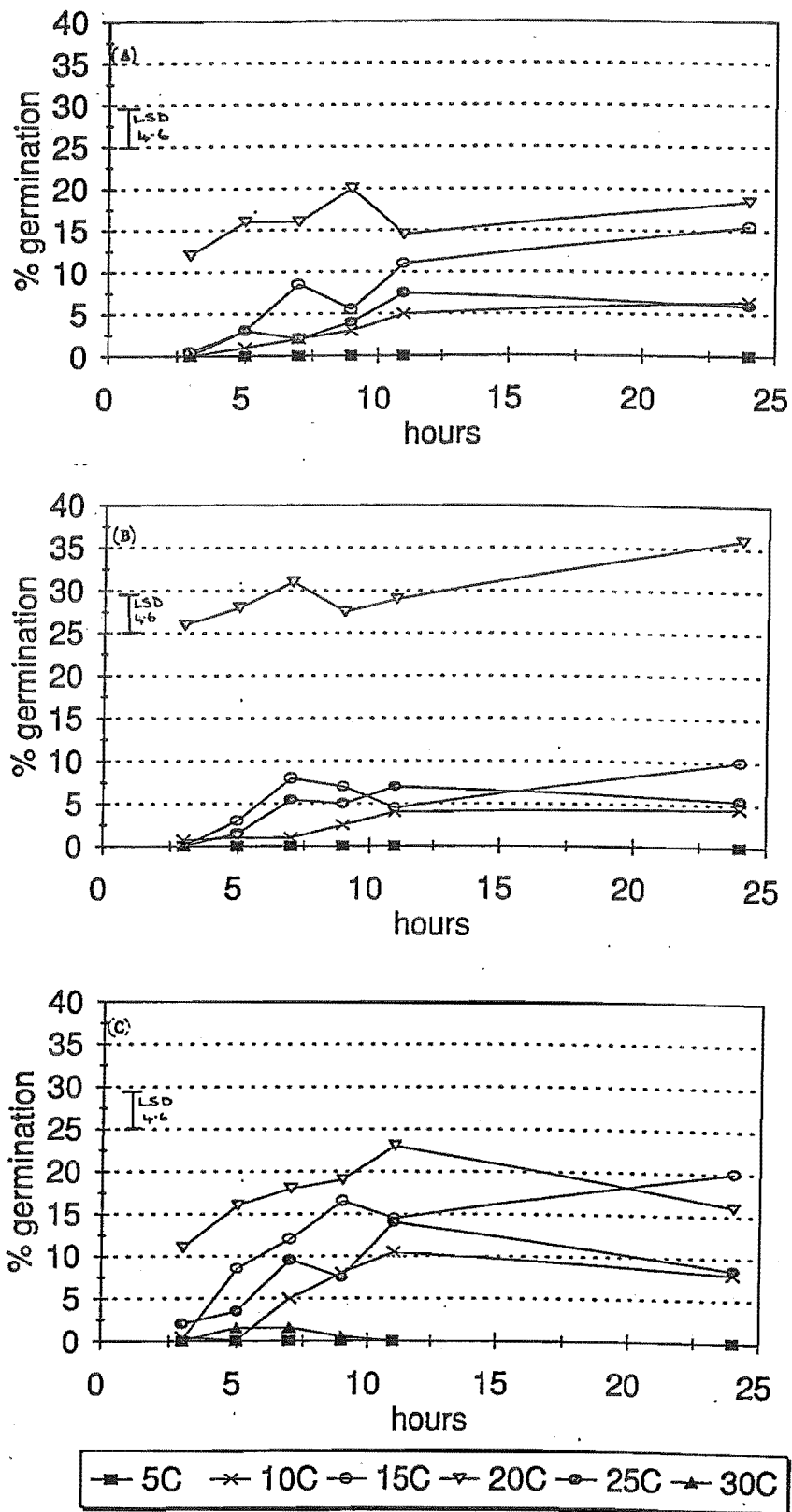


Fig. 8 The effect of temperature on germination of sample 1 (A, ex *R. echinatus*, Rakaia), sample 2 (B, ex *R. procerus*, Rakaia) and sample 3 (C, ex *R. echinatis*, Springston)

4.4 DISCUSSION

The percentage germination of the urediospores in this experiment reflected the normal range observed by the author in previously tested field samples of mixed populations. Samples taken from the field and different sample sites may have a range of ages but this range is usually very narrow (Dinoor, person. com.). The older spores are dislodged by wind movement daily, and therefore there is usually no accumulation of spores in pustules over several days in the field. The populations of the three samples in this experiment were of mixed ages and sources and therefore the results are comparative only and can not be attributed only to either differences in isolate type or to spore source.

As germination of *P. violaceum* urediospores is inhibited by daylight, and free moisture is required (Roelfs and Bushnell 1985), temperature and leaf wetness during the night are crucial to disease development of *P. violaceum*. In summer Canterbury has an average of seven hours darkness and night temperatures average between 12-15°C, according to the data collected at the Lincoln Climate Station. Since urediospores are produced in summer when wet periods suitable for infection to occur are not frequent the ability of spores to germinate quickly at 12-15°C may give a race or strain of the rust having this ability an advantage in establishing infection. Samples 1 and 3 reached their maximum germination within 7hr at the temperatures experienced in Canterbury summers and plants at both collection sites were severely infected. Sample 2 would be expected to infect the same as samples 1 and 3 in most ambient conditions, but would be at an advantage at the higher temperatures (i.e. 20°C).

5. TELIOSPORE GERMINATION AND HOST INFECTION

5.1 INTRODUCTION

It has been shown that the teliospores of many rusts germinate only after a period of dormancy (Mengden 1983). Dormancy appears to be an inherent property of the spore, requiring an activating process to break it. In nature, dormancy is often an asset as it allows the rust to overwinter and survive periods of weather unfavourable for growth or when the host has no leaves. Conditions required for many species of rust to break teliospore dormancy, and to initiate germination, have been investigated by Anikster (1986) under experimental conditions. He successfully induced germination of 4000 teliospore samples isolated from 59 hosts and representing 27 rust species by floating the spores on distilled or tap water at 16-18°C for 24-144h.

Where biological control of a host is desired an understanding of the factors relating to initiation of germination of teliospores could be of great importance. For example, having the ability to inoculate plants with germinating teliospores when temperatures are low and before the usual sexual cycle occurs might allow the earlier start of an epidemic than would be normal.

As no reports could be found in the literature concerning the germination of *P. violaceum* teliospores the following experiments were undertaken.

5.2 GERMINATION OF TELIOSPORES *IN VITRO*

Method

Teliospores which had overwintered from the previous season were collected from Site 1 (Table 2) ex *R. echinatus*, on 28th October 1994 to determine if teliospores could be germinated *in vitro* and if so what the optimum temperature for germination was and to observe basidiospore formation.. The spores were held in the dark floating on tap water at 5°C for 2 weeks as recommended by Anikster (1986) and modified by Dinoor (pers. comm.) The spores were then placed on 1cm² squares of 1.5% water agar on sterilised glass slides placed in sterile 90mm. Petri dishes lined with damp filter paper. The spores were incubated in the dark at 5, 10, 15, 20, 25 and 30°C. After 45 hours the spores were examined microscopically (x10 magnification) to assess germination. A teliospore was considered to have germinated when at least one promycelium had been formed and was as long as the width of the teliospore.

Results

Germination occurred after 45h at all temperatures between 5 and 25°C. The optimum temperature was 10°C. Basidiospore germination was also observed on the slides at 5-15°C. Promycelia were produced in the dark and orange pigment migrated along the promycelia as they extended. Three transverse septa were clearly visible and the pigment migrated through sterigmata into the 4 developing basidiospores (Plate 11). On 2 occasions germ tubes were observed to fuse to form dikaryotic hyphae (Plate 12)

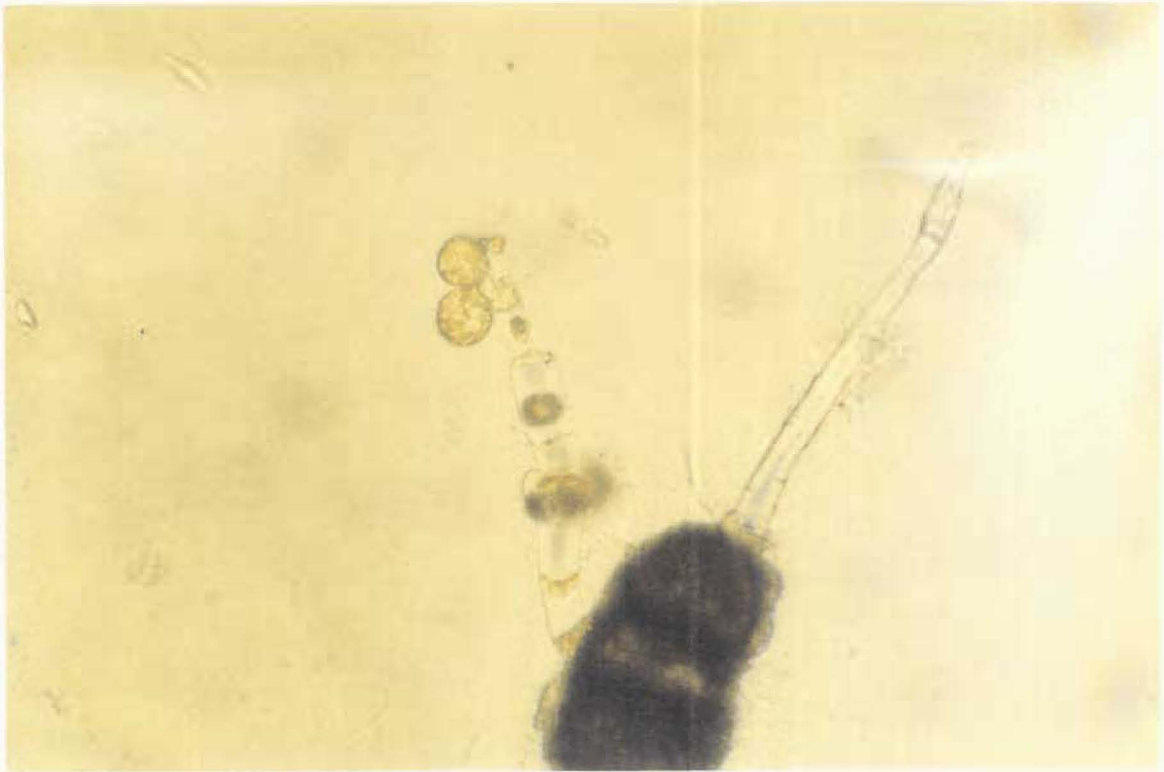


Plate 11. Germinating teliospore with transverse septa in promycelium, sterigmata and developing basidiospores (x400)

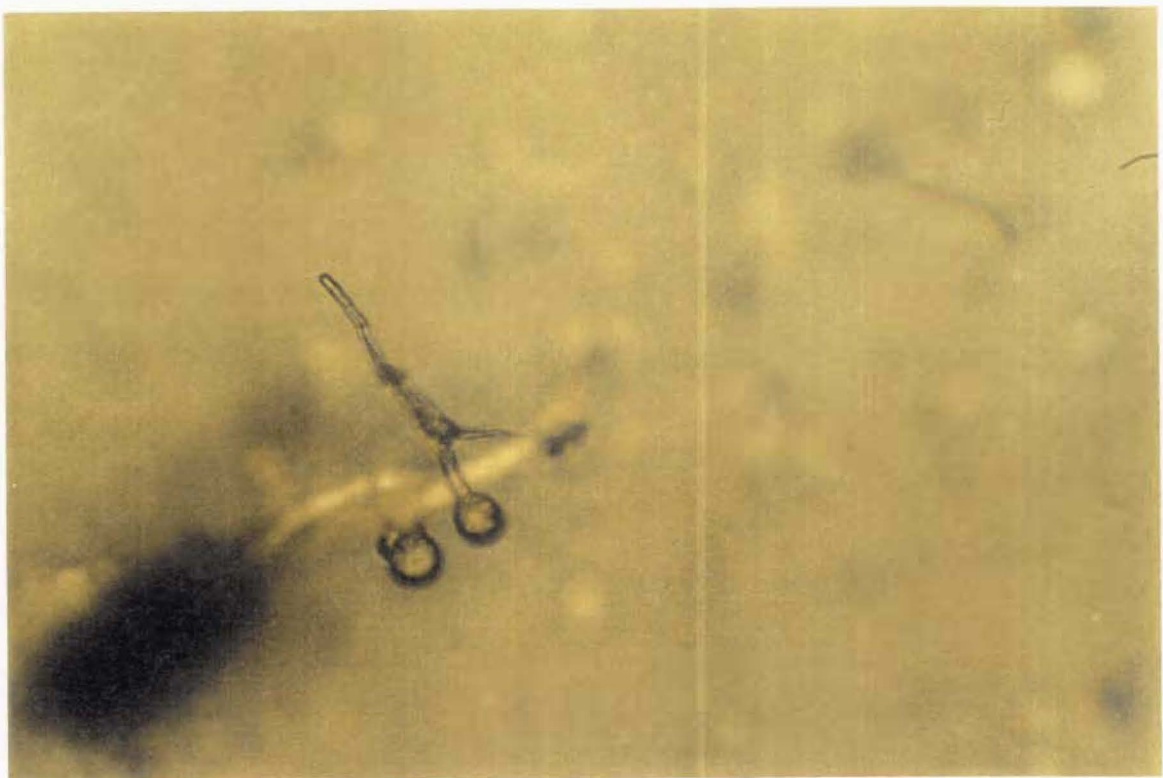


Plate 12. Dikaryotic hyphae formed from two germinating basidiospores (x400)

5.3 TELIOSPORE INOCULATION OF WHOLE PLANTS

Method

Having established that some of the teliospores were viable and that they would germinate after cold-water treatment, an attempt was made to induce the disease in potted plants by inoculation with teliospores.

Two glasshouse grown plants of each host (*R. cissburiensis*, *R. laciniatus*, *R. echinatus*, *R. ulmifolius*), which had at least four fully expanded leaves per cane, were inoculated with the cold-treated *P. violaceum* teliospores. The second youngest fully expanded leaf on each cane was tagged then misted with tap water. Two 20µl drops of teliospore suspension were applied to the adaxial surface of the leaf. Each plant was enclosed in a large, clear plastic bag and the plants were grown in a controlled temperature room. One plant of each host was incubated at 18°C day: 10°C night and the other at 13°C day: 5°C night (+/-2°C) both with a 16h photoperiod. After 48h the plastic bags were removed and the plants were grown in the controlled temperature rooms for a further four weeks. A visual assessment of infection was made every 4 days.

Results

No infection occurred on any plant at any temperature. It would appear that the conditions were unsuitable for either germination of the spores or penetration of the host to occur. Host resistance could not have been a determining factor as one of the species, *R. echinatus*, was known to be highly susceptible in the field and in detached leaf studies.

5.4 TELIOSPORE INOCULATION OF DETACHED LEAVES

Method

Detached leaves from *R. cissburiensis*, *R. laciniatus*, *R. echinatus*, *R. ulmifolius* were inoculated with spore suspensions of cold water-treated teliospores collected from each of the field sites (Table 2). The detached leaves of 3 ages (youngest on cane, middle leaf and oldest on cane) were placed on moist filter paper in 90mm Petri dishes as before. To determine which side of the leaf penetration occurred most readily a single 30 μ l drop of spore suspension was applied to both surfaces of leaves on different samples. The leaves were incubated in the dark at 10°C for 48hr to enhance germination then at 5, 10, 15, 20, 25 and 30°C with a 16hr photoperiod. A visual assessment of infection was made every 4 days for four weeks.

Results

As no infection was observed on any of the leaves the conclusion drawn from the whole plant experiment was reinforced. While it was possible to show at the end of the experiment that many of the cells of the teliospores were still viable, by staining with lactic blue stain (Dinoor, pers. comm.), it is uncertain why no infection occurred. It is clear that further investigation is needed to elucidate this problem.

5.5 DISCUSSION

The optimum temperatures for germination of teliospores and urediospores *in vitro* differed by about 10°C, reflecting the difference in ambient temperatures at the time of infection by each of

these spore stages. This difference in temperature optima for different stages of the rust has also been recorded for *Phragmidium rubi-idaei* (DC.) Karst. (Anthony *et al* 1985) and for *Cronartium ribicola* J.C. Fischer (Doran, 1919). The failure to induce infection with *P. violaceum* teliospores on either whole plants or *in vitro* may have been due to a number of factors which were not investigated. For example, teliospores are important means of perennation for many rust species and tests have shown that many lose germinability outdoors after exposure to direct sunlight, as well as in shaded sites (Anikster 1986). Anthony *et al* (1985) successfully infected 3-month old plants of raspberry with *P. rubi-idaei* by rubbing both the abaxial and adaxial leaf surfaces with teliospore-bearing leaflets that had overwintered on the canes. Spermogonia had appeared 7 days after inoculation only on inoculated surfaces and were more numerous on the abaxial surfaces. However, they found that the wavelength emitted by the lights in the incubators was crucial to the germination of the teliospores and the development of basidiospores. It has been reported that in some rust species not all teliospores germinate in the same season (Dinoor, person. com.) extending the “bridge of infection” over several seasons. If teliospores could be induced to germinate readily and uniformly then there is a possibility of enhancing *P. violaceum* as a biological control agent by inoculating with teliospores early in the season while temperatures are still low and applying urediospores when temperatures are higher.

6. CONCLUSIONS

Matters for further investigation

The results reported here come from a limited investigation of a disease first observed by the author in 1990 and found to be new to New Zealand. Among the more interesting questions that need to be answered are

- the need to clarify the confused state of the taxonomy of the genus *Rubus* especially in relation to the species present in New Zealand and Australia
- resistance to *P. violaceum* among species of *Rubus*
- factors effecting spore germination and host infection by *P. violaceum*

The ability to identify members of the *Rubus fruticosus* agg. positively is essential before more studies can be undertaken. The use of morphological characters is of limited effectiveness because of the extensive phenotypic plasticity and hybridisation which occurs between species. Minisatellite DNA “fingerprinting” has been successfully used to distinguish apple cultivars (Nybom and Schaal 1990), and *Rubus* and *Ribes* cultivars (Nybom, *et al.*, 1989, Nybom and Hall 1990,). This method could be used to study the genotype diversity and distribution of natural populations of wild blackberry, and their relatedness, which might explain the resistance of certain species. For further understanding of the relationship between host and pathogen it will also be necessary to have a simple and dependable method of identifying strains of the fungus. The use of RAPD’s and PCR “fingerprinting” techniques could probably distinguish the races of the rust which are present in New Zealand and ascertain if physiologically different races have evolved here.

P. violaceum has been present on wild blackberry in New Zealand for at least six years.

Regular observations on sites near Lincoln University indicate that the rust is causing substantial damage to localised thickets of *R. echinatus* but little damage to other species of *R. fruticosus* agg. Whether the present strains of rust will continue to spread and inoculum levels increase sufficiently to challenge and even control these other species is a matter for speculation. Australian scientists working on this disease have endeavoured to enhance the control of blackberry in their country by the release of a more virulent strain of the rust (Bruzzeze 1992).

While identification of strains present in New Zealand has begun in this report further work comparing strains both from New Zealand and Australia and also from Chile and Europe could well yield isolates that would be more virulent towards species of *R. fruticosus* not at present being severely affected by *P. violaceum* in New Zealand. While *P. violaceum* cannot be expected to eliminate wild blackberry, severe infections over a number of years can have a marked effect on its vigour and competitiveness. Infected plants have been observed to stop “tip-rooting”, reducing the ability of the plant to spread and making blackberry a much more manageable weed than at present.

From the field and laboratory observations it is clear that *P. violaceum* has potential as a biological control agent for at least one or two species of the presently difficult to control blackberry weed in New Zealand. In order to exploit the pathogenic properties of *P. violaceum* as a control agent it will be necessary to explore further the host range of the strains already present here and compare them with strains from overseas that may show even greater virulence. More research is also required into the ecology of the fungus to determine optimum conditions for its spread to ensure that the maximum infection occurs in the early spring and

season of the blackberry plant. If it was decided to utilise *P. violaceum* and enhance the control of this weed then the recommendations from this study are that:

- further studies are undertaken to determine the host range of the strains of the rust presently in New Zealand,
- to determine if a more pathogenic strain of the rust (specific to our host species) be found and released and,
- to continue investigating the possibility of early season inoculation with teliospores to enhance infection.

ACKNOWLEDGEMENTS

I would like to thank the following people for making this project and my Masterate possible:

Prof. Paul Mulcock, Emeritus Professor Lincoln University, who has been my mentor and friend for the last nine years and proof read the endless essays and versions of this dissertation.

Prof. Roy Gaunt, Plant Pathology, Lincoln University, for taking over as my supervisor after Dr Close's retirement and suggesting a summer project instead of more essays.

Prof. Amos Dinoor, Hebrew University of Jerusalem, Israel, for taking such an interest in my project and the many helpful comments on the writing up stage via the internet.

Dr. Alison Stewart, Lincoln University, my line manager and assistant supervisor, for allowing me the time from my daily duties to complete this project.

Mrs. Marlene Jaspers, Lincoln University, for the friendship and encouragement through all the highs and lows and for leading by her example of hard work and dedication.

Finally, to my long-suffering partner, Ken, who spent many cold evenings working in the garage while I studied in the warmth of the lounge. Welcome back into the warm.

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APPENDIX I

Monthly summary data from the Lincoln Climate Station

Month	Sunshine (h)	Rainfall (mm)	Mean Temp °C		Comments
			Max	Min	
Oct '94	216	19.0	14.9	6.9	Drier than average
Nov '94	270	22.7	17.2	6.9	Warmer, windier than average
Dec '94	259	24.7	22.9	11.7	Drier, sunnier than average
Jan '95	239.2	32.2	21.8	11.4	Lower than average temps.
Feb '95	163.5	25.3	21.3	12.5	Northerlies, drier
Mar '95	223	32.6	21.7	9.9	Drier than average
Apr '95	114	30.9	16.2	9.2	Nor-easterlies daily

APPENDIX II

Univariate and multivariate repeated measures analysis comparing the effect of temperature and time on germination of uredospores.

SOURCE OF VARIATION	DF	SS	MS	F	P
Between Samples					
Temperature	5	21943.993	4388.799	238.236	0.000
Error	18	331.597	18.422		
Within samples					
Sample	2	366.792	183.396	2.488	0.097
Sample x temperature	10	3182.236	318.224	4.317	0.001
Error	36	2653.528	73.709		
Time	5	1634.993	326.999	36.620	0.000
Time x temperature	25	1369.410	54.776	6.134	0.000
Error	90	803.653	8.929		
Sample x time	10	237.236	23.724	2.194	0.020
Sample x time x temperature	50	589.569	11.791	1.091	0.334
Error	180	1945.972	10.811		

Note: Split plot design for sample and time