STUDIES OF
MARSSONINA AND *DREPANOPEZIZA*
SPECIES PATHOGENIC TO POPLARS

A thesis presented in partial fulfilment of
the requirements for the degree of Doctor
of Philosophy at Massey University

ADRIAN SPIERS

1981

Seedling of *Populus trichocarpa* (2 years old) ex California attacked by *Marssonina brunnea*. Note that as typically observed with this disease defoliation of the lower foliage has occurred.

ACKNOWLEDGEMENTS

I am indebted to Dr. H. T. Wenham for acting as supervisor.

I also wish to thank:

Professor K. S. Milne.

Mr C.W.S. van Kraayenoord.

Mr D. H. Hopcroft and Mr R. Bennett of the Electron Microscope Laboratory, D.S.I.R. for their invaluable assistance with transmission and scanning electron microscope studies and for printing the numerous micrographs.

Mr H. F. Neilson for technical assistance and valuable discussions.

Mr L. Maiden and Staff of the Central Photographic Unit, Massey University for photographs of disease symptoms and photographic processing respectively.

Mr N. A. Trustrum and Ms M. F. Christensen-Widt of the Aokautere Science Centre for shooting and printing the frontispiece respectively.

Staff of the Draughting Office, Ministry of Works and Development, Palmerston North for preparation of Figures 7, 11, 12, 22, 23, 26, 31, 49, 103, 104 and 115.

Ms J. A. Nielsen for typing the thesis.

Mr J. C. Chandler for Latin translations.

The Ministry of Works and Development, New Zealand for financing this study.

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ABSTRACT

A taxonomic study was conducted of *Marssonina* species pathogenic to poplars. From descriptions of conidial morphology in the literature and examination of type material 3 species were recognised viz: *M. populi*, *M. castagnei* and *M. brunnea*. Study of worldwide collections of 280 herbarium specimens confirmed the existence of the three species and further established the validity of type specimens as species representatives. The stability of selected differential taxonomic criteria was evaluated both in the laboratory and field under varying environmental conditions. Subsequent comparative morphological studies under defined laboratory conditions established that features of conidium morphology (shape, mean dimensions and septum location) were valid differential taxonomic criteria. Further, the existence of 'large-conidium variants' was demonstrated. Microconidia were of no value in species delimitation. Host range and pathogenicity tests revealed two host specific forms of *M. brunnea:*

(i) *M. brunnea* f. sp. *trepidae* pathogenic to *P. tremula* and *P. tremuloides*,
(ii) *M. brunnea* f. sp. *brunnea* pathogenic to *P. deltoides* and *P.* x *euramericana*.

Conidium and microconidium ontogeny of the three *Marssonina* species was annellidic and not phialidic as previously reported.

A taxonomic study of *Drepanopeziza* species pathogenic to poplars established the synonymy of *D. tremulae* and *D. punctiformis* and the close morphological similarity of *D. tremulae*, *D. populorum* and *D. populi-albae*. Production of apothecia of *D. tremulae* was induced in the laboratory and the rate of maturation shown to be temperature dependent. Incubation temperature had no significant effect on dimensions of asci and ascospores.

Seed transmission studies established that *M. brunnea* was transmitted on imported poplar seedlines as a contaminant. Seed-borne contamination was effectively controlled by dusting seed with a number of fungicides, the benzimidazole derived compounds being particularly effective.

Studies on the pathogenesis of *Marssonina* species to poplars showed that germtubes penetrated poplar leaves directed by enzymatic activity, the infection peg being naked. Within host tissue hyphae ramified indiscrim-

inately and completely disrupted cellular contents. The resistance of leaves to infection increased significantly with maturity, this being attributed to the ultrastructure of the mature cell wall.

In depth host range studies of New Zealand and overseas isolates of *M. brunnea* established the wide host range and strong pathogenicity of this species. In many instances gross differences in pathogenicity were revealed between isolates. The solution to this disease as seen in the breeding of resistant material is discussed.

INTRODUCTION

The genus *Populus* includes approximately 40 species indigenous to North America, Mexico, Europe, North Africa and Asia. In New Zealand *P. deltoides* cv. Frimley, *P. nigra* cv. Italica (Lombardy poplar), cultivars of *P. nigra* x *P. deltoides* (*P.* x *euramericana*), and *P. alba* (silver poplar) have been used extensively for soil conservation purposes, amenity plantings nursery and orchard shelterbelts.

In 1973, wind-borne urediniospores from Australia of the two rust species *Melampsora larici-populina* and *M. medusae* established infections on poplars in the North Island. Currently *M. larici-populina* occurs throughout New Zealand whereas *M. medusae* has a very limited distribution (Wilkinson & Spiers, 1976). All poplar clones then in cultivation (1973), with the exception of *P. alba* were susceptible to both *Melampsora* species and many trees have since died as a result of successive premature defoliations.

Since poplars are primarily used for soil conservation purposes in New Zealand, chemical control of *Melampsora* species was considered impracticable due to the prohibitive cost of fungicide application. The solution to this disease complex was therefore seen in the use of resistant clones. To this end imported seed from rust-resistant trees in the United States, Holland, Italy, Belgium and Japan were sown and resistant seedlings selected (Wilkinson & van Kraayenoord, 1975). Prior to sowing all seed was dusted with thiram as a routine precaution against possible seed-borne pathogens. The seedlings were raised in isolation from the main poplar nursery and were regularly inspected for disease.*

On February 19, 1976, *Marssonina* infections were observed on seedlings of *P. deltoides*, *P. deltoides* x *P. nigra* and *P. deltoides* x *P. trichocarpa* raised from Dutch and Italian seed lines imported during June/July 1975. This constituted a first record of this disease in New Zealand and the Southern Hemisphere. Because *Marssonina* is a devastating disease of Euramerican poplars in Europe (Castellani & Cellerino, 1964) the outbreak was viewed with concern and every effort was made to eradicate the disease

* All research in New Zealand pertaining to poplars is conducted by the Aokautere Science Centre, Ministry of Works & Development, Palmerston North.

by destroying infected seedlings and fallen leaves, and by pruning the remaining uninfected seedlings to ground level. One year later (January 1977), further *Marssonina* infections were detected in the research nursery and in a plantation of poplars (*P.* x *euramericana* cv. Regenerata) 400 metres from the initial outbreak, indicating that attempts to eliminate the disease had been unsuccessful. The disease is now well established on poplars at the Research Nursery and in the surrounding countryside.

The fact that *Marssonina* was first observed in New Zealand in seedlings raised from imported seed collected from plantations in countries known to have *Marssonina* strongly suggested seed as the source of primary inoculum. Although the genus *Populus* has worldwide distribution and a considerable literature exists pertaining to *Marssonina* diseases of this host genus there are no published reports of the disease being seed-borne.

Identification of the particular species of *Marssonina* present in New Zealand has proven difficult due to the confusion in the literature concerning the taxonomy of *Marssonina* and *Drepanopeziza* species (perfect state) pathogenic to poplars. Six species of *Marssonina* have been described, namely:

M. populi (Lib.) Magn.
M. castagnei (Desm. & Mont.) Magn.
M. brunnea (Ell. & Ev.) Magn.
M. tremulae (Lib.) Kleb.
M. tremuloidis Kleb.
M. populina (Schnabl.) Magn.

These species have been erected on the basis of conidial features, (conidium morphology, dimensions, septum location), host specificity and symptom expression. However the validity and/or synonymy of each of the above species has not been established and accordingly various authors continue to recognise different species. Working independently, Thompson (1937) and Boyer (1961) concluded that the various species were taxonomically inseparable and consequently recognised a single species, *M. populi*. This interpretation has not been accepted by later authors (Rimpau, 1962; Gremmen, 1965a; Pirozynski, 1974; Cellerino, 1979) who variously recognise some of the six described species.

Similar confusion exists concerning the taxonomy of the *Drepanopeziza* state and four species have been erected, namely:

D. populorum (Desm.) v. Hohn.

D. populi-albae (Kleb.) Nannf.

D. tremulae Rimpau.

D. punctiformis Gremmen.

Thompson (1937) concluded that apothecia of *D. populorum* and *D. populi-albae* were not significantly different and accordingly recognised only one species, *D. populorum* (Desm.) V. Hohn. Both Rimpau (1962) and Gremmen (1965a) accepted *D. populi-albae* and *D. populorum* as the perfect states of *M. castagnei* and *M. populi* respectively. There is disagreement however, as regards the perfect state of *M. brunnea*, Rimpau (1962) regarding it as *D. tremulae*, and Gremmen (1965 a, b) considering it to be *D. punctiformis*.

The present work constitutes a study of the *Marssonina* - poplar disease complex, with particular emphasis on the taxonomy of *Marssonina* and *Drepanopeziza* species and the possible seed-borne nature of the disease.

CHAPTER 1

TAXONOMY OF MARSSONINA SPECIES

A. INTRODUCTION

A considerable literature exists on the taxonomy of the genus *Marssonina*, which has recently been summarised by Sutton (1980). Since this is the most recent authoritative statement on the genus, Sutton's review, including his definition of the genus, is now stated:

'*Marssonina* Magnus nom. nov., Hedwigia 45: 2 (1906), nom. cons. prop. *Gloeosporium* Desm. & Mont., Ann. Sci. nat. 12: 295 (1849), nom. rej. prop. *Marssonina* Fischer, Fungi europaei exsiccati cent. XIX, no. 1857 (1874). *Marssoniella* Höhn., Sber. Akad. Wiss. Wien 125: 108 (1916). Sp. typ.: *M. fragariae* (Lib.) Kleb. (syn. *Leptothyrium fragariae* Lib., *M. potentillae* (Desm.) Magnus .

Mycelium immersed, hyaline to pale brown, branched, septate. *Conidiomata* acervular, subcuticular, dark brown to black, amphigenous, separate, rarely confluent, sometimes associated with a dendroid subcuticular subicle, pseudoparenchymatic. Dehiscence irregular. *Conidiophores* hyaline, branched irregularly, mostly lateral, septate, smooth, comprising the subcuticular acervuli. *Conidiogenous cells* holoblastic, annellidic, sympodial and indeterminate, or monoblastic and determinate, integrated or discrete, doliiform or cylindrical, hyaline, smooth. *Conidia* hyaline, 1-septate, separating equal or unequal cells, smooth, base truncate, guttulate, apical cells obtuse or subacute, sometimes straight or curved, ± constricted at the septum.

Höhnel (1910d, 1916b) showed that the type of *Gloeosporium*. *G. castagnei* Desm. & Mont., has 1-septate conidia and that it had been referred to *Marssonina* by Magnus (1906). *Gloeosporium* antedates *Marssonina* by several years, and if the respective type species are congeneric, as seems likely, the fungi at present placed in *Marssonina* should be transferred to *Gloeosporium* in accordance with nomenclatural priority. Arx (1957a) considered the two genera to be identical but argued for the conservation of *Marssonina* against *Gloeosporium* to preserve current usage. The fungi hitherto placed in *Gloeosporium*, the majority of which are not congeneric with *G. castagnei*, have been removed to other genera, notably by Höhnel (1916b) and Arx (1957a). There has not been any modern revision of *Marssonina* or the more than 150 taxa described in it. There is wide variation in conidial morphology, structure of conidiomata and their relationships with the substrates in which they occur. It therefore seems probable that *Marssonina* will eventually be fragmented into smaller generic units particularly if differences in conidiogenesis are correlated with such morphological distinctions.

Some species have been correlated with *Diplocarpon* states (Wolf 1912, 1924) others with *Drepanopeziza* (Rimpau, 1962).'

A survey of the literature revealed that the following 6 *Marssonina* species have been described as pathogenic to poplars with species differentiation being based on conidium shape and dimensions, with some regard to host specificity and symptom expression:

(i) *Marssonina populi* (Lib.) Magn.
(ii) *Marssonina castagnei* (Desm. & Mont.) Magn.
(iii) *Marssonina tremulae* (Lib.) Kleb.
(iv) *Marssonina brunnea* (Ell. & Ev.) Magn.
(v) *Marssonina populina* (Schnabl.) Magn.
(vi) *Marssonina tremuloidis* Kleb.

The following is an account of the historical background of these species, a statement of their original description, their current taxonomic status, host range and distribution.

1. *MARSSONINA POPULI* (Lib.) Magn. - Hedwigia 45, (1906). Synonymy (after Rimpau, 1962).

Leptothyrium populi Lib. - Plant. Crypt. Ard. Nr. 257 (1834).

Gloeosporium populi (Lib.) Mont. & Desm. - Exs. Nr. 2129 (1856).

Gloeosporium populi (Lib.) Magn. - in Saccardo, Michelia 1, 533 (1878).

Marssonina populi (Lib.) Sacc. - Syll. fung. 3. 767 (1884).

Marssonina populi-nigrae (Lib.) Kleb. - Haupt. u. Nebenfruchtf. Ascomyc. p.357 (1918).

Gloeosporium berkeleyi Mont. - Ann. Sci. Nat., ser. 3, 12, 296 (1849).

Asteroma labes Berk. - Exs. Nr. 346 (1843).

Gloeosporium labes Berk. et Br. - in Cooke, Handb. Brit. Fung. 1, 474 (1871).

This species was originally described by Libert (1834) as *Leptothyrium populi* from *P. nigra* in France. Her description is as follows:

'Epiphyllum. Maculis primo orbiculatis subradiatis, demum irregularibus confluentibus fuscis; peritheciis sic planiusculis, pallidis, basi circumscissis; pulpa alba; ascellis subclavatis; Sporidiis 3-4 pellucidis. Ad folia *Populi*. Autumno.

Translation:

Epiphyllous spots initially circular and radiate, becoming irregular and confluent, dark brown; flat, pale perithecia with circumscissile bases; with white masses of conidia, conidia subclavate, translucent with 3-4 guttules.

On leaves of *Populus* in the autumn.'

This species has since been described by Rimpau (1962), Gremmen (1965a), O'Riordain & Kavanagh (1965), Pirozynski (1974) and Cellerino (1979). Conidial dimensions cited by these authors and Saccardo (1884) are presented in Table 1. Both Rimpau (1962) and Pirozynski (1974) correctly followed Magnus (1906) referring to this species as *M. populi* (Lib.) Magn. whereas Gremmen (1965a), O'Riordain & Kavanagh (1965) and Cellerino (1979) followed Klebahn (1918) naming the fungus *M. populi-nigrae* (Lib.) Kleb. This epithet was introduced by Klebahn (1918) for the *Marssonina* species pathogenic to *P. nigra, P. pyramidalis, P. balsamifera* and *P. berolinensis* and hence species delimitation was based on host specificity.

Synonymy of *M. populi* (Lib.) Magn. and *M. populi-nigrae* (Lib.) Kleb. is supported by the comparable descriptions of these species by Rimpau (1962), Pirozynski (1974) (*M. populi*) and Gremmen (1965a), O'Riordain & Kavanagh (1965), Cellerino (1979) (*M. populi-nigrae*) and possession of a common perfect state, *Drepanopeziza populorum* (Desm.) v. Höhn.

M. populi is pathogenic to species of the Sections Aigeiros (*P. nigra, P. nigra* x *P. deltoides*) and Tacamahaca (*P. tacamahaca, P. trichocarpa, P. balsamifera*) and induces roundish tan brown blotches. This species is recorded from Europe, England, Russia, Canada and the United States (Rimpau, 1962; Gremmen, 1964; Pirozynski, 1974; Cellerino, 1979).

2. *MARSSONINA CASTAGNEI* (Desm. & Mont.) Magn. - Hedwigia 45 (1906)
Synonymy (after Rimpau, 1962).

Gloeosporium castagnei Desm. & Mont. - Ann. Sci. Nat., sér 3, 12, 295 (1849).
Marsonia castagnei (Desm. & Mont.) Sacc. - Syll. fung. 3, 768 (1884).
Leptothyrium circinans Fuck. - Symb. mycol. p383 (1867).
Gloeosporium circinans (Fuck.) Sacc. - Syll. fung 3, 712 (1884).
Didymosporium piriforme Riess. - Hedwigia 1, 24 (1853).
Marsonia piriformis (Riess.) Sacc. - Syll. fung. 3, 767 (1884).
Marssonina piriformis (Riess.) Magn. - Hedwigia 45, 89 (1906).
Gloeosporium populi-albae Desm. - Bull Soc. Bot. France 4, 799 (1857).

According to Von Arx (1970) and Pirozynski (1974), *Leptothyrium circinans* Fuckel and *Gloeosporium populi-albae* Desm. are identical and in fact are species of another genus (*Titaeosporina tremulae* (Lib.) v. Luyk). *Marssonina castagnei* was originally described by Desmazières and Montagne (1849) from *P. alba* in France. Their description is as follows:

'*Gloeosporium castagnei* Desm. et Montag. MSS: epiphyllum, maculis orbicularibus confluentibus brunneis, sporis ovoideis aut pyriformibus brevipedicellatis, cirris niveis crassis, - HAB. In foliorum vivorum *Populi albae* pagina superiori circa Montaud et Aix (Bouches-du Rhone a cl. Castagne lectum et ad Montagne sub n. 1081 missum."

Translation

Epiphyllous, with circular, confluent, brown spots, conidia ovoid to pear shaped, borne on short pedicels, spore tendrils thick and white. On the upper surface of living leaves of *Populus alba* near Montaud and Aix (Bouches-du-Rhone, collected by Castagnei and sent to Montagne under No 1081.'

Dezmazières and Montagne (1849) observed that conidia of *G. castagnei* and *Leptothyrium populi* (Libert, 1834) were morphologically similar being pyriform to lunate and borne on short pedicels. These authors commented that the main difference between the two species was the enormous volume of mucilage enveloping conidia of *G. castagnei*.

The morphology of *M. castagnei* (Desm. & Mont.) Magn. has recently been described by Rimpau (1962), Gremmen (1965a); Pirozynski (1974), and Cellerino (1979). Conidial dimensions cited by these authors and Saccardo (1884) are listed in Table 1. Criteria used for delimiting *M. castagnei* from other *Marssonina* species pathogenic to poplars have been conidial dimensions, septum location and host specificity (Gremmen 1965a).

The perfect state of *M. castagnei* is *Drepanopeziza populi-albae* (Kleb.) Nannf. (Rimpau, 1965; Gremmen, 1965a).

M. castagnei is pathogenic only to *P. alba* and has been recorded from Italy, France, Germany, Spain, Yugoslavia, Iran, Canada and The United States (Cellerino, 1979).

3. *MARSSONINA TREMULAE* (Lib.) Kleb. - Haupt-u. Nebenfruchtf. Ascomyc. p.357 (1918).
Synonymy (after Rimpau, 1962).

Leptothyrium tremulae Lib. - Plant Crypt. Ard. Nr. 161 (1832).

Gloeosporium tremulae (Lib.) Pass. - Hedwigia 13, 187 (1874).

Gloeosporium populi-albae Desm. var. *tremulae* Sacc. - Syll. fung. 3, 712 (1884).

Gloeosporium tremulae v. Höhn. - Sitzber. Akad. Wiss. Wien, Math - Nat. Kl., Abt. 1, 125, 69 (1916).

Titaeosporina tremulae (Lib.) v. Luyk. - Ann. mycol. 17, 112 (1919).

Gloeosporium brunneum Ell. et Ev. - J. Mycol. 5, 154 (1889).

Marsonia brunnea (Ell. et Ev.) Sacc. - Syll. fung. 10, 478 (1892).

Marssonina brunnea (Ell. et Ev.) Magn. - Hedwigia 45, 89 (1906).

Depazea frondicola Fr. - Obs. mycol. 2, 365 (1818).

Examination of the original description of *Leptothyrium tremulae* Libert by the writer revealed that this fungus was not a species of *Marssonina*. Previously Pirozynski (1974) considered *L. tremulae* to be synonymous with *Titaeosporina tremulae* (Lib.) van Luyk. This was later confirmed by Sutton (1980). It should also be noted that Libert (1832) regarded *Depazea frondicola* Fr. as a synonym of *L. tremulae* Lib.

Examination of the original descriptions of *Gloeosporium tremulae* (Lib.) Pass. and *G. populi-albae* Desm. var. *tremulae* Sacc. by the writer established that these species were also not species of *Marssonina*. Both are listed by Sutton (1980) as synonyms of *Asteroma frondicola* (Fr. ex. Ficinus & Schubert) Morelet = (*Titaeosporina tremulae*).

Examination of the original description of *Gloeosporium brunnewn* Ell. & Ev. by the writer established that the fungus was in fact a species of *Marssonina*.

The original description by Ellis & Everhart (1889) is as follows:

'*Gloeosporium* (*Marsonia*) *brunneum*, n.s. On leaves of *Populus candicans*, New Field, N J., August, 1889. Leaf mottled above with small black spots which soon become confluent in large areas, especially around the margin, the entire lower surface of the leaf soon assuming a uniform bronze-brown color. Acervuli 1-3 in each of the minute black spots, pale, erumpent on both sides of the leaf, finally nearly black. Conidia clavate obpiriform, hyaline, 1-septate below the middle, 14-16 by 5-7u. On account of the smaller conidia and different habit this seems sufficiently distinct from *G. populi* and *C. castagnei*. There are no well defined spots, only the small black specks soon confluent and blackening finally the greater part of the leaf.'

Saccardo (1892) renamed this species *Marsonia brunnea* (Ell. & Ev.) Sacc. which was later amended by Magnus (1906) to *Marssonina brunnea* (Ell. & Ev.) Magn., the current epithet. Following its discovery *M. brunnea* remained obscure for many years until Gremmen (1964) reported the occurrence of an apparently new *Marssonina* disease of poplars in Holland, distinguished from the then more common species *M. populi* by having smaller conidia and punctiform leaf spots rather than blotches. Gremmen (1965a) described the perfect stage of this fungus as *Drepanopeziza punctiformis* and later established that this apparently new *Marssonina* was in fact synonymous with *M. brunnea* (Ell. & Ev.) Magn. (Gremmen 1965b). Since its initial discovery in Holland *M. brunnea* has become widespread throughout Europe and has caused serious economic loss to commercial plantations of poplars (Cellerino, 1979).

Conidial dimensions of *M. brunnea* cited by Ellis & Everhart (1889), Gremmen (1965a), Pirozynski (1974) and Cellerino (1979) are listed in Table 1. This species is pathogenic to most species of poplar and has been recorded from the British Isles, Europe, Asia, Canada and the United States of America (Cellerino, 1979).

Examination by the writer of the descriptions of the remaining fungi cited as synonyms of *M. tremulae* (Lib.) Kleb. by Rimpau (1962) revealed that only *M. tremulae* (Lib.) Kleb. described briefly by Klebahn (1918), was a species of *Marssonina*. In view of their comparable conidial dimensions (Table 1), *M. brunnea* (Ell. & Ev.) Magn. and *M. tremulae* (Lib.) Kleb. are possibly synonymous. *M. tremulae* (Lib.) Kleb. is recognised by Rimpau (1962) and Cellerino (1979) and according to the latter is specifically pathogenic to *P. tremula*.

The perfect state of *M. tremulae* was described by Rimpau (1962) as *Drepanopeziza tremulae*. Later Gremmen (1965a) cited *D. punctiformis* as the perfect state of *M. brunnea*. If the two imperfect states are proven synonymous then in view of *D. tremulae* being first described this binomial will take precedence.

4. *MARSONINA POPULINA* (Schnabl.) Magn. Hedwigia 45, 89 (1906).
Synonymy (after Rimpau 1962).

Marsonia populina Schnabl. - Ber. Bayer. Bot. Ges. 2, 68 (1892).

This species was originally described in Germany on *P. nigra* as follows:

'*Marsonia populina* Schnabl. nov. spec.

Maculis amphigenis, rotundis vel. irregularibus, saepius fuscomarginatis, ca. 1 cm diam. subinde confluentibus; acervulis epiphyllis, punctiformibus, fulvescentibus; conidiis oblongis, utrinque rotundatis, 1-septatis, medio constrictis, hyalinis, 9-11u long. 4-5u cross.

Hab. in foliis *Populi nigrae* prope Munchen Der Pilz trat im Herbste 1892 an 2- und 3 jahrigen Schwarzpappeln in einer Gärtnerei in Sendling auf.

Translation:

Amphigenous spots, round to irregular and often with a dark margin, approximately one cm in diameter then confluent; epiphyllous dot-like tan coloured; conidia oblong, uniseptate, constricted in the middle, hyaline, 9-11u by 4-5u.

On leaves of *P. nigra* collected near Munich. The fungus occurred in Autumn 1892 on 2 and 3 year old black poplars at a nursery in Sendling.'

Rimpau (1962) examined specimens of *M. populina* and concluded it was morphologically similar to *M. tremuloidis*. Due to the absence of further reports of this species and in view of its comparable conidial dimensions with *M. brunnea* (Table 1), the author regards these two species as possibly being synonymous.

5. *MARSSONINA TREMULOIDIS* Kleb. Haupt. - und Nebenfruchtf. Ascomyc. p.357 (1918).

This species was originally described briefly by Klebahn (1918) from leaves of *P. tremuloides* from the United States. Klebahn (1918) cited conidial dimensions as 12.5-16 x 4-5u and noted that possibly the same species infected leaves of *P. grandidentata* from Canada (10-14.5 x 3.7-4.2u). Klebahn (1918) must have used host specificity as the criterion for differentiating these *Marssonina* species since conidial dimensions he cited for *M. tremuloidis* (*P. tremuloides*) and *M. tremulae* (*P. tremula*) were essentially similar. Rimpau (1962) examined material of *M. tremuloidis*, observing that conidia were morphologically similar to those of *M. populina*. In view of that fact that *M. tremuloidis* was erected on the basis of host specificity and since conidia of this species and *M. brunnea* are virtually

identical (Table 1), they are probably synonymous. Possible synonymy is further supported by the fact that in recent years *M. tremuloidis* has been recognised only by Rimpau (1962). Pirozynski (1974) regarded *M. brunnea* as the *Marssonina* species pathogenic to *P. tremuloides* and *P. grandidentata*, which implies that he also rejected *M. tremuloidis*.

The above review of the original descriptions of the six described *Marssonina* species tentatively supports the contentions of Gremmen (1965 a,b) and Pirozynski (1974) that recognition of only three species is warranted, namely:

M. populi (Lib.) Magn.
M. castagnei (Desm. & Mont.) Magn.
M. brunnea (Ell. & Ev.) Magn.

Further, the review strongly suggests that *M. tremulae* (Lib.) Kleb., *M. populina* (Schnabl.) Magn. and *M. tremuloidis* Kleb. are synonymous with *M. brunnea* (Ell. & Ev.) Magn. If synonymy is proven then *M. brunnea* has priority.

An alternative interpretation of the taxonomy of *Marssonina* species pathogenic to poplars has been suggested by Thompson (1937) and Boyer (1961). Following study of the *Marssonina* poplar disease complex Thompson (1937) concluded that - 'no sufficiently distinctive criteria existed for the separation of forms into species'. Boyer (1961) supported this contention stating that - 'as a group isolates appeared to consist of a series of closely related fungi often separable on both pathological and cultural bases but too highly variable to be distinguished clearly taxonomically.' Accordingly these authors recognised the one species *M. populi* (Lib.) Magn. perfect state *Drepanopeziza populi* (Desm.) v. Höhn.

In view of the expressed belief by the writer that only three species of *Marssonina* are pathogenic to poplars an attempt was made to resolve the problem by examination of:

(i) type material of the six described species (where possible),

(ii) herbarium material and field collections.

TABLE 1: Conidial dimensions of *Marssonina* species cited by various authorities.

Marssonina Species	Conidial Dimensions (u) Length	Breadth	Authority
M. populi	20	12	Saccardo (1884)
	18.5-20.5-23	10-11-12	Rimpau (1962)
	17-25	6-11	Gremmen (1965a)
	15.5-24.8	5.5-9.5	O'Riordain & Kavanagh (1965)
	16.5-(18.5-24.5)-27	7.5-(8.5-10.5)-13	Pirozynski (1974)
M. castagnei	18-20	7-8	Saccardo (1884)
	15.5-17.5-19	8-8.5-9.5	Rimpau (1962)
	18-20	5-9	Gremmen (1965a)
	15-(16.5-20.5)-23.5	5.5-(6.3-7.5)-8	Pirozynski (1974)
	17.5-22.5	7.5-10	Cellerino (1979)
M. brunnea	14-16	5-7	Ellis & Everhart (1889)
	13-19	5-9	Gremmen (1965a)
	11-(13-18)-21	3.5-(4.5-5.7)-7	Pirozynski (1974)
	12-(14-15)-19	4-5-7	Cellerino (1979)
M. tremulae	14-15.5-17	4-4.5-5.5	Rimpau (1962)
	15-18.4	4.5-5.5	Cellerino (1979)
M. tremuloidis	12.5-16	4-5	Klebahn (1918)
	9-11.5-13	4-4.5-5	Rimpau (1962)
M. populina	9-11	4-5	Schnabler (1892)
	9-11	4-5	Rimpau (1962)

B. EXAMINATION OF TYPE MATERIAL

Type material of *M. populi*, *M. castagnei*, *M. brunnea* and lectotype specimens of *M. tremulae* were assembled and examined. Although type material of *M. tremuloidis* (Klebahn 1918, Nr. 17) was not examined, the species from *P. grandidentata* (Nr. 18) which Klebahn (1918) noted was probably synonymous with *M. tremuloidis* was examined. To confirm this possible synonymy a specimen of *M. tremuloidis* from *P. tremuloides* was examined. Despite extensive overseas enquiries the type specimen of *M. populina* was not located.

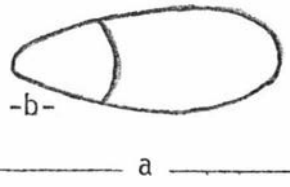
In each instance symptoms were noted and conidia were mounted in lactophenol and stained with 0.5% acid fuchsin. Sixty conidia from each specimen were measured using a Leitz micrometer (500x). Dimensions recorded were length, breadth, and distance from the conidium base to the septum. From these measurements the following dimensions and ratios were recorded and calculated:

(i) Conidium length - the shortest and longest conidium, the mean length and standard deviation of the mean.

(ii) Conidium breadth - the most narrow, the widest, the mean breadth and standard deviation of the mean.

(iii) Length to breadth ratio (L:B ratio) - the ratio of conidium length to breadth.

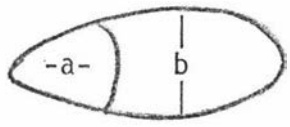
(iv) The ratio of conidium length to the distance from the conidium base to the septum (TL:S ratio).



ratio of a:b

(v) The distance from the conidium base to the septum, expressed as a percentage of the total conidium length (% Sept.).

(vi) The ratio of the distance from the conidium base to the septum to conidium breadth (LS:B ratio).



ratio of a:b

Additionally, conidium shape was recorded.

1. *Marssonina populi* (Lib.) Magn.

Type specimens were received from Jardin Botanique National de Belgique; The Herbarium, Royal Botanic Gardens, Kew, England and Botanischer Garten und Institut für Systematische Botanik der Universität, Switzerland. The material consisted of entire leaves of *P. nigra* and exhibited on the adaxial surface large (1-2 cm diam.), circular to irregular tan blotches (Fig. 1), often confluent forming extensive tan patches dotted with whitish masses of conidia. Conidia were hyaline, broadly obovoid to pyriform, usually curved and divided by a single septum into a smaller lower cell and a larger, rounded upper cell (Fig. 1). Guttules were often present in both cells. Conidial dimensions and ratios of the three specimens examined are presented in Table 2.

Because Libert collected the type specimens in the autumn of 1834 abundant microconidia were present. Microconidia were unicellular, hyaline, ellipsoid to bacillate and somewhat flattened at the base (Fig. 1). They measured 4.0 - 6.0 - 8.0 x 1.0 - 1.3 - 1.6u, and were produced from ampulliform microconidiophores (5 - 15 x 2 - 5u).

The symptoms exhibited by type material were in agreement with the original description by Libert (1834). Dimensions of conidia and microconidia were not cited by Libert (1834).

2. *Marssonina castagnei* (Desm. & Mont.) Magn.

Type material was obtained from the Jardin Botanique National de Belgique and consisted of entire leaves of *Populus alba*. Symptoms were mostly epiphyllous being discrete, brown, circular lesions (1-5mm diam.) which were often paler in the centre and dotted with whitish punctiform acervuli. Large irregular blotches were formed by coalescence of adjoining circular lesions (Fig. 2).

Conidia were hyaline, obovoid to broadly obovoid, straight but occasionally curved, and divided by a single septum into a smaller lower cell and a larger rounded upper cell (Fig. 2). Guttules were observed in both cells. Conidia measured 11.0 - $17.2_{1.6}$ - 21.0 x 4.0 - $6.1_{0.4}$ - 7.0u. Values for L:B, TL:S, LS:B ratios and % Sept. were 2.83, 2.44, 1.15 and 41% respectively.

The symptoms exhibited by type material were in agreement with those cited by Desmazières & Montagne (1849). Conidial dimensions were not cited by these authors.

3. *Marssonina brunnea* (Ell. & Ev.) Magn.

Type material was obtained from The National Fungus Collection, U.S.D.A., Maryland, USA, and The Herbarium, Royal Botanic Gardens, Kew, England, and in each instance consisted of single leaves of *P. candicans*. Symptoms exhibited were essentially similar to those described by Ellis & Everhart (1889); that is, - "leaf mottled above with small black spots which soon become confluent in large areas, especially around the margin, the entire lower surface of the leaf soon assuming a uniform bronze-brown colour." The spots were amphigenous, punctiform, black, circular to angular and usually 1 mm in diameter (Fig. 3). Conidia were hyaline, obovoid, straight to slightly curved, divided by a single septum into a smaller lower cell and a larger rounded upper cell (Fig. 3). Guttules were often observed in both cells. Conidial dimensions and ratios of the type specimens examined are presented in Table 3.

The writers observations were in agreement with those of Ellis & Everhart (1889), with the exception of conidium breadth.

4. *Marssonina tremulae* Kleb.

The lectotype of *M. tremulae* designated by G. L. Hennebert (13.10.1965) referred to the specimen:

Sydow - Mycotheca Germanica Nr. 834. *Marsonia populi* (Lib.) Sacc., *P. tremula*, Brandenburg, Funkenkrug bei Berlin, Leg. H. Sydow, 29.9.1908.

Lectotype material was obtained from: Botanischer Garten and Institut für Systematische Botanik der Universitat., Switzerland; Rijksherbarium, Leiden, The Netherlands; Farlow Herbarium and Reference Library, Harvard

TABLE 2: Dimensions of conidia from type material of *M. populi* (Lib.) Magn.

Specimen Number	Conidial Dimensions (u) Length Range	Length Mean	Breadth Range	Breadth Mean	Ratio L:B	Ratio TL:S	Ratio LS:B	% Sept.
1[a]	15.0-26.0	20.5$_{2.0}$[b]	5.0-10.0	6.5$_{0.8}$	3.14	3.55	0.88	28.1
2	15.0-30.0	20.7$_{2.5}$	5.0-8.0	6.7$_{0.7}$	3.10	3.70	0.83	27.0
3	15.0-26.0	19.8$_{2.3}$	4.5-9.0	6.6$_{0.8}$	3.00	3.73	0.80	27.0
MEAN	15.0-30.0	20.3$_{2.3}$	4.5-10.0	6.6$_{0.8}$	3.08	3.66	0.84	27.4

[a] 1. Botanischer Garten, Zurich, Switzerland.
2. Jardin Botanique National de Belgique.
3. The Herbarium, Kew, England.

[b] Standard deviation

TABLE 3: Dimensions of conidia from type material of *M. brunnea* (Ell. & Ev.) Magn.

Specimen Number	Conidial Dimensions (u) Length Range	Length Mean	Breadth Range	Breadth Mean	Ratio L:B	Ratio TL:S	Ratio LS:B	% Sept.
1[a]	12.0-20.0	15.1$_{1.0}$[b]	3.5-6.0	4.5$_{0.4}$	3.35	3.01	1.10	33.1
2	11.0-18.0	14.5$_{1.2}$	4.0-7.0	4.7$_{0.6}$	3.10	3.17	1.00	31.5
MEAN	11.0-20.0	14.8$_{1.1}$	3.5-7.0	4.6$_{0.5}$	3.22	3.09	1.05	32.3

[a] 1. The National Fungus Collection, Maryland, USA.
2. The Herbarium, Kew, England.

[b] Standard deviation,

University, Massachusetts, USA; Jardin Botanique National de Belgique.

The material consisted of entire leaves of *P. tremula* and exhibited on the adaxial surface, small (approx. 1 mm), discrete punctiform, black, circular to angular lesions. Irregular blotches were formed by coalescence of adjoining lesions (Fig. 4). On the abaxial surface circular black spots covered with amber masses of conidia were present. Conidia were hyaline, obovoid, straight to slightly curved, divided by a single septum into a smaller lower cell and a larger rounded upper cell (Fig. 4). Guttules were often observed in both cells. Conidial dimensions and ratios of the four lectotype specimens examined are presented in Table 4.

5. *Marssonina tremuloidis* Kleb.

(i) *P. grandidentata* (Klebahn 1918, Nr. 18). This specimen was received from the National Mycological Herbarium, Canada Agriculture Ontario, Canada under the following title:

M. brunnea (Ell. & Ev.) Magn. D.A.O.M. 130530. *P. grandidentata* Michx., Guelph, Ontario, Collected by J. Dearness, 8 August 1913, Identified by D.B.O. Savile. (Previously issued as *M. castagnei* in Elam Bartholomew, Fungi Columbia Nr. 4235).

The material consisted of entire leaves and exhibited on the adaxial surface, small (approx. 1 mm), discrete punctiform, black, circular to angular lesions and irregular necrotic blotches (Fig. 5). On the abaxial surface circular black spots covered with amber spore masses were present (Fig. 5).

Conidia were hyaline, narrowly obovoid, straight to slightly curved, divided unequally by a single septum (Fig. 5). Guttules were commonly observed in both cells. Conidial dimensions and ratios are presented in Table 5.

The writers observations were essentially in agreement with those of Klebahn (1918).

(ii) *P. tremuloides*. The single specimen of *M. tremuloidis* examined was received from The Farlow Herbarium and Reference Library, Harvard University, Massachusetts, USA under the following title:

TABLE 4: Dimensions of conidia from lectotype material of *M. tremulae* Kleb.

Specimen Number	Conidial Dimensions (u) Length		Breadth		Ratios L:B	TL:S	LS:B	% Sept.
	Length	Mean	Range	Mean				
1[a]	11.0-20.0	$15.2^{b}_{1.6}$	3.5-7.0	$4.8_{0.6}$	3.16	3.08	1.03	32.4
2	11.0-20.0	$15.7_{1.6}$	3.5-6.5	$5.1_{0.7}$	3.09	2.95	1.04	33.8
3	11.0-20.0	$15.6_{1.3}$	3.5-6.5	$5.0_{0.5}$	3.14	3.02	1.04	33.1
4	11.0-20.0	$15.2_{1.5}$	3.5-7.0	$5.1_{0.5}$	2.94	2.90	1.01	34.3
MEAN	11.0-20.0	$15.4_{1.5}$	3.5-7.0	$5.0_{0.5}$	3.08	3.00	1.03	33.4

[a] 1. Jardin Botanique National de Belgique.
2. Farlow Herbarium, Harvard University, USA.
3. Botanischer Garten, Zurich, Switzerland.
4. Rijksherbarium, Leiden, The Netherlands.

[b] Standard Deviation

TABLE 5: Dimensions of conidia of *M. tremuloidis* Kleb. from *P. grandidentata* and *P. tremuloides*

Specimen Number	Conidial Dimensions (u) Length		Breadth		Ratios L:B	TL:S	LS:B	% Sept.
	Range	Mean	Range	Mean				
1[a]	11.0-20.0	$14.2^{b}_{1.2}$	3.5-6.5	$3.8_{0.2}$	3.70	3.07	1.20	32.6
2	11.0-20.0	$15.0_{1.4}$	3.5-6.5	$4.1_{0.3}$	3.66	3.23	1.13	31.0
MEAN	11.0-20.0	$14.6_{1.3}$	3.5-6.5	$3.9_{0.3}$	3.68	3.15	1.16	31.8

[a] 1. *P. gradidentata* Ex. National Mycological Herbarium, Ontario, Canada.
2. *P. tremuloides* Farlow Herbarium, Harvard University, USA.

[b] Standard Deviation

TABLE 6: Comparative conidial dimensions of *Marssonina* species from type and other material.

Marssonina Species	Conidial Dimensions				Ratios			% Sept.
	Length		Breadth		L:B	TL:S	LS:B	
	Range	Mean	Range	Mean				
M. populi	15.0-30.0	$20.3^{a}_{2}{}_{.3}$	4.5-10.0	$6.6_{0.8}$	3.08	3.66	0.84	27.4
M. castagnei	11.0-21.0	$17.2_{1.6}$	4.0-7.0	$6.1_{0.4}$	2.83	2.44	1.15	41.0
M. brunnea	11.0-20.0	$14.8_{1.1}$	3.5-7.0	$4.6_{0.5}$	3.22	3.09	1.05	32.3
M. tremulae	11.0-20.0	$15.4_{1.5}$	3.5-7.0	$5.0_{0.5}$	3.08	3.00	1.03	33.4
M. tremuloidis	11.0-20.0	$14.6_{1.3}$	3.5-6.5	$3.9_{0.3}$	3.68	3.15	1.16	31.8

[a] Standard deviation

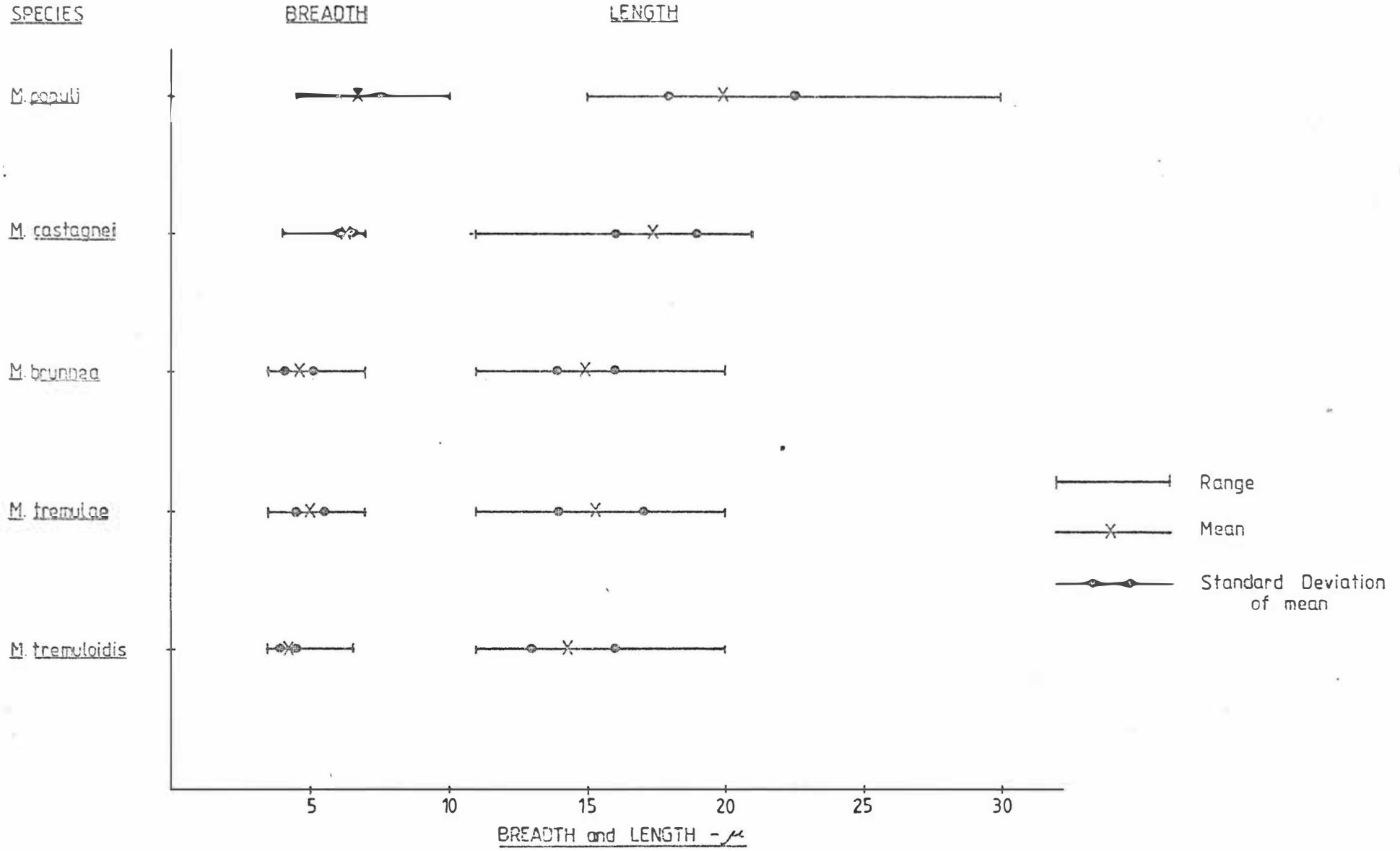


FIG. 7: Range, mean and standard deviation of conidial dimensions of Marssonina species from type and other material

Herbarium J. J. Davis, *Marsonia brunnea* (Ell. & Ev.) Sacc., *P. tremuloides*, Raciue Wisconsin, collected by J. J. Davis, 25 September 1894.

Symptoms exhibited were essentially similar to those described above for *P. grandidentata*. That is, on the adaxial surface small (approx. 1 mm) discrete punctiform black lesions and irregular necrotic blotches were observed. On the abaxial surface, black spots, covered with amber spore masses were evident (Fig. 6). Conidia were morphologically inseparable from those observed on *P. grandidentata* being hyaline, narrowly obovoid, straight to slightly curved and divided unequally by a single septum (Fig. 6). Conidial dimensions and ratios are presented in Table 5 along with those from *P. grandidentata*.

The comparative conidial dimensions and ratios of the five *Marssonina* species are listed in Table 6, and length and breadth dimensions depicted graphically in Fig. 7. These results show that conidia of *M. brunnea*, *M. tremulae* and *M. tremuloidis* are morphologically indistinguishable.

Discussion

The type material was examined with a view to determining the accuracy of the original descriptions and evaluating whether the five collections warrant recognition as separate species. In all cases symptoms were as originally described, the symptoms expressed by *P. nigra* (*M. populi*) and *P. alba* (*M. castagnei*) being essentially similar and consisting of circular to irregular tan blotches. The symptoms exhibited by *P. candicans* (*M. brunnea*) were quite distinct from those exhibited by *P. nigra* (*M. populi*) and *P. alba* (*M. castagnei*), consisting of small black spots with some coalescence. Symptoms exhibited by *P. tremula* (*M. tremulae*) *P. grandidentata* and *P. tremuloides* (*M. tremuloidis*) were essentially similar to those exhibited by *P. candicans* (*M. brunnea*), although coalescence was greater. Symptom expression was used partly by Ellis & Everhart (1889) to justify the erection of *M. brunnea* in addition to *M. populi* and *M. castagnei*. It seems likely that Desmazières & Montagne (1849) justified the erection of *M. castagnei* on the basis of host specificity since they acknowledged the close morphological similarity between conidia of this species and *M. populi*. Klebahn (1918) also justified the erection of *M. tremulae* and *M. tremuloidis* on the basis of host specificity. However it is now widely accepted that

stable morphological criteria should be used for species delimitation rather than unstable physiologic features such as symptom expression and host specificity.

In the present study on the basis of conidium morphology (shape, dimensions, ratios, septum location) *M. brunnea*, *M. tremulae* and *M. tremuloidis* were morphologically indistinguishable and accordingly, *M. tremulae* and *M. tremuloidis* are regarded as being synonymous with *M. brunnea* which has priority. Although type material of *M. populina* was not examined the author considers this species to be synonymous with *M. brunnea* in view of the reported close morphological similarity between conidia of *M. populina* and *M. tremuloidis* (Rimpau, 1962).

The contention of Thompson (1937) and Boyer (1961) that there is only one *Marssonina* species pathogenic to poplars was not supported since conidia of *M. populi*, *M. castagnei* and *M. brunnea* were morphologically distinct. Although conidia of all three species were hyaline, uniseptate and guttulate, conidia of *M. populi* were readily distinguished from those of *M. castagnei* and *M. brunnea* on the basis of shape and dimensions. That is, conidia of *M. populi* were long (20u mean), curved to strongly curved to pyriform and broadly obovoid (6.5u mean) whereas conidia of *M. castagnei* and *M. brunnea* were shorter (17u, 15u, respectively), straight to slightly curved, obovoid to broadly obovoid (6.0u *M. castagnei*), or narrowly obovoid to obovoid (4.3u, *M. brunnea*). Although of similar shape, conidia of *M. castagnei* and *M. brunnea* were readily distinguished by septum location since within *M. castagnei* the septum was located approximately 40% along the length of the conidium whereas in *M. brunnea* septum location was 32%.

It must be emphasised that each type specimen represents a single field collection at one point in time and accordingly cannot be truly representative of the species as no allowance is made for genetic or environmentally induced variability. That is, it is possible for two apparently separate type specimens to merely represent extremes of the same continuum. In the present study the three type specimens appeared to be morphologically distinct. To confirm that each was in fact representative of the species, a worldwide collection of herbarium specimens was examined and species identification attempted on the basis of conidial morphological features. Such studies also enabled confirmation of the validity of conidium shape, dimensions and septum location as differential criteria.

C. EXAMINATION OF HERBARIUM MATERIAL

Two hundred and eighty collections of *Marssonina* species on poplars were received on short term loan from 13 overseas institutions (Appendix 1). Included in the material examined were local collections of infected poplars. For each collection conidia mounted in lactophenol and stained with 0.5% acid fuchsin were measured with a Leitz optical micrometer as detailed previously for examination of type specimens. In each instance identification was attempted on the basis of conidial morphological features as expressed by type material.

RESULTS

Specimens were readily identified on the basis of conidium morphology. Eighty six were identified as *M. populi*, 80 as *M. castagnei* and 114 as *M. brunnea*. The specimens examined and their dimensions are listed in Appendices 1 and 2 respectively. The species of *Marssonina* pathogenic to poplars in New Zealand was identified as *M. brunnea*.

Features of conidium morphology (shape, dimensions and septum location) exhibited by representatives of the three species are considered, as follows:

(a) Conidium Shape

Conidium shape was relatively constant, enabling ready delimitation of species. Conidia of *M. populi* were typically curved to strongly curved, and broadly obovoid to pyriform (Fig. 8). Conidia of *M. castagnei* were typically straight to slightly curved, and obovoid to broadly obovoid (Fig. 9). Conidia of *M. brunnea* were straight to slightly curved, and narrowly obovoid (Fig. 10).

(b) Conidium Dimensions

(i) Length and Breadth

The length and breadth dimensions of the three *Marssonina* species are presented in Table 7, and depicted graphically in Fig. 11. These results show that conidial dimensions of all three species overlapped, particularly those of *M. populi* and *M. castagnei*. Accordingly it was not possible to delimit the three species strictly on the basis of conidial dimensions. The low values recorded for the coefficients of variation for mean conidium

TABLE 7: Conidial dimensions of *Marssonina* species from herbarium material.

Species	Conidium Length (u) Range	Range of Mean	Mean	CVa	Conidium Breadth (u) Range	Range of Mean	Mean	CV
M. populi	13.0-30.0	$16.3^{b}_{1.5}$-$24.3_{1.6}$	$19.6_{1.4}$	7.1%	4.0-11.0	$5.3_{0.4}$-$9.0_{0.9}$	$6.7_{0.5}$	7.4%
M. castagnei	12.0-30.0	$14.6_{0.9}$-$19.4_{1.8}$	$17.9_{1.2}$	6.7%	4.0-9.0	$4.8_{0.3}$-$6.4_{0.6}$	$5.9_{0.4}$	6.8%
M. brunnea	10.0-23.0	$13.2_{1.0}$-$17.2_{1.8}$	$15.0_{0.8}$	5.3%	3.5-7.0	$3.7_{0.4}$-$5.5_{0.6}$	$4.4_{0.3}$	6.8%

a CV (Coeff. of variation) = $\frac{\text{standard deviation}}{\text{mean}}$ x 100.

b Standard deviation

TABLE 8: Conidial ratios of *Marssonina* species from herbarium material.

Species	L:B Ratio Range	Mean	CVa	TL:S Ratio Range	Mean	CV	LS:B Ratio Range	Mean	CV
M. populi	2.42-3.59	$2.93^{b}_{0.2}$	6.8%	3.04-4.08	$3.53_{0.2}$	5.6%	0.64-1.14	$0.83_{0.1}$	12.0%
M. castagnei	2.57-3.34	$3.02_{0.1}$	3.3%	2.30-2.90	$2.53_{0.1}$	3.9%	1.03-1.56	$1.20_{0.1}$	8.3%
M. brunnea	2.91-3.90	$3.42_{0.2}$	5.8%	2.60-3.70	3.10	6.4%	0.90-1.36	$1.10_{0.1}$	9.0%

a CV (Coeff. of variation) = $\frac{\text{Standard deviation}}{\text{mean}}$ x 100.

b Standard deviation

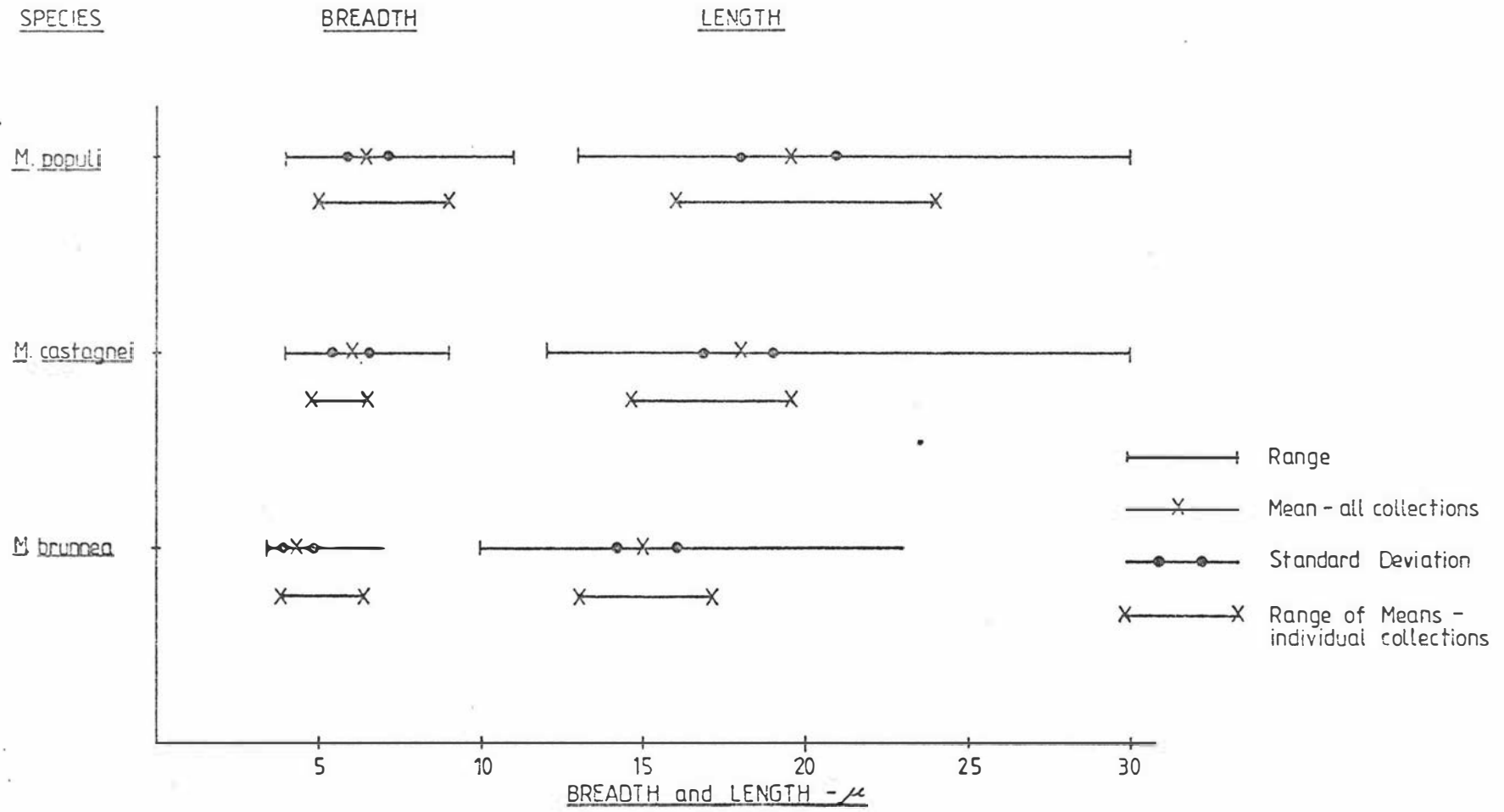


FIG. 11: Range, mean, standard deviation and range of means of conidial dimensions of Marssonina species from examination of field collections

TABLE 9: Location of the conidial septum of *Marssonina* species from herbarium material.

Species	Septum Location[a]		CV[b]
	Range	Mean	
M. populi	24.5-32.8	$28.1_{3.6_2}$	11.3%
M. castagnei	35.0-44.0	$39.6_{2.2}$	5.5%
M. brunnea	27.1-38.6	$32.4_{1.5}$	4.6%

[a] Expressed as a percentage of total conidium length measured from the conidium base.

[b] CV (Coeff. of variation) = $\frac{\text{standard deviation}}{\text{mean}} \times 100.$

[c] Standard deviation

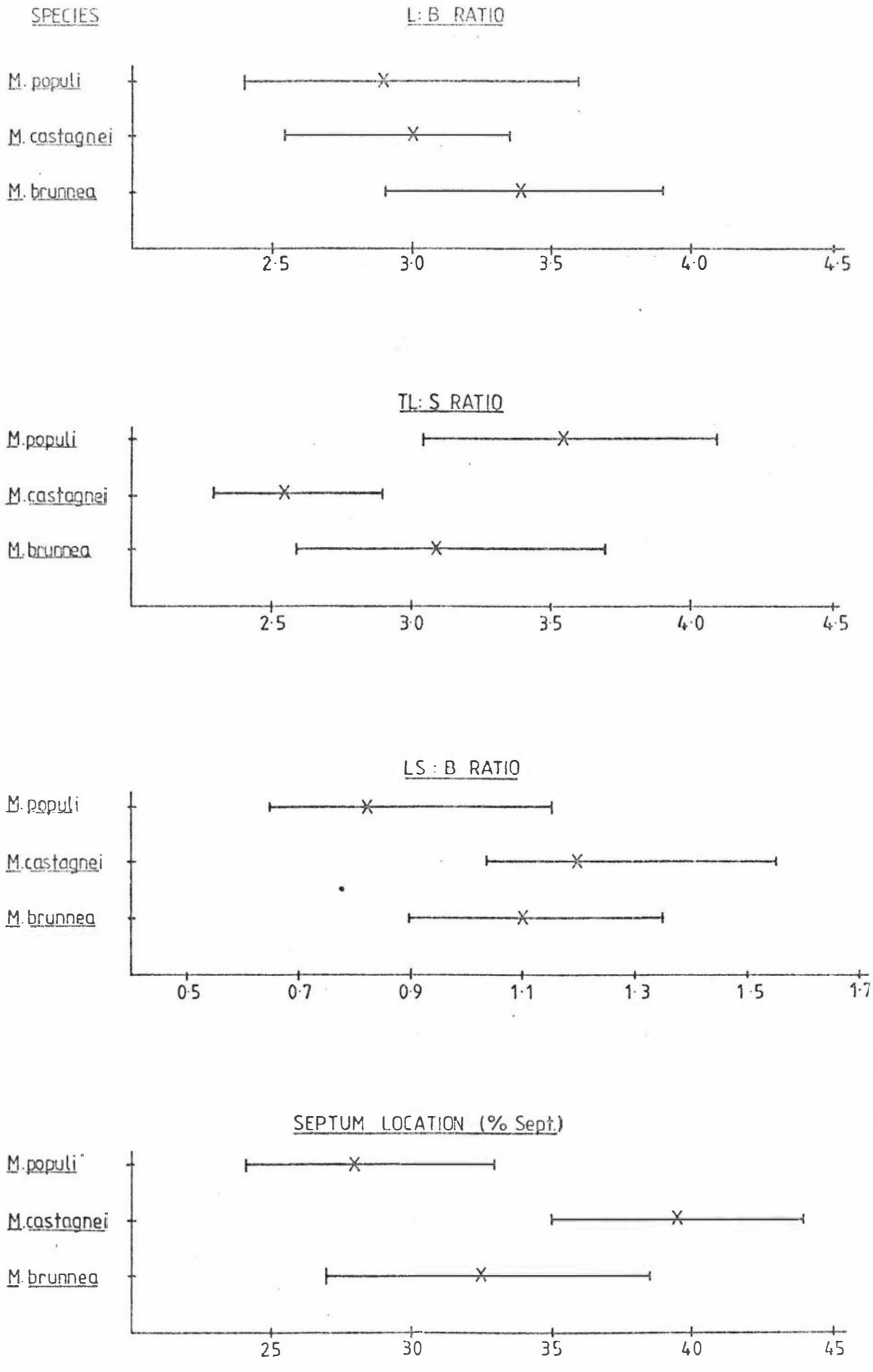


FIG. 12: Range (⊢——⊣) and mean (⊢—X—⊣) values for conidium ratios and septum location of Marssonina species from field collections

TABLE 10: Comparative conidial dimensions of *Marssonina* species from type and herbarium specimens.

Species	Conidium Length Range	Conidium Length Mean	Conidium Breadth Range	Conidium Breadth Mean	Ratios L:B	Ratios TL:S	Ratios LS:B	Septum Location
M. populi								
Type	15.0-30.0	$20.3^{a}_{2}{}_{.3}$	4.5-10.0	$6.6_{0.8}$	3.08	3.66	0.84	27.4
Herbarium	13.0-30.0	$19.6_{1.4}$	4.0-11.0	$6.7_{0.5}$	2.93	3.53	0.83	28.1
M. castagnei								
Type	11.0-21.0	$17.2_{1.6}$	5.0-7.0	$6.1_{0.4}$	2.83	2.44	1.15	41.0
Herbarium	12.0-30.0	$17.9_{1.2}$	4.0-9.0	$5.9_{0.4}$	3.02	2.53	1.20	39.6
M. brunnea								
Type	11.0-20.0	$14.8_{1.1}$	3.5-7.0	$4.6_{0.5}$	3.22	3.09	1.05	32.3
Herbarium	10-23.0	$15.0_{0.8}$	3.5-7.0	$4.4_{0.6}$	3.42	3.10	1.10	32.4

[a] Standard deviation

length and breadth within species was surprising in view of the diversity of host species, source countries and collection dates.

(ii) Conidium Ratios (L:B, TL:S, LS:B)

The values obtained are presented in Table 8 and depicted graphically in Fig. 12.

The L:B ratios of all three species overlapped strongly and were therefore of no value in distinguishing species (Fig. 12A).

The TL:S ratio enabled *M. populi* to be distinguished *M. castagnei*, but neither species from *M. brunnea* (Fig. 12B).

The LS:B ratios of the three species overlapped (Fig. 12C). However, the mean LS:B ratio of *M. populi* was distinct from those of *M. castagnei* and *M. brunnea*. Accordingly the LS:B ratio enabled identification of only those specimens of *M. populi* with a LS:B ratio of less than 0.90.

(c) Septum Location

The location of the conidial septum enabled separation of *M. castagnei* from *M. populi* and *M. brunnea* (Table 9, Fig. 12D).

Table 10 reveals that conidial features of type and herbarium specimens were closely comparable.

DISCUSSION

Following examination of 280 collections of poplars from throughout the world, dating from mid-18th century until the present day, three species were recognised on the basis of morphological features exhibited by the type specimens thus supporting recognition of three species. Furthermore these studies confirmed within each species the close morphological similarity between type and field collections, thereby establishing the validity of the type specimens as suitable species representatives.

As regards conidium shape, the above results are in agreement with Pirozynski (1974) who described conidia of *M. populi* as being curved, broadly obovoid to pyriform, *M. castagnei* as straight to slightly curved, obovoid and *M. brunnea* as being straight and narrowly obovoid.

Conidial dimensions have been used in part by Rimpau (1962), Gremmen (1965a) and Pirozynski (1974) to delimit *Marssonina* species. However in the present study conidial dimensions of the three species overlapped so as to preclude delimitation on the basis of this feature alone.

The L:B, TL:S and LS:B ratios have not previously been used to delimit species of *Marssonina* and although the L:B ratio was of no value, the TL:S ratio enabled separation of conidia of *M. populi* and *M. castagnei* but neither of these species from *M. brunnea*. Conidia of many but not all specimens of *M. populi* were distinguished from those of other *Marssonina* species by a LS:B ratio of <0.9.

The location of the conidial septum enabled separation of *M. castagnei* from other *Marssonina* species as reported by Gremmen (1965a). However in this species the septum was uniformily located 40% along the length of the conidium and not 'nearly half of total length', as reported by Gremmen (1965a). By contrast the septum in *M. populi* and *M. brunnea* was commonly located 28% and 33% along the conidium respectively, or 'about one third of total length' (Gremmen, 1965a).

In summary, conidium shape in combination with length and breadth dimensions, TL:S and LS:B ratios and septum location were criteria for differentiating *Marssonina* species pathogenic to poplars. The opinions of Thompson (1937) and Boyer (1961), that strains of *Marssonina* were too variable to be taxonomically distinguishable proved to be invalid. In fact the low values recorded within species for the coefficients of variation for conidial features clearly reflected the stability of conidium morphology. This uniformity was remarkable considering the diverse origins of specimens.

D. INFLUENCE OF ENVIRONMENTAL FACTORS ON CONIDIUM MORPHOLOGY

In practice a species should include individuals separated from other groups of individuals by well marked discontinuities, preferably in several unrelated characters (Hawksworth, 1974). Interpretation of what constitutes 'a well marked discontinuity' however, must rest with the taxonomist studying the specific fungus in question. In this regard the writer agrees with the concept of speciation as proposed by Snyder & Hansen (1954) namely, that species delimitation should be based only on relatively stable morphological characters selected following extensive experimental studies to first determine the full extent of morphologic variability.

The Snyder & Hansen (1954) classification of species of the genus *Fusarium* provides a working example of both their philosophy and the current trend towards consolidation of species, and suggests the pattern that could well be followed in the revision of any genus. The first step is 'collection' and involves the assembly of isolates from as many geographic regions as possible. The second step is 'analysis' whereby isolates are cultured on various media or hosts under varying conditions to identify those morphological characters least prone to environmentally induced variability. The third and final step of 'synthesis' involves selection of those morphologic features serving to best differentiate species.

As regards *Marssonina* species pathogenic to the genus *Populus*, Boyer (1961) found that 'spore size and distribution' on PDA and host tissue were influenced to such a degree by environmental factors so as to render this criterion of no value in species delimitation. Obviously Rimpau (1962), Gremmen (1965a) and Pirozynski (1974) disagreed since they in part used conidial dimensions (as expressed on host tissue) for delimitation of *M. brunnea, M. populi* and *M. castagnei*. However since the work of Boyer (1961) there have been no further indepth studies reporting the influence of environmental factors on expression of conidial morphological features of *Marssonina* species pathogenic to poplars.

A further precept of modern taxonomy is that organisms be identifiable to species, 'on the basis of what they are, not upon where they occur or what they do' (Snyder & Hansen, 1954). It follows that morphological criteria identified as suitable for species differentiation on culture media must be equally valid in the field situation. Ideally

then concurrent studies should be conducted on culture media and host tissue to determine initially the range of variability expressed by conidial features under varying environmental conditions and hence identify those features which are relatively stable and secondly to determine the environmental conditions under which these diagnostic features are most clearly expressed. The agar leaf-disc technique (Spiers, 1978) readily enables such parallel taxonomic studies.

Experiments were conducted to determine in culture and host tissue the influence of environmental factors on the range of conidium variability expressed by the three *Marssonina* species ('analysis' *sensu* Snyder & Hansen, 1954). Environmental factors studied were:

(i) media, pH, incubation temperature and photoperiod (in culture),

(ii) leaf age, leaf surface, host resistance, infection level, incubation temperature and photoperiod (on host tissue).

In a third experiment conducted under field conditions the influence of environmental factors on conidium morphology of *M. brunnea* was determined. Only *M. brunnea* was studied since it was the only species established in New Zealand.

1. LABORATORY STUDIES ON THE INFLUENCE OF CULTURAL FACTORS

Materials and Methods

Monosporous isolates (Table 11) were obtained from infected poplar leaves by streaking conidia onto potato dextrose agar (PDA). The plates were dark-incubated at 20°C for two days and single germinating conidia transferred to fresh plates. Isolates were held on PDA and 15%V8 juice agar (15%V8) slants at 5°C. Experiments were conducted in two series, the first being exploratory.

TABLE 11: Isolates of *Marssonina* examined in morphological studies on culture media

Species and Isolate	Host Species	Origin	Collector	Comments/Collection Date
M. brunnea				
Br 1	*P. yunnanensis*[a]	Palmerston North, NZ	Author	10/1/1978
Br 2	*P.* x *euramericana*	Pahiatua, NZ	Author	10/1/1978
Br 3	*P. fremontii* cv. ANU61/48	Palmerston North, NZ	Author	10/1/1978
Br 4	*P. frem.* x *P. nigra* Sempervirens cv. ANU66/9	Palmerston North, NZ	Author	10/1/1978
Br 5	*P.* x *eura.* cv. I214	Palmerston North, NZ	Author	10/1/1978
Br 6	*P. alba*	Palmerston North, NZ	Author	10/1/1978
Br 7	*P.* x *eura.* cv. Flevo	Palmerston North, NZ	Author	10/1/1978
Br 8	*P.* x *eura.* cv. NL2194	Palmerston North, NZ	Author	10/1/1978
Br 9	*P. delt.* x *P. maxi.* cv. I83/58	Palmerston North, NZ	Author	10/1/1978
Br 10	*P. deltoides*	Urbana, Illinois, USA	J. J. Jokela	No. 78079, 7/7/1978
Br 11	*P. deltoides*	Urbana, Illinois, USA	J. J. Jokela	No. 78071, 7/7/1978
Br 12	*P. deltoides*	Urbana, Illinois, USA	J. J. Jokela	No. 78085, 7/7/1978
Br 13	*P. deltoides*	Iowa, USA	A. L. Schipper	24/11/1978
Br 14	*P. trichocarpa*	St. Paul, Minnesota, USA	A. L. Schipper	8/1978
Br 15	*P. deltoides*	Colorado, USA	T. E. Hinds	30/10/1979
Br 16	*P.* x *eura.* cv. Robusta	Kew, Surrey, England	D. A. Burdekin	27/7/1979
Br 17	*P.* x *eura.* cv. Robusta	Dublin, Ireland	F. O'Riordain	31/10/1979
Br 18	*P.* x *eura.*	Nancy, France	J. Pinon	11/5/1978
Br 19	*P. deltoides*	Hees, Holland	Author	Ex poplar seed
Br 20	*P.* x *eura.* cv. I214	Ankara, Turkey	M. Vural	5/12/1979
Br 21	*P. tremuloides*	Vail, Colorado, USA	T. E. Hinds	8/1978
Br 22	*P. tremuloides*	Vail, Colorado, USA	T. E. Hinds	8/1978
Br 23	*P. tremuloides*	Fairbanks, Alaska, USA	T. E. Hinds	3/8/1977
Br 24	*P. tremuloides*	Rosemount, Minnesota, USA	M. E. Ostry	19/7/1979

CONTINUED ON PAGE 34

TABLE 11 CONTINUED: Isolates of *Marssonina* examined in morphological studies on culture media

Species and Isolate	Host Species	Origin	Collector	Comments/Collection Date
M. castagnei				
Cs 1	*P. alba*	Zurich, Switzerland	R. H. Rimpau	12/5/1961, ETH4551
Cs 2	*P. alba*	Dublin, Ireland	F. O'Riordain	31/10/1979
Cs 3	*P. alba*	Ankara, Turkey	M. Vural	/12/1979
M. populi				
Po 1	*P.* x *canadensis*	Herts., England	J.S.W. Dickens	13/11/1979
Po 2	*P. nigra* cv. Italica	Bletchley, Milton, England	A. G. Bailey	10/11/1979
Po 3	*P. nigra* cv. Italica	Kew, Surrey, England	A. G. Bailey	12/11/1979
Po 4	*P. nigra* cv. Italica	Dublin, Ireland	F. O'Riordain	31/10/1979
Po 5	*P.* x *berolinensis*	Munchen, Germany	P. Schutt	
Po 6	*P. nigra*	Zurich, Switzerland	R. H. Rimpau	5/5/1961, ETH M4548
Po 7	*P. nigra*	Zurich, Switzerland	R. H. Rimpau	31/7/1960, ETH S2474

[a] *P. frem.* = *P. fremontii*
P. x *eura.* = *P.* x *euramericana* (*P. nigra* x *P. deltoides*)
P. delt. = *P. deltoides*
P. maxi. = *P. maximowiczii*

Series I

Single isolates of *M. populi*, *M. castagnei* and *M. brunnea* were incubated at 20°C under 12 hours light/12 hours dark on 18 growth media (pH 6.5). The growth media tested were: 5%, 10%, 15%, 20% V8 juice agar (%V8); cornmeal dextrose agar, (CDA); neopeptone glucose agar, (NPG); Czapek Dox agar, (CD); glucose peptone agar, (GPA); prune agar, (PA); malt agar, (MA); Leonian malt agar, (LMA); Sabouraud agar (SA); tryptone glucose yeast agar, (TGYA); Tochinai agar, (THA); oatmeal agar (OA); potato carrot agar, (PCA); potato carrot agar + 10%V8 juice, (PC-10); and potato dextrose agar, (PDA). Features of conidium morphology were evaluated following 10 days incubation.

The most suitable media for production of regular conidia were CDA, PDA, PCA, PC-10 and 15%V8.

To determine the influence of media pH, incubation temperature and photoperiod on conidial morphology, the three *Marssonina* species were incubated under the following conditions on PDA and 15%V8 for 10 days:

The influence of media pH was assessed on isolates of the 3 species incubated at 20°C under a 12 hour white light photoperiod on both media adjusted to pH levels of: 5.5, 6.0, 6.5, 7.0 and 7.5.

The influence of incubation temperature was assessed on PDA and 15%V8, pH 6.5, incubated under a 12 hour white light photoperiod at temperatures of: 12°C, 16°C, 20°C, 24°C and 28°C.

The influence of photoperiod was evaluated on PDA and 15%V8, pH 6.5, incubated at 20°C under light regimes of:

(a) continuous dark,
(b) 12 hour cool white fluorescent light/12 hour dark,
(c) 12 hour near ultra-violet (General Electric (BLB) NUV) light/12 hour dark.

From these exploratory experiments the optimum cultural conditions for production of uniform conidia of the three species were:

media	-	PDA, CDA, PCA, PC-10, 15%V8,
pH	-	pH 6.0, 6.5,
temperature	-	20°C and 24°C,
photoperiod	-	12 h white light or 12 h NUV light.

For routine use, 15%V8, pH 6.5, 20°C and a 12 hour white light photoperiod were selected. It should be noted that mycelial growth and sporulation intensity of all three species was acceptable under these conditions.

Series II

In this series the influence of a specific environmental factor on conidial morphology was determined by growing isolates (Table 11) at optimum conditions for the other three environmental factors, as follows:

(A) The influence of media

Isolates were incubated on PDA, CDA, PCA, PC-10 and 15%V8, pH 6.5, at 20°C under a 12 hour, white light photoperiod.

(B) The influence of media pH

Isolates were incubated on 15%V8 at pH 5.5, 6.0, 6.5, 7.0, 7.5 at 20°C, under a 12 hour, white light photoperiod.

(C) The influence of incubation temperature

Isolates were incubated on 15%V8, pH 6.5, at 12, 16, 20, 24, 28°C under a 12 hour, white light photoperiod.

(D) The influence of photoperiod

Isolates were incubated on 15%V8, pH 6.5 at 20°C under: continuous dark; 12 hour, white light; and 12 hour, NUV light (General Electric BLB) photoperiod.

Following 10 days incubation, conidia mounted in lactophenol and stained with 0.5% acid fuchsin were measured with a Leitz optical micrometer (500X). The lengths and breadths of sixty conidia per treatment were measured and the TL:S, LS:B ratios and location of the conidial septum determined. These calculations enabled the influence of environmental factors on conidium morphology to be assessed with some precision.

RESULTS

The raw data for the influence of growth media, media pH, incubation temperature and photoperiod on conidial morphology are presented in Appendices 3-6 respectively.

A. GROWTH MEDIA

(a) Conidium Shape

Conidium shape of the three species was similarly affected by culture media. Within species, conidium morphology on PDA and CDA, was different from that on PCA, PC-10 and 15%V8 (Figs. 13, 14, 15).

(b) Conidium Dimensions

(i) Length and Breadth

Conidial dimensions of *M. brunnea* were markedly affected by culture media (Table 12), the length and breadth dimensions on PDA and CDA being significantly different (probability, $P > 0.05$, using a t-test) to those on PCA, PC-10 and 15%V8, whereas the dimensions on PCA, PC-10 and 15%V8 were not significantly different ($P>0.05$), from each other.

Conidial lengths of neither *M. populi* nor *M. castagnei* were significantly different ($P>0.05$) on the various media. However the breadth of conidia of *M. populi* on CDA and of *M. castagnei* on PDA and CDA were significantly greater ($P>0.05$) than on other media (Table 12).

(ii) TL:S and LS:B ratios

The TL:S and LS:B ratios of the 3 species varied between media, particularly the LS:B ratio of *M. brunnea* and *M. castagnei*, and the TL:S ratio of *M. populi* (Table 12).

(c) Septum Location (% Sept.)

Septum location was not markedly influenced by culture media (Table 12).

B. MEDIA PH

Media pH had no appreciable effect on conidium shape, dimensions, TL:S and LS:B ratios and septum location, as evidenced by the small values obtained for the coefficients of variation (Table 13).

TABLE 12: Influence of culture media on mean conidial dimensions of *Marssonina* species following 10 days incubation at 20°C under 12 hours white light photoperiod.

Species and Treatment	Conidial Dimensions (u) Mean Length	Mean Breadth	TL:S Ratio	LS:B Ratio	% Sept.
M. brunnea					
CDA	$17.3^{a}_{3.3}$	$5.1_{0.5}$	3.00	1.06	33.3
PDA	$17.3_{3.3}$	$5.0_{0.5}$	3.01	1.08	33.1
PCA	$14.9_{0.3}$	$4.2_{0.4}$	3.13	1.20	32.0
PC-10	$15.1_{0.8}$	$4.2_{0.4}$	3.06	1.21	32.7
15%V8	$15.3_{0.7}$	$4.2_{0.4}$	3.05	1.22	32.7
Mean	$16.0_{1.2}$	$4.5_{0.5}$	$3.05_{0.05}$	$1.15_{0.07}$	$32.7_{0.5}$
CV[b]	7.5%	11.1%	1.6%	6.0%	1.5%
M. castagnei					
CDA	$21.8_{4.2}$	$6.6_{0.9}$	2.62	1.30	39.0
PDA	$21.0_{4.4}$	$6.6_{0.8}$	2.50	1.25	40.1
PCA	$20.0_{2.8}$	$5.9_{0.3}$	2.43	1.42	41.0
PC-10	$20.4_{2.5}$	$5.9_{0.5}$	2.47	1.41	41.0
15%V8	$21.5_{3.0}$	$5.7_{0.3}$	2.45	1.43	41.0
Mean	$21.0_{0.7}$	$6.1_{0.4}$	$2.50_{0.07}$	$1.36_{0.08}$	$40.4_{0.9}$
CV	3.3%	6.5%	2.8%	5.9%	2.2%
M. populi					
CDA	$22.3_{3.9}$	$6.7_{0.5}$	3.14	1.00	32.0
PDA	$23.0_{4.0}$	$6.1_{2.1}$	3.20	1.00	31.4
PCA	$22.7_{4.4}$	$6.3_{0.7}$	3.50	1.03	28.7
PC-10	$22.6_{4.1}$	$6.1_{0.7}$	3.40	1.06	29.6
15%V8	$22.6_{4.1}$	$6.2_{0.7}$	3.50	1.01	29.0
Mean	$22.6_{0.6}$	$6.3_{0.2}$	$3.35_{0.17}$	$1.02_{0.02}$	$30.1_{1.5}$
CV	0.9%	3.1%	5.0%	2.0%	5.0%

[a] Standard deviation

[b] CV (coefficient of variation) = $\frac{\text{standard deviation}}{\text{mean conidial dimension}} \times 100$

TABLE 13: Influence of media pH on mean conidial dimensions of *Marssonina* species following 10 days incubation on 15%V8 at 20°C under 12 hours white light photoperiod.

Species and Treatment	Conidial Dimensions (u) Mean Length	Mean Breadth	TL:S Ratio	LS:B Ratio	% Sept.
M. brunnea					
pH 5.5	$15.3^{a}_{0.9}$	$4.2_{0.4}$	3.03	1.23	33.0
6.0	$15.4_{0.8}$	$4.2_{0.5}$	3.04	1.24	32.8
6.5	$15.3_{0.8}$	$4.2_{0.5}$	3.05	1.22	32.7
7.0	$15.3_{0.8}$	$4.2_{0.4}$	3.02	1.25	33.0
7.5	$15.2_{0.7}$	$4.2_{0.4}$	3.03	1.23	33.0
Mean	$15.3_{0.07}$	$4.2_{0.0}$	$3.03_{0.01}$	$1.23_{0.01}$	$33.0_{0.1}$
CV[b]	0.4%	0.0%	0.3%	0.8%	0.3%
M. castagnei					
pH 5.5	$21.0_{2.2}$	$6.2_{0.2}$	2.42	1.40	41.4
6.0	$21.3_{2.8}$	$6.1_{0.3}$	2.47	1.44	41.0
6.5	$21.4_{3.0}$	$6.1_{0.3}$	2.43	1.46	41.3
7.0	$21.6_{2.7}$	$6.0_{0.3}$	2.38	1.49	41.4
7.5	$21.1_{3.0}$	$6.2_{0.3}$	2.46	1.40	41.2
Mean	$21.3_{0.2}$	$6.1_{0.08}$	$2.43_{0.03}$	$1.44_{0.04}$	$41.3_{0.16}$
CV	0.9%	1.3%	1.2%	2.8%	0.4%
M. populi					
pH 5.5	$22.3_{4.0}$	$6.2_{0.6}$	3.48	1.01	29.0
6.0	$22.6_{4.0}$	$6.2_{0.6}$	3.46	1.01	29.0
6.5	$22.5_{4.1}$	$6.2_{0.5}$	3.47	1.00	29.0
7.0	$22.6_{4.0}$	$6.2_{0.6}$	3.43	1.04	29.3
7.5	$22.5_{4.0}$	$6.1_{0.6}$	3.43	1.03	29.3
Mean	$22.5_{0.12}$	$6.2_{0.04}$	$3.45_{0.02}$	$1.02_{0.01}$	$29.1_{0.16}$
CV	0.5%	0.6%	0.6%	1.0%	0.5%

[a] Standard deviation

[b] CV (coefficient of variation) = $\frac{\text{standard deviation}}{\text{mean conidial dimension}} \times 100$

TABLE 14: Influence of incubation temperature on mean conidial dimensions of *Marssonina* species following 10 days incubation on 15%V8 agar (pH 6.5) under 12 hours white light photoperiod.

Species and Treatment	Conidial Dimensions (u) Mean Length	Mean Breadth	TL:S Ratio	LS:B Ratio	% Sept.
M. brunnea					
12°C	$15.6^{a}_{0.8}$	$4.2_{0.5}$	3.03	1.25	32.9
16°C	$15.6_{0.8}$	$4.2_{0.5}$	3.03	1.25	33.0
20°C	$15.4_{0.8}$	$4.2_{0.5}$	3.02	1.25	33.1
24°C	$15.4_{0.8}$	$4.2_{0.5}$	3.01	1.25	33.2
28°C	$15.1_{0.9}$	$4.3_{0.5}$	3.01	1.21	33.2
Mean	$15.4_{0.2}$	$4.2_{0.04}$	$3.02_{0.01}$	$1.24_{0.02}$	$33.1_{0.13}$
CV[b]	1.3%	0.9%	0.3%	1.6%	0.4%
M. castagnei					
12°C	$21.5_{2.8}$	$6.1_{0.2}$	2.40	1.44	42.1
16°C	$21.0_{2.7}$	$6.0_{0.2}$	2.41	1.46	42.0
20°C	$21.4_{3.0}$	$6.1_{0.2}$	2.41	1.46	41.5
24°C	$21.0_{2.4}$	$6.1_{0.3}$	2.43	1.38	40.6
28°C	No growth				
Mean	$21.2_{0.26}$	$6.1_{0.05}$	$2.41_{0.01}$	$1.43_{0.04}$	$41.5_{0.7}$
CV	1.2%	0.8%	0.4%	2.8%	1.7%
M. populi					
12°C	$23.1_{3.8}$	$6.2_{0.6}$	3.25	1.10	30.9
16°C	$22.8_{3.6}$	$6.2_{0.5}$	3.32	1.07	30.3
20°C	$22.7_{3.9}$	$6.2_{0.5}$	3.40	1.04	29.5
24°C	$22.8_{3.8}$	$6.2_{0.5}$	3.44	1.03	28.5
28°C	No growth				
Mean	$22.8_{0.17}$	$6.2_{0.0}$	$3.35_{0.08}$	$1.06_{0.03}$	$30.0_{1.0}$
CV	0.7%	0.0%	2.4%	2.8%	3.3%

[a] Standard deviation

[b] CV (coefficient of variation) = $\frac{\text{standard deviation}}{\text{mean conidial dimension}} \times 100$

TABLE 15: Influence of photoperiod on mean conidial dimensions of *Marssonina* species following 10 days incubation on 15%V8 agar (pH 6.5) at 20°C.

Species and Treatment	Conidial Dimensions (u) Mean Length	Mean Breadth	TL:S Ratio	LS:B Ratio	% Sept.
M. brunnea					
Dark	$15.4_{0.7}$[a]	$4.2_{0.4}$	3.04	1.25	32.8
12 h white light	$15.5_{0.8}$	$4.2_{0.4}$	3.05	1.25	32.8
12 h NUV	$15.5_{0.8}$	$4.2_{0.4}$	3.05	1.25	32.8
Mean	$15.5_{0.06}$	$4.2_{0.0}$	$3.05_{0.006}$	$1.25_{0.0}$	$32.8_{0.0}$
CV[b]	0.4%	0.0%	0.002%	0.0%	0.0%
M. castagnei					
Dark	$21.8_{2.7}$	$6.1_{0.2}$	2.40	1.46	41.6
12 h white light	$21.5_{2.5}$	$6.0_{0.2}$	2.45	1.47	41.0
12 h NUV	$21.6_{2.4}$	$6.2_{0.2}$	2.35	1.48	42.4
Mean	$21.6_{0.16}$	$6.1_{0.1}$	$2.40_{0.05}$	$1.47_{0.01}$	$41.7_{0.7}$
CV	0.7%	1.6%	2.0%	0.07%	1.7%
M. populi					
Dark	$22.6_{3.7}$	$6.2_{0.5}$	3.34	1.05	30.1
12 h white light	$22.8_{3.8}$	$6.1_{0.5}$	3.44	1.05	29.1
12 h NUV	$22.6_{4.0}$	$6.2_{0.7}$	3.50	1.00	29.1
Mean	$22.7_{0.1}$	$6.2_{0.06}$	$3.43_{0.08}$	$1.03_{0.03}$	$29.4_{0.6}$
CV	0.4%	0.9%	2.3%	2.9%	2.0%

[a] Standard deviation

[b] CV (coefficient of variation) = $\frac{\text{standard deviation}}{\text{mean conidial dimension}} \times 100$

TABLE 16: Influence of cultural and environmental factors on conidium morphology of *Marssonina* species (All units expressed as coefficients of variation).

Species and Treatment	Conidial Dimensions Length	Breadth	TL:S Ratio	LS:B Ratio	% Sept.	Mean per factor
M. brunnea						
Media	7.5 [a]	11.1	1.6	6.0	1.5	5.5
pH	0.4	0.0	0.3	0.8	0.3	0.4
Temperature	0.01	0.9	0.3	1.6	0.4	0.6
Photoperiod	0.4	0.0	0.0	0.0	0.0	0.1
Mean/factor	2.1	3.0	0.5	2.1	0.5	
M. castagnei						
Media	3.3	6.5	2.8	5.9	2.2	4.1
pH	0.9	1.3	1.2	2.8	0.4	1.3
Temperature	1.2	0.8	0.4	2.8	1.7	1.4
Photoperiod	0.7	1.6	2.0	0.07	1.7	1.2
Mean/factor	1.5	2.5	1.6	2.9	1.5	
M. populi						
Media	0.9	3.1	5.0	2.0	5.0	3.2
pH	0.5	0.6	0.6	1.0	0.5	0.6
Temperature	0.7	0.0	2.4	2.8	3.3	1.8
Photoperiod	0.4	0.9	2.3	2.9	2.0	1.7
Mean/factor	0.6	1.1	2.6	2.2	2.7	

[a] CV (coefficient of variation) = $\frac{\text{standard deviation of overall mean}}{\text{overall mean of conidial feature}} \times 100$

C. INCUBATION TEMPERATURE

Incubation temperature had no appreciable effect on conidium shape, dimensions, TL:S and LS:B ratios and septum location as reflected by the low values recorded for the coefficient of variation for each factor (Table 14).

D. PHOTOPERIOD

Photoperiod had no appreciable effect on conidium shape, dimensions, TL:S and LS:B ratios and septum location. For each factor the coefficient of variation was small (Table 15).

The influence of media, media pH, incubation temperature and photoperiod on the coefficient of variation is summarised in Table 16. These results show that conidium morphology was most markedly affected by media. By contrast, the influences of media pH, incubation temperature and photoperiod were minor, in most instances less than 3.0%. The best conditions for expression of differential taxonomic criteria were incubation on 15%V8 agar, pH 6.5, 20°C under a 12 hour white light photoperiod for at least 10 days.

In summary, the above cultural studies have convincingly demonstrated the stability of conidium shape, dimensions, ratios and septum location under varying environmental conditions (pH, temperature, photoperiod) on a single specified medium. These results were somewhat surprising in view of the many reports of the expression of morphological features of other fungi being modified by media, pH, temperature and light (Williams, 1959; Ruppel, 1974; Harding, 1975; Misaghi *et al*, 1978).

2. LABORATORY STUDIES ON THE INFLUENCE OF HOST FACTORS

Materials and Methods

The influence of host and environmental factors on conidium morphology of single isolates of *M. brunnea* (Br 5), *M. populi* (Po 6), and *M. castagnei* (Cs 2) were determined in the laboratory. Leaf discs (2.5 cm diam) of *P. nigra* cv. Italica 'Aurea' inserted into plates of 2% WA (Spiers, 1978) were inoculated with conidial suspensions of each isolate. Except where specified, leaf discs from fully expanded, mature leaves were inoculated on the adaxial surface with 5000 conidia/disc.

A. HOST FACTORS

(i) Influence of Leaf Surface

The adaxial and abaxial leaf surfaces of mature leaves were inocu'ated and incubated at 20°C with a 12 hour white light photoperiod.

(ii) Influence of Leaf Age

The adaxial surface of leaf discs from:

(a) very soft, expanding leaves,
(b) soft, expanded leaves,
(c) hard, mature leaves,
(d) overmature, senescing leaves,

were inoculated and incubated at 20°C with a 12 hour white light photoperiod.

(iii) Influence of Infection Level

Leaf discs were inoculated with 1000 conidia/disc and 100,000 conidia/disc which resulted in light and extremely heavy necrotic disease reactions following incubation at 20°C with a 12 hour white light photoperiod.

(iv) Influence of Host Resistance

Hosts of varying resistance were inoculated and incubated at 20°C under a 12 hour white light photoperiod.

B. ENVIRONMENTAL FACTORS

(i) Influence of Temperature

Following inoculation, leaf discs were incubated at temperatures of 12°C, 16°C, 20°C, 24°C, 28°C and 24/8°C, all with a 12 hour white light photoperiod. With the temperature regime 24/8°C the incubator was programmed to give 24°C for 14 hours then a temperature change to 8°C for 10 hours.

(ii) Influence of Photoperiod

Following inoculation leaf discs were incubated at 20°C under:

(a) continuous dark,
(b) 12 hour cool white, fluorescent light/12 hour dark,
(c) 12 hour NUV light/12 hour dark.

For each treatment, eight leaf discs were inoculated. Twelve days later conidia mounted in lactophenol and stained with 0.5% acid fuschin were measured (60 conidia, 500x, Leitz micrometer).

RESULTS

The influence of host and environmental factors on conidium morphology is summarised in Tables 17-24. In summary, these results show that conidium shape, dimensions, ratios and septum location were affected only to a minor degree and hence were of taxonomic value.

A. HOST FACTORS

(i) Influence of Leaf Surface

Leaf surface had no appreciable effect on conidium shape, conidial dimensions, TL:S and LS:B ratios and septum location (Table 17).

(ii) Influence of Leaf Age

Leaf age had no appreciable effect on conidium shape, conidial dimensions, TL:S and LS:B ratios and septum location (Table 18).

(iii) Influence of Infection Level

Infection level had no appreciable effect on conidium shape, conidial dimensions, TL:S and LS:B ratios and septum location (Table 19).

(iv) Influence of Host Resistance

Within species, conidial dimensions often differed significantly ($P>0.05$) between hosts (Tables 20, 21, 22). However the differences were small, less than 2 microns for conidium length and 0.5 microns for conidium breadth. In no instance was conidium shape greatly affected (Figs. 16, 17, 18). In all instances, the coefficients of variation for conidium length and breadth were small (<3%), confirming the stability of conidial dimensions on different hosts.

B. ENVIRONMENTAL FACTORS

(i) Influence of Temperature

Temperature had no appreciable effect on conidium shape, dimensions, TL:S and LS:B ratios and septum location (Table 23).

TABLE 17: Influence of leaf surface on conidial dimensions of *Marssonina* species following 12 days incubation at 20°C under 12 hours white light photoperiod.

Species and Treatment	Conidial Dimensions (u) Mean Length	Mean Breadth	TL:S Ratio	LS:B Ratio	% Sept.
M. brunnea					
Adaxial	$14.4^{a}_{0.6}$	$4.1_{0.2}$	3.20	1.09	31.3
Abaxial	$14.4_{0.5}$	$4.1_{0.2}$	3.20	1.09	31.3
Mean	$14.4_{0.0}$	$4.1_{0.0}$	3.20	$1.09_{0.0}$	$31.3_{0.0}$
CV[b]	0.0%	0.0%	0.0%	0.0%	0.0%
M. castagnei					
Adaxial	$17.3_{1.3}$	$5.3_{0.3}$	3.06	1.06	32.6
Abaxial	$17.2_{1.2}$	$5.2_{0.2}$	3.05	1.07	32.8
Mean	$17.2_{0.07}$	$5.2_{0.07}$	$3.05_{0.0}$	1.06	$32.7_{0.14}$
CV	0.4%	1.3%	0.0%	0.0%	0.4%
M. populi					
Adaxial	$19.3_{1.2}$	$6.0_{0.3}$	3.40	0.95	29.4
Abaxial	$19.5_{1.0}$	$6.0_{0.3}$	3.61	0.91	27.7
Mean	$19.4_{0.14}$	$6.0_{0.0}$	$3.51_{0.15}$	$0.93_{0.3}$	$28.5_{1.2}$
CV	0.7%	0.0%	4.2%	3.2%	4.2%

[a] Standard deviation

[b] CV (Coeff. of variation) = $\frac{\text{standard deviation}}{\text{mean conidial dimension}} \times 100$

TABLE 18: Influence of leaf age on conidial dimensions of *Marssonina* species following 12 days incubation at 20°C under 12 hours white light photoperiod.

Species and Treatment	Conidial dimensions (u) Mean Length	Mean Breadth	TL:S Ratio	LS:B Ratio	% Sept.
M. brunnea					
Soft 1 [a]	$14.1_{0.6}$ [b]	$4.1_{0.2}$	3.25	1.06	30.7
2	$14.3_{0.5}$	$4.1_{0.2}$	3.20	1.08	31.3
Mature	$14.2_{0.6}$	$4.1_{0.2}$	3.27	1.06	30.6
Senescent	$14.1_{0.6}$	$4.0_{0.2}$	3.18	1.09	31.4
Mean	$14.3_{0.1}$	$4.1_{0.02}$	$3.21_{0.04}$	$1.08_{0.015}$	$31.0_{0.4}$
CV [c]	0.7%	0.5%	1.3%	1.3%	1.3%
M. castagnei					
Soft 1	$17.0_{1.0}$	$5.4_{0.3}$	2.85	1.10	34.0
2	$16.8_{1.2}$	$5.4_{0.3}$	2.80	1.14	35.7
Mature	$16.7_{0.8}$	$5.3_{0.3}$	2.94	1.07	33.9
Senescent	$16.5_{1.0}$	$5.3_{0.3}$	3.12	1.06	34.2
Mean	$16.5_{0.2}$	$5.3_{0.06}$	$2.93_{0.14}$	$1.09_{0.03}$	$34.4_{0.8}$
CV	1.2%	1.1%	4.8%	3.2%	2.4%
M. populi					
Soft 1	$19.6_{1.0}$	$6.0_{0.3}$	3.56	0.92	28.0
2	$19.3_{1.2}$	$6.0_{0.4}$	3.52	0.94	28.4
Mature	$19.3_{1.3}$	$6.0_{0.3}$	3.52	0.94	29.4
Senescent	$19.6_{1.0}$	$6.0_{0.4}$	3.56	0.92	28.5
Mean	$19.5_{0.16}$	$6.0_{0.0}$	$3.54_{0.02}$	$0.93_{0.01}$	$28.6_{0.6}$
CV	0.8%	0.0%	0.6%	1.2%	2.0%

[a] Soft 1 Soft expanding leaf tissue
2 Soft expanded leaf tissue

[b] Standard deviation

[c] CV (coeff. of variation) = $\frac{\text{standard deviation}}{\text{mean conidial dimension}} \times 100$

TABLE 19: Influence of infection level on conidial dimensions of *Marssonina* species following 12 days incubation at 20°C under 12 hours white light photoperiod.

Species and Treatment	Conidial dimensions (u) Mean Length	Mean Breadth	TL:S Ratio	LS:B Ratio	% Sept.
M. brunnea					
Light [a]	$14.2_{0.6}$ [b]	$4.1_{0.2}$	3.20	1.07	31.2
Necrotic	$14.4_{0.6}$	$4.1_{0.2}$	3.15	1.11	31.7
Mean	$14.3_{0.1}$	$4.1_{0.0}$	$3.18_{0.03}$	$1.09_{0.03}$	$31.4_{0.3}$
CV [c]	0.7%	0.0%	1.1%	2.6%	1.1%
M. castagnei					
Light	$17.1_{0.9}$	$5.2_{0.3}$	2.95	1.11	33.8
Necrotic	$17.0_{0.9}$	$5.1_{0.2}$	3.08	1.09	32.5
Mean	$17.0_{0.07}$	$5.1_{0.07}$	$3.02_{0.09}$	$1.10_{0.01}$	$33.1_{0.9}$
CV	0.4%	1.4%	3.0%	0.01%	2.8%
M. populi					
Light	$19.6_{1.1}$	$6.0_{0.3}$	3.43	0.95	29.1
Necrotic	$19.6_{1.0}$	$6.0_{0.4}$	3.57	0.90	27.0
Mean	$19.6_{0.0}$	$6.0_{0.0}$	$3.50_{0.1}$	$0.93_{0.03}$	$28.0_{1.5}$
CV	0.0%	0.0%	2.8%	3.8%	5.3%

a Light-inoculated with 1000 conidia/disc
Necrotic-inoculated with 100,000 conidia/disc

b Standard deviation

c CV (coeff. of variation) = $\frac{\text{standard deviation}}{\text{mean conidial dimension}} \times 100$

TABLE 20: Influence of host tissue on conidial dimensions of *Marssonina brunnea* following 12 days incubation at 20°C under 12 hours white light photoperiod.

Host species	Conidial dimensions (u) Mean Length	Mean Breadth	TL:S Ratio	LS:B Ratio	% Sept.
P. deltoides cv. ANU A60/129	$13.4_{0.6}$[a]	$4.2_{0.2}$	3.41	0.94	29.3
P. alba cv. I42/57	$13.5_{0.7}$	$4.1_{0.2}$	3.36	0.97	29.7
P. deltoides cv. ANU A60/110	$13.8_{0.7}$	$4.1_{0.2}$	3.35	1.00	29.7
P. alba x *P. tremula cv.* Canescens B	$14.0_{0.6}$	$4.4_{0.3}$	3.27	1.00	30.5
P. x *euramericana cv.* Flevo	$14.0_{0.8}$	$4.0_{0.2}$	3.30	1.07	30.2
P. deltoides cv. ANU A60/135	$14.0_{0.7}$	$4.1_{0.2}$	3.39	1.01	29.5
P. nigra cv. Italica	$14.2_{0.8}$	$4.1_{0.2}$	3.22	1.07	31.1
P. alba var. pyramidalis	$14.2_{0.8}$	$4.1_{0.2}$	3.28	1.07	30.4
P. Rochester	$14.3_{0.6}$	$4.0_{0.2}$	3.21	1.12	31.1
P. x *euramericana cv.* Robusta	$14.5_{1.0}$	$4.1_{0.2}$	3.05	1.15	32.7
P. x *euramericana cv.* 'Regenerata Great-ford'	$14.8_{1.0}$	$4.1_{0.2}$	3.22	1.07	31.1
Mean	$14.1_{0.4}$	$4.1_{0.1}$	$3.28_{0.1}$	$1.04_{0.06}$	$30.4_{0.9}$
CV[b]	2.8%	2.4%	3.0%	5.8%	2.9%

[a]Standard deviation

[b]CV (Coeff. of variation) = $\frac{\text{standard deviation}}{\text{mean conidial dimension}} \times 100$

TABLE 21: Influence of host tissue on conidial dimensions of *Marssonina castagnei* following 12 days incubation at 20°C under 12 hours white light photoperiod.

Host Species	Conidial dimensions (u) Mean Length	Mean Breadth	TL:S Ratio	LS:B Ratio	% Sept.
P. nigra cv. TR56/52	$15.3_{1.0}$ [a]	$5.3_{0.3}$	3.10	0.92	32.2
P. alba x *P. tremula cv.* Canescens B	$15.5_{0.9}$	$5.5_{0.3}$	3.08	0.91	32.5
P. trichocarpa CF	$15.8_{0.9}$	$5.4_{0.3}$	2.84	1.03	35.2
P. maximowiczii cv. OJP M108	$15.9_{1.1}$	$5.4_{0.2}$	2.92	1.01	34.2
P. alba cv. Pyramidalis	$16.0_{0.6}$	$5.3_{0.2}$	2.80	1.08	35.7
P. nigra cv. TR42/80	$16.0_{0.8}$	$5.4_{0.3}$	3.10	0.95	32.2
P. deltoides x *P. alba cv.* Delmak 16	$16.3_{0.8}$	$5.3_{0.2}$	2.95	1.04	33.8
P. nigra cv. Thevestina	$16.4_{1.2}$	$5.4_{0.4}$	3.11	0.96	32.1
P. alba cv. Hickeliana	$16.5_{1.2}$	$5.6_{0.3}$	2.94	1.00	34.0
P. deltoides x *P. yunnanensis cv.* NZ5004	$16.6_{0.9}$	$5.2_{0.2}$	2.86	1.11	34.9
P. delt. x *P. alba cv.* Delmak 26	$16.6_{1.0}$	$5.2_{0.2}$	2.96	1.07	33.7
P. fremontii x *P. nigra* Sempervirens cv. ANU66/9	$16.7_{1.2}$	$5.1_{0.3}$	3.01	1.08	33.2
P. alba cv. B02	$16.8_{1.2}$	$5.5_{0.3}$	2.90	1.04	34.5
P. alba x *P. glandulosa cv.* K66-20-1	$16.9_{1.2}$	$5.4_{0.3}$	2.90	1.08	34.5
P. alba 72/8/3	$17.1_{1.2}$	$5.5_{0.3}$	2.84	1.10	35.2
Mean	$16.3_{0.5}$	$5.4_{0.1}$	$2.95_{0.1}$	$1.02_{0.06}$	$33.8_{1.2}$
CV [b]	3.0%	1.8%	3.4%	5.6%	3.5%

[a] Standard deviation

[b] CV (coeff. of variation) = $\frac{\text{standard deviation}}{\text{mean conidial dimension}} \times 100$

TABLE 22: Influence of host tissue on conidial dimensions of *Marssonina populi* following 12 days incubation at 20°C under 12 hours white light photoperiod.

Host species	Conidial dimensions (u) Mean Length	Mean Breadth	TL:S Ratio	LS:B Ratio	% Sept
P. deltoides cv. Frimley	$18.6_{1.1}$ [a]	$6.3_{0.4}$	4.05	0.72	24.7
P. trichocarpa cv. V235	$19.0_{1.1}$	$6.4_{0.4}$	3.60	0.82	28.0
P. simonii	$19.2_{1.3}$	$6.4_{0.4}$	3.56	0.84	28.0
P. maximowiczii cv. OJP 1016	$19.2_{1.4}$	$6.2_{0.4}$	4.09	0.76	24.4
P. x *euramericana cv.* Robusta	$19.4_{1.2}$	$6.1_{0.3}$	3.55	0.93	29.0
P. nigra cv. Italica	$19.5_{1.4}$	$6.0_{0.4}$	3.36	0.98	29.7
P. x *euramericana cv.* Marilandica F	$19.5_{1.1}$	$6.1_{0.4}$	3.80	0.84	26.4
P. delt. x *P. maxi. cv.* I83/58	$19.5_{1.0}$	$6.3_{0.4}$	3.60	0.86	27.7
Mean	$19.2_{0.3}$	$6.2_{0.1}$	$3.70_{0.2}$	$0.84_{0.08}$	$27.2_{1.0}$
CV [b]	1.5%	1.6%	5.4%	9.5%	7.0%

[a] Standard deviation

[b] CV (coeff. of variation) = $\frac{\text{standard deviation}}{\text{mean conidial dimension}} \times 100$

TABLE 23: Influence of incubation temperature on conidial dimensions of *Marssonina* species on host tissue following 12 days incubation under 12 hours white light photoperiod.

Species and Treatment	Conidial Dimensions (u) Mean Length	Mean Breadth	TL:S Ratio	LS:B Ratio	% Sept.
M. brunnea					
12°C	$14.4_{0.7}$ [a]	$4.1_{0.2}$	3.23	1.08	30.9
16°C	$14.4_{0.6}$	$4.1_{0.2}$	3.20	1.10	31.2
20°C	$14.2_{0.8}$	$4.1_{0.2}$	3.22	1.07	31.1
24°C	$14.4_{0.6}$	$4.1_{0.2}$	3.26	1.07	30.6
24/8°C	$14.2_{0.6}$	$4.1_{0.2}$	3.20	1.08	31.3
28°C	$14.5_{0.8}$	$4.1_{0.2}$	3.26	1.08	30.6
Mean	$14.3_{0.1}$	$4.1_{0.0}$	$3.23_{0.03}$	$1.08_{0.01}$	$30.9_{0.3}$
CV [b]	0.7%	0.0%	0.9%	0.9%	1.0%
M. castagnei					
12°C	$16.8_{0.9}$	$5.1_{0.3}$	3.05	1.07	32.7
16°C	$16.8_{1.0}$	$5.0_{0.3}$	3.03	1.13	33.4
20°C	$17.2_{1.2}$	$5.2_{0.3}$	3.05	1.07	32.8
24°C	$17.2_{1.2}$	$5.2_{0.3}$	2.85	1.15	35.0
24/8°C	$17.0_{1.0}$	$5.2_{0.3}$	2.99	1.10	33.4
28°C	No infection				
Mean	$17.0_{0.2}$	$5.1_{0.1}$	$2.99_{0.08}$	$1.10_{0.04}$	$33.4_{0.8}$
CV	1.2%	2.0%	2.7%	3.6%	2.3%
M. populi					
12°C	$19.2_{1.4}$	$6.0_{0.5}$	3.36	0.96	29.7
16°C	$19.2_{1.4}$	$6.0_{0.4}$	3.26	0.98	30.7
20°C	$19.5_{1.5}$	$6.0_{0.6}$	3.50	0.97	28.8
24°C	$19.6_{1.0}$	$6.0_{0.4}$	3.50	0.94	28.7
24/8°C	$19.5_{1.4}$	$6.0_{0.4}$	3.50	0.96	28.7
28°C	No infection				
Mean	$19.5_{0.3}$	$6.0_{0.0}$	$3.42_{0.1}$	$0.96_{0.01}$	$29.3_{0.9}$
CV	1.5%	0.0%	2.9%	1.0%	3.1%

[a] Standard deviation

[b] CV (Coeff. of variation) = $\frac{\text{standard deviation}}{\text{mean conidial dimension}} \times 100$

TABLE 24: Influence of photoperiod on conidial dimensions of *Marssonina* species on host tissue following 12 days incubation at 20°C.

Species and Treatment	Conidial dimensions (u) Mean Length	Mean Breadth	TL:S Ratio	LS:B Ratio	% Sept.
M. brunnea					
Dark	$14.4_{0.7}$[a]	$4.1_{0.2}$	3.14	1.10	31.8
12 hr. white light	$14.2_{0.5}$	$4.1_{0.2}$	3.22	1.08	31.0
12 hr. NUV	$14.4_{0.7}$	$4.1_{0.2}$	3.30	1.06	30.3
Mean	$14.3_{0.1}$	$4.1_{0.0}$	$3.22_{0.08}$	$1.08_{0.02}$	$31.0_{0.7}$
CV [b]	0.7%	0.0%	2.5%	1.8%	2.2%
M. castagnei					
Dark	$17.0_{0.9}$	$5.2_{0.3}$	2.94	1.09	34.0
12 hr. white light	$17.2_{1.2}$	$5.2_{0.3}$	3.05	1.07	32.8
12 hr. NUV	$17.2_{1.2}$	$5.4_{0.3}$	2.88	1.13	34.6
Mean	$17.1_{0.1}$	$5.3_{0.1}$	$2.95_{0.08}$	$1.10_{0.03}$	$33.8_{0.9}$
CV	0.6%	1.9%	2.7%	2.7%	2.7%
M. populi					
Dark	$19.5_{1.5}$	$6.0_{0.4}$	3.56	0.90	28.1
12 hr. white light	$19.5_{1.4}$	$6.0_{0.4}$	3.36	0.98	29.7
12 hr. NUV	$19.7_{1.4}$	$6.0_{0.5}$	3.53	0.93	28.3
Mean	$19.5_{0.1}$	$6.0_{0.0}$	$3.48_{0.1}$	$0.93_{0.04}$	$28.7_{0.9}$
CV	0.5%	0.0%	2.9%	4.3%	3.1%

[a] Standard deviation

[b] CV (coeff. of variation) = $\frac{\text{standard deviation}}{\text{mean conidial factor}} \times 100$

TABLE 25: Influence of host and environmental factors on conidium morphology of *Marssonina* species. (All units expressed as coefficients of variation).

Species and Treatment	Conidial Dimensions Length	Breadth	TL:S Ratio	LS:B Ratio	% Sept.	Mean per factor
M. brunnea						
Leaf surface	0.0 [a]	0.0	0.0	0.0	0.0	0.0
Leaf age	0.7	0.5	1.3	1.3	1.3	1.0
Infection level	0.7	0.0	1.1	2.6	1.1	1.1
Host resistance	2.8	2.4	3.0	5.8	2.9	3.4
Temperature	0.7	0.0	0.9	0.9	1.0	0.5
Photoperiod	0.7	0.0	2.5	1.8	2.2	1.4
Mean/factor	0.9	0.5	1.5	2.1	1.4	-
M. castagnei						
Leaf surface	0.4	1.3	0.0	0.0	0.4	0.4
Leaf age	1.2	1.1	4.8	3.2	2.4	2.5
Infection level	0.4	1.4	3.0	0.01	2.8	1.5
Host resistance	3.0	1.8	3.4	5.6	3.5	3.5
Temperature	1.2	2.0	2.7	3.6	2.3	2.4
Photoperiod	0.6	1.9	2.7	2.7	2.7	2.1
Mean/factor	1.1	1.6	2.8	2.5	2.3	-
M. populi						
Leaf surface	0.7	0.0	4.2	3.2	4.2	2.5
Leaf age	0.8	0.0	0.6	1.2	2.0	0.9
Infection level	0.0	0.0	2.8	3.8	5.3	2.4
Host resistance	1.5	1.6	5.4	9.5	7.0	5.0
Temperature	1.5	0.0	2.9	1.0	3.1	1.7
Photoperiod	0.5	0.0	2.9	4.3	3.1	2.2
Mean/factor	0.8	0.3	3.1	3.8	4.1	-
Overall mean/factor	0.9	0.8	2.5	2.8	2.6	-

[a] CV (Coeff. of variation) = $\frac{\text{standard deviation}}{\text{mean conidial dimension}} \times 100$

(ii) Influence of Photoperiod

Photoperiod had no appreciable effect on conidium shape, dimensions, TL:S and LS:B ratios and septum location (Table 24).

The influences of both host and environmental factors on conidium morphology are summarised in Table 25. They suggest that host tissue was the factor inducing greatest conidium variance. This result was essentially similar to that observed in cultural studies where greatest variance was induced by media.

Best conditions for expression of features of conidial morphology were inoculation of the adaxial leaf surface of leaf discs of *P. nigra cv.* Italica 'Aurea' (from mature leaves), with 5000 conidia/disc (2.5 cm diam) and incubation at 20°C under a 12 hour white light photoperiod for at least 10 days.

3. FIELD STUDIES ON THE INFLUENCE OF COLLECTION DATE ON CONIDIUM MORPHOLOGY OF M. BRUNNEA

Materials and Methods

To determine the influence of widely varying host and environmental factors on conidium morphology of *M. brunnea*, conidial collections were made from the same trees of two poplar clones (*P.* x *euramericana cv.* NL 2194 and *P. fremontii* x *P. nigra* Sempervirens cv. ANU 66/9). The first collection was made on the first of October 1978 and thereafter at monthly intervals until the first of June 1979.

RESULTS

(a) Conidium Shape

Conidium shape remained constant throughout the growing season on both clones. Conidia were straight, occasionally slightly curved and narrowly obovoid.

(b) Conidium Dimensions

(i) Length and Breadth

Length and breadth dimensions were remarkably stable as evidenced by the small coefficients of variation obtained for conidium length and breadth dimensions (Table 26). Conidium lengths on *P.* x *euramericana* cv. NL 2194 did not differ significantly ($P>0.05$, t-test) between successive collections.

On *P. fremontii* x *P. nigra* Sempervirens cv. ANU 66/9 conidium lengths differed significantly (P>0.05, t test) on two occasions. On both clones conidium breadths often differed significantly (P> 0.05, t-test) between successive collections.

Between monthly collections throughout the growing season on both host species the coefficients of variation for mean conidium length and breadth dimensions remained constant at 7% and 5% respectively, indicating that neither host nor collection date were important.

(ii) TL:S and LS:B ratios

As with length and breadth dimensions, TL:S and LS:B ratios varied only slightly between successive collections, the coefficient of variation being less than 5% in both instances (Table 26).

(c) Septum Location (% Sept.)

Septum location also remained relatively constant throughout the year, as evidenced by the coefficient of variation being less than 3% (Table 26).

The above field observations were in agreement with results obtained in the laboratory on culture media and host tissue, confirming the stability of conidium shape, length and breadth dimensions, TL:S and LS:B ratios and septum location.

TABLE 26: Conidial dimensions of *M. brunnea* sampled monthly from two poplar clones growing under field conditions.

Host Collection Date	*P.* x *euramericana cv.* NL 2194 Conidial Dimensions (u) Mean Length	Mean Breadth	TL:S Ratio	LS:B Ratio	% Sept	*P. fremontii* x *P. nigra Sempervirens cv.* ANU 66/9. Conidial Dimensions (u) Mean Length	Mean Breadth	TL:S Ratio	LS:B Ratio	% Sept.
1.10.78	$15.0_{1.0}$ [a]	$4.0_{0.2}$	3.15	1.17	31.7	$14.8_{1.2}$	$4.0_{0.2}$	3.13	1.17	32.0
1.11.78	$14.7_{0.8}$	$4.1_{0.2}$	3.00	1.20	33.3	$14.8_{1.0}$	$4.1_{0.2}$	3.01	1.20	33.2
1.12.78	$14.5_{1.0}$	$4.0_{0.2}$	3.31	1.08	30.2	$14.4_{1.0}$	$4.0_{0.2}$	3.23	1.11	31.0
1.1.79	$14.5_{0.9}$	$4.2_{0.2}$	3.16	1.10	31.6	$14.6_{0.8}$	$4.1_{0.2}$	3.20	1.11	31.3
1.2.79	$14.8_{0.8}$	$4.1_{0.2}$	3.05	1.20	32.7	$14.5_{0.9}$	$4.1_{0.2}$	3.06	1.16	32.6
1.3.79	$14.7_{0.9}$	$4.2_{0.2}$	3.15	1.11	31.7	$14.6_{0.9}$	$4.0_{0.2}$	3.24	1.12	30.8
1.4.79	$14.6_{1.0}$	$4.2_{0.3}$	3.23	1.09	30.9	$14.6_{0.9}$	$4.1_{0.2}$	3.15	1.13	31.7
1.5.79	$14.8_{0.8}$	$4.0_{0.2}$	3.15	1.16	31.7	$15.1_{0.9}$	$4.2_{0.2}$	3.06	1.17	32.6
1.6.79	$15.0_{0.8}$	$4.1_{0.2}$	3.13	1.21	31.9	$14.8_{1.0}$	$4.1_{0.2}$	3.13	1.24	31.9
Overall mean	$14.7_{0.2}$	$4.1_{0.09}$	$3.15_{0.09}$	$1.14_{0.05}$	$31.7_{0.9}$	$14.7_{0.2}$	$4.1_{0.06}$	$3.13_{0.08}$	$1.15_{0.04}$	$31.9_{0.8}$
CV [b]	1.4%	2.2%	2.8%	4.4%	2.8%	1.4%	1.5%	2.5%	3.4%	2.5%

[a] Standard deviation

[b] CV (coeff. of variation) = $\frac{\text{standard deviation}}{\text{mean}} \times 100$

> significantly greater ($P>0.05$, t test) between successive collections

E. COMPARATIVE CONIDIUM MORPHOLOGY ON HOST TISSUE AND 15%V8 AGAR UNDER DEFINED LABORATORY CONDITIONS

Preceeding studies (Section D 'analysis') confirmed the stability of morphological features and identified those environmental conditions under which they were best expressed. However, there remained the need to determine (under defined environmental conditions) the relative value of these specific morphological features (conidium shape, length and breadth dimensions, TL:S and LS:B ratios and septum location) for species delimitation. That is, 'synthesis' *sensu* Snyder & Hansen (1954). Furthermore, there was also the need to establish whether specific morphological features expressed on the two substrates (host, 15%V8) were comparable under the one set of environmental conditions.

Materials and Methods

During cultural studies (Section D 'analysis') low numbers of abnormally large conidia were observed in isolates of all three species. Monosporous isolates of these conidia in no instance reverted to forming typical conidia. In the following experiment monosporous isolates of both abnormally large and typical conidia (Table 27) were used to inoculate leaf discs and 15%V8. The former monosporous isolates were designated 'large conidium variants' and are indicated by the suffix 'L'.

To investigate conidium morphology on host tissue, leaf discs (2.5 cm diam) from mature leaves of *P. nigra* cv. Italica 'Aurea' were inoculated on the adaxial surface with a conidial suspension (5000 conidia/disc) obtained from 10 day 15%V8 cultures. Following incubation the leaf discs were incubated at 20°C under a 12 hour white light photoperiod for 10 days.

To investigate conidium morphology in culture, isolates were streak-plated onto 15%V8 juice agar (pH 6.5) and incubated at 20°C under a 12 hour white light photoperiod for 10 days.

Conidia from both substrates were mounted in lactophenol and stained with 0.5% acid fuchsin. Conidium shape was noted and length and breadth dimensions, TL:S and LS:B ratios and septum location were recorded and calculated, as previously outlined.

TABLE 27: Isolates of *Marssonina* used to inoculate 15%V8 and host tissue in comparative laboratory studies.

Species and Isolate	Host [a]	Origin	Collector	Comments
M. brunnea				
Br1	*P. yunnanensis*	Palmerston North, NZ	Author	10/1/1978
Br 2	*P.* x *euramericana*	Pahiatua, NZ	Author	10/1/1978
Br 3	*P. fremontii* cv. ANU61/48	Palmerston North, NZ	Author	10/1/1978
Br 4	*P. frem.* x *P. nigra* Sempervirens cv. ANU66/9	Palmerston North, NZ	Author	10/1/1978
Br 5	*P.* x *eura.* cv. I-214	Palmerston North, NZ	Author	10/1/1978
5L1		15%V8	Author	Large conidium variant
5L2		15%V8	Author	" " "
Br 6	*P. alba*	Palmerston North, NZ	Author	10/1/1978
Br 7	*P.* x *eura.* cv. Flevo	Palmerston North, NZ	Author	
Br 8	*P.* x *eura.* cv. NL2194	Palmerston North, NZ	Author	10/1/1978
Br 9	*P. delt.* x *P. maxi.* cv. I83/58	Palmerston North, NZ	Author	10/1/1978
Br 10	*P. deltoides*	Urbana, Illinois, USA	J. J. Jokela	No. 78070, 7/7/1978
Br 11	*P. deltoides*	Urbana, Illinois, USA	J. J. Jokela	No. 78071, 7/7/1978
Br 12	*P. deltoides*	Urbana, Illinois, USA	J. J. Jokela	No. 78075, 7/7/1978
12L		15%V8		Large conidium variant
Br 13	*P. deltoides*	Iowa, USA	A. L. Schipper	24/11/1978
Br 14	*P. trichocarpa*	St Paul, Minnesota, USA	A. L. Schipper	/8/1978
14L		15%V8	Author	Large conidium variant
Br 15	*P. deltoides*	Colorado, USA	T. E. Hinds	30/10/1979
Br 16	*P.* x *eura.* cv. Robusta	Kew, Surrey, England	D. A. Burdekin	27/7/1979
Br 17	*P.* x *eura.* cv. Robusta	Dublin, Ireland	F. O'Riordain	31/10/1979
Br 18	*P.* x *euramericana*	Nancy, France	J. Pinon	11/5/1978
18L		15%V8	Author	Large conidium variant
Br 19	*P. deltoides*	Hees, Holland	Author	Ex poplar seed
Br 20	*P.* x *eura.* cv. I214	Ankara, Turkey	M. Vural	5/12/1979
Br 21	*P. tremuloides*	Vail, Colorado, USA	T. E. Hinds	/8/1978

CONTINUED

Table 27 Continued: Isolates of *Marssonina* used to inoculate 15%V8 and host tissue in comparative laboratory studies.

Species and Isolate	Host	Origin	Collector	Comments
Br 22	*P. tremuloides*	Vail, Colorado, USA	T. E. Hinds	/8/1978
Br 23	*P. tremuloides*	Fairbanks, Alaska, USA	T. E. Hinds	3/8/1977
Br 24	*P. tremuloides*	Rosemount, Minnesota, USA	M. E. Ostry	19/9/1979
24L		15%V8	Author	Large conidium variant
Br 25L	*Populus* sp.	Edinburgh, Scotland	A. J. Hayes	CMI. IMI. 211287
M. castagnei				
Cs 2	*P. alba*	Dublin, Ireland	F. O'Riordain	31/10/1979
Cs 3	*P. alba*	Ankara, Turkey	M. Vural	/12/1979
3L		15%V8	Author	Large conidium variant
M. populi				
Po 1	*P. canadensis*	Herts, England	J.S.W. Dickens	13/11/1979
1L		15%V8		
Po 2	*P. nigra* cv. Italica	Bletchley, Milton, England	A. G. Bailey	10/11/1979
Po 3	*P. nigra* cv. Italica	Kew, Surrey, England	A. G. Bailey	12/11/1979
Po 4	*P. nigra* cv. Italica	Dublin, Ireland	F O'Riordain	31/10/1979
Po 5	*P.* x *berolinesis*	Munchen, Germany	P. Schutt	
Po 6	*P. nigra*	Zurich, Switzerland	R. H. Rimpau	5/5/1961, ETH M4548
6L		15%V8	Author	Large conidium variant
Po 7	*P. nigra*	Zurich, Switzerland	R. H. Rimpau	31/7/1960, ETH.2474
Po 8	*P. nigra*	Ankara, Turkey	M. Vural	/12/1979

[a] *P.* x *eura.* = *P.* x *euramericana* (*P. deltoides* x *P. nigra*)
P. delt. = *P. deltoides*
P. maxi. = *P. maximowiczii*

RESULTS

Morphological features of conidia of the three *Marssonina* species were similarly expressed on *P. nigra* cv. Italica 'Aurea' and 15%V8 (Tables 28, 29) thereby confirming the suitability of 15%V8 as a culture medium for conducting comparative morphological studies.

(a) Conidium Shape

Conidia of *M. brunnea* were straight to slightly curved and narrowly obovoid. Conidia of the 'large conidium variants' were straight to slightly curved and obovoid (Fig. 19). Conidia of the normal forms and 'large conidium variants' of *M. castagnei* were straight to slightly curved and broadly obovoid (Fig. 20). Conidia of the normal forms and 'large conidium variants' of *M. populi* were curved to strongly curved to pyriform and broadly obovoid (Fig. 21). Typical conidia of the three species on both substrates were quite distinct, enabling species identification on the basis of conidium shape alone.

(b) Conidium Dimensions

(i) Length and Breadth

Length and breadth dimensions within isolates of all three species often differed significantly ($P>0.05$, t-test) between substrates (Tables 28, 29). Generally however conidia on host tissue were shorter and broader than conidia from 15%V8. The range and mean overall conidial dimensions of the three species on the two substrates are presented in Table 30 and depicted graphically in Fig. 22. These results show that conidial dimensions of all three species overlap strongly, particularly *M. castagnei*, *M. populi* and the 'large conidium variants' of *M. brunnea*. Despite the overlap, the mean overall length and breadth dimensions of *M. brunnea* were quite distinct from those of *M. populi* and *M. castagnei* which in turn were comparable. Accordingly, conidial dimensions had limited value in delimiting species. The relationship between the normal and 'large conidium variants' of each species on host tissue is shown in Fig. 23. In each instance the length and breadth dimensions of the two forms are quite separate. In fact the difference in conidial dimensions between the normal and the 'large conidium variants' of each species was greater than the differences between normal forms of the three species.

TABLE 28: Comparative mean conidial dimensions of *M. brunnea* on 15%V8 and *P. nigra* cv. Italica 'Aurea' following 10 days incubation at 20°C under a 12 hour white light photoperiod.

Isolate	Conidium Length (u)			Conidium Breadth (u)			TL:S Ratio		LS:B Ratio		Location of Septum	
	15%V8		Host	15%V8		Host	15%V8	Host	15%V8	Host	15%V8	Host
Br 1	15.3		15.0	4.0	<	4.2	2.90	3.24	1.30	1.10	34.4	30.8
Br 2	14.9	>	14.4	4.0		4.1	2.90	3.06	1.30	1.13	34.4	32.6
Br 3	14.1		14.4	4.0	<	4.2	3.20	3.18	1.16	1.05	31.2	31.4
Br 4	15.0		15.0	4.0		4.1	3.04	3.26	1.24	1.12	32.8	30.6
Br 5	15.6	>	14.6	4.3	>	4.1	2.92	3.09	1.25	1.16	34.3	32.3
Br 6	17.4		17.3	4.2	<	4.4	3.00	3.12	1.37	1.12	33.3	32.0
Br 7	15.0	<	15.4	4.1		4.2	2.74	3.07	1.20	1.18	36.4	32.7
Br 8	16.4		16.1	4.2		4.2	2.96	3.06	1.33	1.20	33.7	32.7
Br 9	15.5	>	14.6	4.0		4.0	3.05	3.12	1.26	1.12	32.7	32.0
Br 10	14.1		14.1	4.1	<	4.4	3.00	3.13	1.09	1.08	33.0	32.8
Br 11	14.7		14.6	4.2	<	4.4	2.94	2.90	1.17	1.14	34.0	34.5
Br 12	15.0	>	14.4	4.2	<	4.5	3.07	3.00	1.17	1.07	32.5	33.3
Br 13	14.8		14.5	4.1		4.2	3.02	3.15	1.21	1.20	31.2	31.7
Br 14	15.1	>	14.7	4.1		4.1	3.15	3.14	1.21	1.16	33.0	31.8
Br 15	14.6		14.3	4.0		4.1	3.04	3.16	1.16	1.10	30.0	31.6
Br 16	16.0	>	15.4	4.1		4.2	3.07	3.25	1.19	1.18	30.8	30.8
Br 17	15.0	>	14.1	4.3		4.2	3.15	3.24	1.06	1.03	32.5	30.8
Br 18	15.4	>	14.8	3.8	<	4.0	3.01	3.13	1.34	1.17	33.2	31.9
Br 19	14.8		14.7	4.0	<	4.2	3.13	3.22	1.17	1.08	32.0	31.0
Br 20	15.6	>	14.4	4.4	>	4.2	3.05	3.25	1.14	1.04	31.8	30.7
Br 21	15.8	>	14.0	3.9		3.8	3.05	3.06	1.32	1.20	32.7	32.6
Br 22	15.7	>	14.2	4.0	>	3.7	3.12	2.96	1.26	1.30	32.0	33.7
Br 23	15.6	>	14.5	3.9		3.8	3.23	2.93	1.34	1.17	34.0	30.0
Br 5L1	21.4	>	20.0	4.9		4.8	2.97	3.21	1.30	1.49	33.6	31.1
Br 5L2	22.0	>	20.2	5.0		4.9	3.10	3.32	1.46	1.24	32.2	30.1
Br 12L	19.7	>	18.5	5.1	<	5.5	3.07	2.99	1.24	1.13	32.5	33.4
Br 14L	22.5	>	22.0	4.8	<	5.0	3.27	3.34	1.44	1.29	30.6	30.0
Br 18L	20.5		20.6	5.5	<	5.7	3.66	3.06	1.00	1.28	28.3	32.6
Br 24L	23.0	>	21.5	4.9	<	5.1	2.75	3.19	1.70	1.30	34.6	29.0
Br 25	23.4	>	21.0	5.5	>	5.2	2.74	3.15	1.58	1.20	36.0	32.5

> Significantly different P 0.05 t-test

L 'Large conidium variants'

TABLE 29: Comparative mean conidial dimensions of *M. castagnei* and *M. populi* on 15%V8 and *P. nigra* cv. Italica 'Aurea' following 10 days incubation at 20°C under a 12 hour white light photoperiod.

Species and Isolate	Conidium Length (u) 15%V8		Host	Conidium Breadth (u) 15%V8		Host	TL:S Ratio 15%V8	Host	LS:B Ratio 15%V8	Host	Location of Septum 15%V8	Host
M. castagnei												
Cs 2	18.0		17.6	5.8	>	5.4	2.27	2.84	1.25	1.15	39.6	35.2
3	20.0	>	17.3	5.9		6.1	2.25	2.55	1.32	1.04	38.3	38.2
3L	27.2	>	26.5	7.2	<	8.0	2.32	2.20	1.62	1.50	40.6	43.6
M. populi												
Po 1	18.4		18.6	5.9	<	6.1	3.31	3.45	0.94	0.90	30.8	29.0
2	19.3		19.6	6.0	<	6.3	3.50	3.28	0.93	0.94	28.2	30.4
3	20.9	>	19.3	6.4	>	6.2	3.80	3.66	0.93	0.84	26.1	27.3
4	21.3	>	18.9	5.7	<	6.4	3.55	3.28	1.04	0.90	26.1	30.5
5	24.0	>	22.0	6.2	<	6.5	2.93	3.21	1.36	1.10	33.0	30.0
6	20.0	>	18.7	5.4	<	6.0	3.63	3.50	1.02	0.90	27.5	28.6
7	21.0	>	20.0	6.0	<	6.7	3.42	3.53	1.03	0.84	29.2	28.3
8	19.0	>	18.5	5.8	<	6.7	3.14	3.59	1.02	0.75	31.5	28.0
1L	30.0	>	25.8	7.0	<	8.5	2.86	3.00	1.49	1.01	35.0	33.4
6L	28.7	>	27.0	7.5	<	8.5	3.23	3.20	1.18	1.00	30.9	31.2

> significantly different (P 0.05, t-test)

L 'Large conidium variants'

TABLE 30: Mean overall conidial dimensions of *Marssonina* species on 15%V8 and *P. nigra* cv. Italica 'Aurea' following 10 days incubation at 20°C under 12 hours white light photoperiod.

Species	Conidium Length (u) min	mean	max	Conidium Breadth (u) min	mean	max	TL:S Ratio	LS:B Ratio	% Sept.
M. brunnea									
15%V8	10.0	15.3	22.0	3.5	4.1	6.0	3.03	1.23	32.9
P. nigra	10.0	14.8	22.0	3.5	4.1	6.0	3.12	1.13	31.9
M. brunnea L									
15%V8	16.0	21.8	30.0	4.0	5.1	7.0	3.08	1.39	32.5
P. nigra	16.0	20.5	30.0	4.0	5.2	7.0	3.18	1.27	31.2
M. castagnei									
15%V8	12.0	19.0	30.0	4.5	5.8	8.0	2.56	1.28	39.0
P. nigra	12.0	17.4	30.0	4.5	5.7	8.0	2.69	1.10	36.7
M. castagnei L									
15%V8	18.0	27.2	40.0	6.0	7.2	12.0	2.32	1.62	40.6
P. nigra	18.0	26.5	40.0	6.0	8.0	12.0	2.20	1.50	43.6
M. populi									
15%V8	12.0	20.5	30.0	4.5	5.9	8.0	3.41	1.03	29.0
P. nigra	12.0	19.4	30.0	4.5	6.3	8.0	3.44	0.90	29.0
M. populi L									
15%V8	18.0	29.3	40.0	6.0	7.2	12.0	3.04	1.33	33.0
P. nigra	18.0	26.4	40.0	6.0	8.5	12.0	3.10	1.00	32.3

> significantly different ($P > 0.05$, t-test)

L 'Large conidium variants'

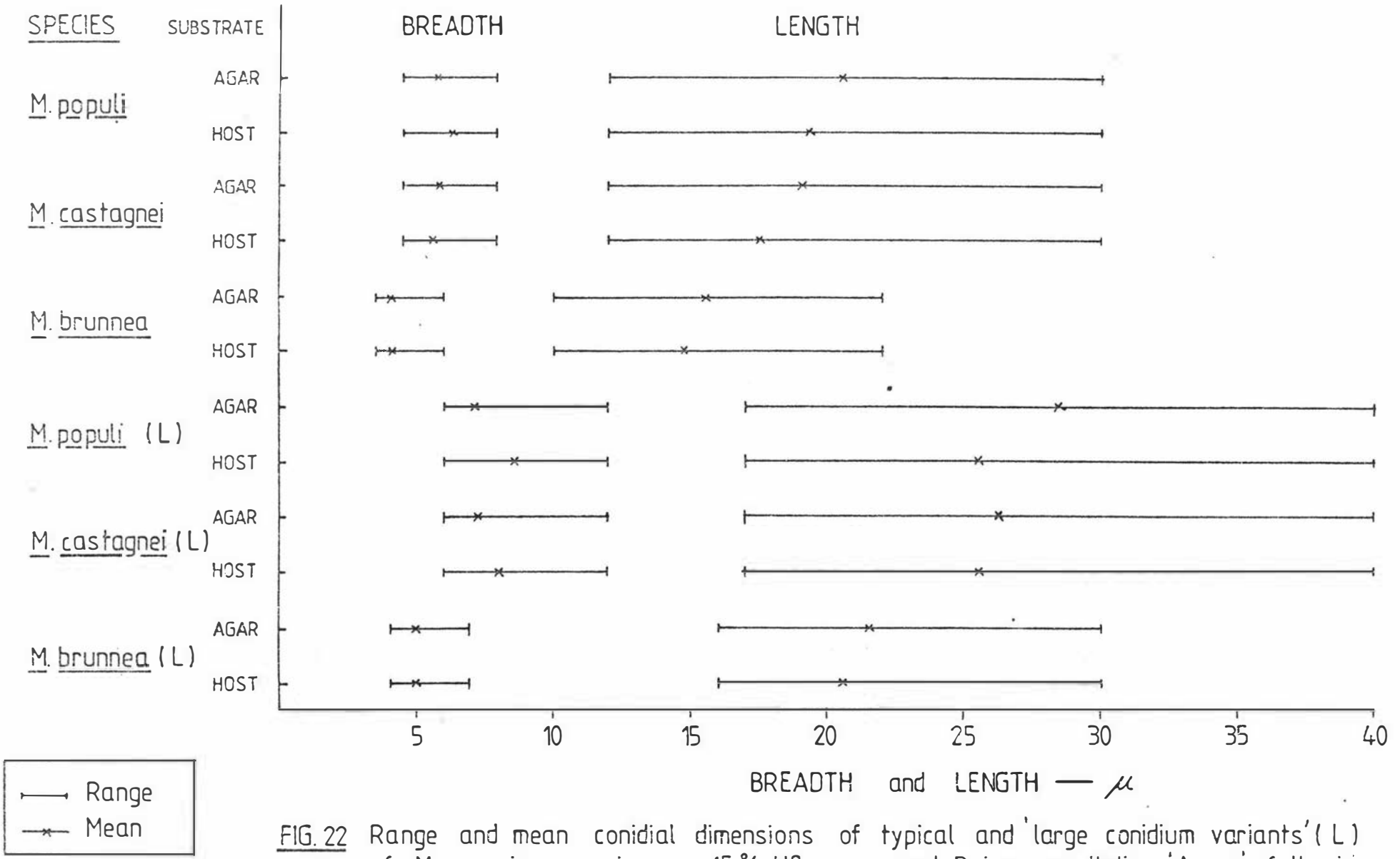


FIG. 22 Range and mean conidial dimensions of typical and 'large conidium variants' (L) of Marssonina species on 15% U8 agar and P.nigra cv Italica 'Aurea' following 10 days incubation at 20°C under a 12h white light photoperiod.

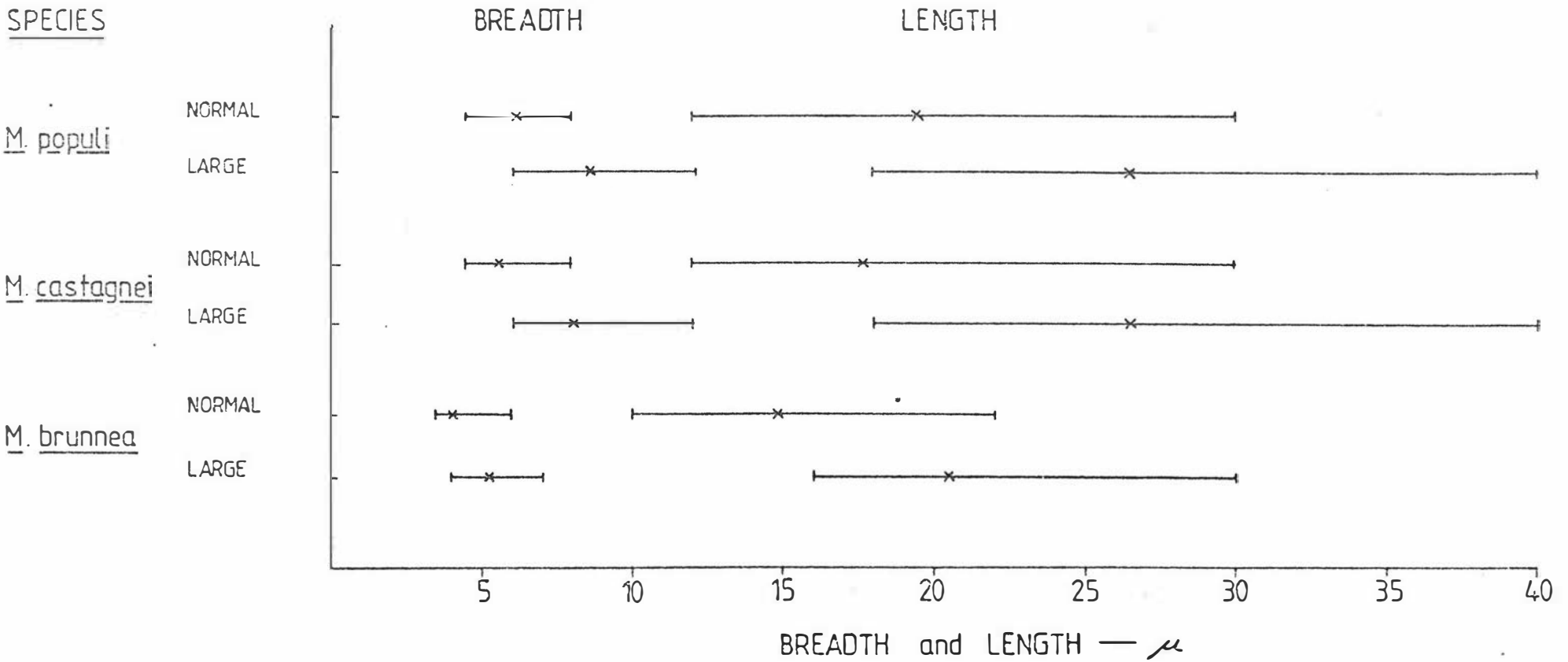


FIG. 23: Range and mean conidial dimensions of normal and 'large conidium variants' of Marssonina species on P. nigra cv Italica 'Aurea' following 10 days incubation at 20 °C under a 12 h white light photoperiod.

(ii) TL:S & LS:B Ratios

Within species, the TL:S and LS:B ratios were essentially similar between substrates (Tables 28, 29, 30). Despite the fact that the values of the TL:S ratios of *M. castagnei* and the LS:B ratios of *M. populi* were generally lower than those of other species, the overlap between species was such that these ratios alone were of no value in delimiting species. However many isolates of *M. populi* were identifiable by a LS:B ratio of >1.00.

(c) Septum Location

Within species septum location on both substrates was essentially similar (Tables 28, 29, 30). Septa of *M. brunnea* and *M. populi* were commonly located approximately 30% along the length of the conidium. In contrast, the septum of *M. castagnei* was regularly positioned 40% along the length of the conidium and accordingly allowed delimitation of *M. castagnei* from *M. brunnea* and *M. populi*. Generally septum location alone was sufficient to identify conidia of *M. castagnei*.

DISCUSSION

The results of this study ('synthesis') established that conidium morphology expressed by each *Marssonina* species both on host tissue and 15%V8 under defined conditions was essentially similar and further showed that on both substrates conidium shape enabled separation of the three species. Conidia of *M. castagnei* alone were identifiable by septum location. Although conidial dimensions overlapped precluding species delimitation on this criteria alone, in combination with conidium shape and septum location mean conidial dimensions assisted species identification. That is, conidia of *M. brunnea* were generally shorter (15v20u) and narrower (4v6u) than those of *M. castagnei* and *M. populi*. Although of comparable dimensions, conidia of *M. castagnei* and *M. populi* were readily identified by conidium shape (straight v curved) and septum location (40 v 30%).

The value of cultural studies for determining the full extent of genetic variability was realized by recognition of 'large conidium variants' in cultures of all three species. This phenonomen was common and it is surprising that it has not been previously reported, probably reflecting the lack of indepth cultural studies of these species. The pathogenicity of the 'large conidium variants' was not reduced, suggesting possible

natural occurrence of such conidia. This was confirmed in the course of examination of a herbarium specimen of *P. alba* infected with *M. castagnei* (J. Eriksson, Stockholm, Sweden, 5/8/1884) where both conidial forms were equally abundant (18.5 x 6u v 23 x 8u). Furthermore, abnormally large conidia were subsequently observed, invariably in low numbers, in local collections of *M. brunnea*. Obviously the 'large conidium variants' are not separate species and their existence illustrates how additional species could be erected on the basis of a single morphological criterion without first conducting detailed studies to determine the limits of pathogen variability.

F. COMPARATIVE MICROCONIDIUM MORPHOLOGY ON HOST TISSUE AND 15%V8 AGAR

Preceeding studies established that the three species of *Marssonina* were readily delimited on the basis of conidium morphology expressed on both agar and host tissue. However during examination of field collections microconidia were often more prevalent than conidia, especially in collections made during late autumn. Since there are no reports detailing comparative morphological studies of microconidia it is unknown whether *Marssonina* species may be identified solely on the basis of morphological features of microconidia. Dimensions of microconidia have been reported only twice from host tissue (Gremmen, 1965a; Pirozynski, 1974).

In the present study the morphology of both microconidiophores and microconidia of *Marssonina* species were compared from field collections, and in the laboratory on culture media and host tissue.

Materials and Methods

(a) Field Collections

Microconidia of *M. brunnea*, *M. castagnei* and *M. populi* from field collections (Appendix 7) were mounted in lactophenol and stained with 0.5% acid fuchsin. The length and breadth dimensions of 60 microconidia from each collection were recorded (Leitz optical micrometer, 1250x, under oil immersion).

(b) Laboratory Studies

Isolates (Table 31) were induced to form microconidia:

(i) In culture - by incubation on 15%V8, pH 6.5, 20°C in darkness for 12 days,

(ii) On host tissue - by inoculation of the adaxial surface of over-mature leaf discs of *P. nigra* cv. Italica 'Aurea' and incubating for 12 days at 20°C in darkmess.

Microconidia from agar and host tissue were measured as for field collections.

In addition to light microscopy, microconidia and microconidiophores of the three species from field collections and laboratory studies were

examined by scanning electron and transmission electron microscopy. Preparation of material for scanning and transmission electron microscopy is outlined in Appendices 8a and 8b respectively.

RESULTS

(a) Field Collections

Microconidiophores of the three species were comparable being narrowly conical to ampulliform, nonbranched or singly branched measuring 5 - (10 - 12) - 16u x 1.5 - (2 - 3) - 5u (Fig. 24).

Microconidia of the three species were also morphologically similar, being hyaline, smooth walled, unicellular, bacillate to obovoid and often somewhat flattened at the base (Fig. 25). Microconidial dimensions are listed in Appendix 9, summarised in Table 32 and depicted graphically in Fig. 26.

On host tissue, microconidiophores and microconidia were formed intraepidermally either alone or in acervuli with conidia (Fig. 27).

(b) Laboratory Studies

On agar and host tissue microconidiophores and microconidia of all isolates of the three species were morphologically indistinguishable, both between species and within species between substrates.

Microconidiophores were narrowly conical to ampulliform, non-branched or singly branched, measuring 5 - (10 - 12) - 15 x 1.5 - (2 - 3) - 5u. In culture, microconidia often accumulated in balls on the apices of microconidiophores (Fig. 28).

On both substrates microconidia of all isolates were hyaline, smooth walled, unicellular, bacillate to obovoid with a truncated base (Fig. 29). Microconidia formed by the 'large conidium variants' were in each instance significantly longer ($P>0.05$, t-test) than microconidia formed by the respective parental forms (Table 33, Fig. 30). Dimensions of microconidia are depicted graphically in Fig. 31.

Isolates of the three species commonly formed microconidia from conidia. They arose from either cell of the conidium and in contrast to the relatively uniformly sized microconidia formed by

TABLE 31: Isolates of *Marssonina* species induced to form microconidia in laboratory studies on 15%V8 and leaf discs.

Isolate	Species	Host	Origin
Br 5	*M. brunnea*	*P.* x *euramericana cv.* I214	Palmerston North, NZ
Br 5L1	*M. brunnea*	'Large conidium variant'	15%V8
Br 24	*M. brunnea*	*P. tremuloides*	Minnesota, USA
Br 24L	*M. brunnea*	'Large conidium variant'	15%V8
Cs 3	*M. castagnei*	*P. alba*	Ankara, Turkey
Cs 3L	*M. castagnei*	'Large conidium variant'	15%V8
Po 1	*M. populi*	*P. canadensis*	Herts., England
Po 1L	*M. populi*	'Large conidium variant'	15%V8

TABLE 32: Dimensions of microconidia of *Marssonina* species from field collections.

Species	Length (u)			Breadth (u)			LS:B Ratio
	Min.	Mean	Max.	Min.	Mean	Max.	
M. brunnea	3.2	$4.5_{0.4}$[a]	8.0	1.0	$1.3_{0.06}$	2.0	$3.5_{0.3}$
M. castagnei	3.2	$4.9_{0.3}$	8.0	1.0	$1.5_{0.1}$	2.5	$3.3_{0.4}$
M. populi	3.4	$5.6_{0.5}$	9.0	1.0	$1.3_{0.1}$	2.0	$4.1_{0.4}$

[a] Standard deviation

TABE 33: Dimensions of microconidia of *Marssonina* species induced in laboratory studies on 15%V8 and leaf discs.

Isolate	Substrate	Length (u) Min.	Mean	Max.	Breadth (u) Min.	Mean	Max.	LS:B Ratio
Br 5	host	3.2	4.1	6.4	1.0	1.3	1.8	3.1
	15V8	3.2	3.8	6.0	1.0	1,2	1.6	3.2
Br 5L1	Host	6.0	8.0	10.0	1,0	1.3	1,8	6.1
	15V8	5.6	8.4	10.5	1,0	1.4	1.8	6.0
Br 24	Host	3.2	4.6	6.8	1.0	1.2	1.6	3.8
	15V8	3.2	4.5	7.0	1,0	1.2	1.6	3.7
Br 24L	Host	5.6	7.7	9.6	1.0	1.2	2.0	6.4
	15V8	5.6	8.0	10.0	1.2	1.6	2.0	5.0
Cs 3	Host	3.4	5.0	7.6	1.0	1.2	1.8	4.2
	15V8	3.4	5.0	7.4	1.0	1.4	1.8	3.6
Cs 3L	Host	5.6	8.7	11.0	1.0	1.5	1.8	5.8
	15V8	5.6	9.0	11.2	1.1	1.4	1.6	6.4
Po 1	Host	4.0	5.0	7.0	1.0	1.2	1.8	4.2
	15V8	3.6	5.0	7.0	1,0	1.3	1.8	3.8
Po 1L	Host	4.8	8.3	10.0	1.0	1.3	1.8	6.4
	15V8	6.0	8.5	12.0	1.0	1.4	1.8	6.1

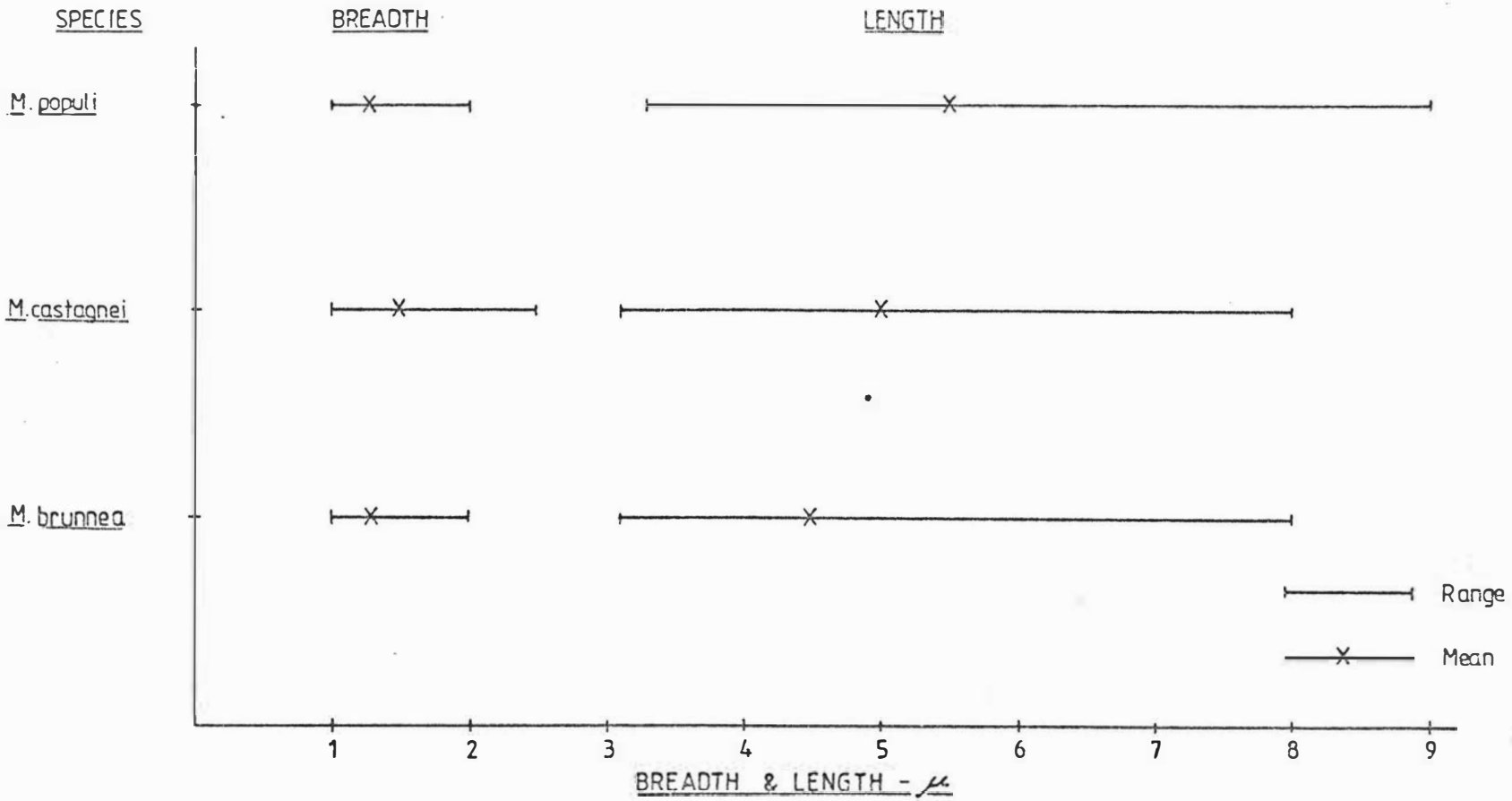


FIG. 26: Range and mean microconidial dimensions of Marssonina species from examination of field collections

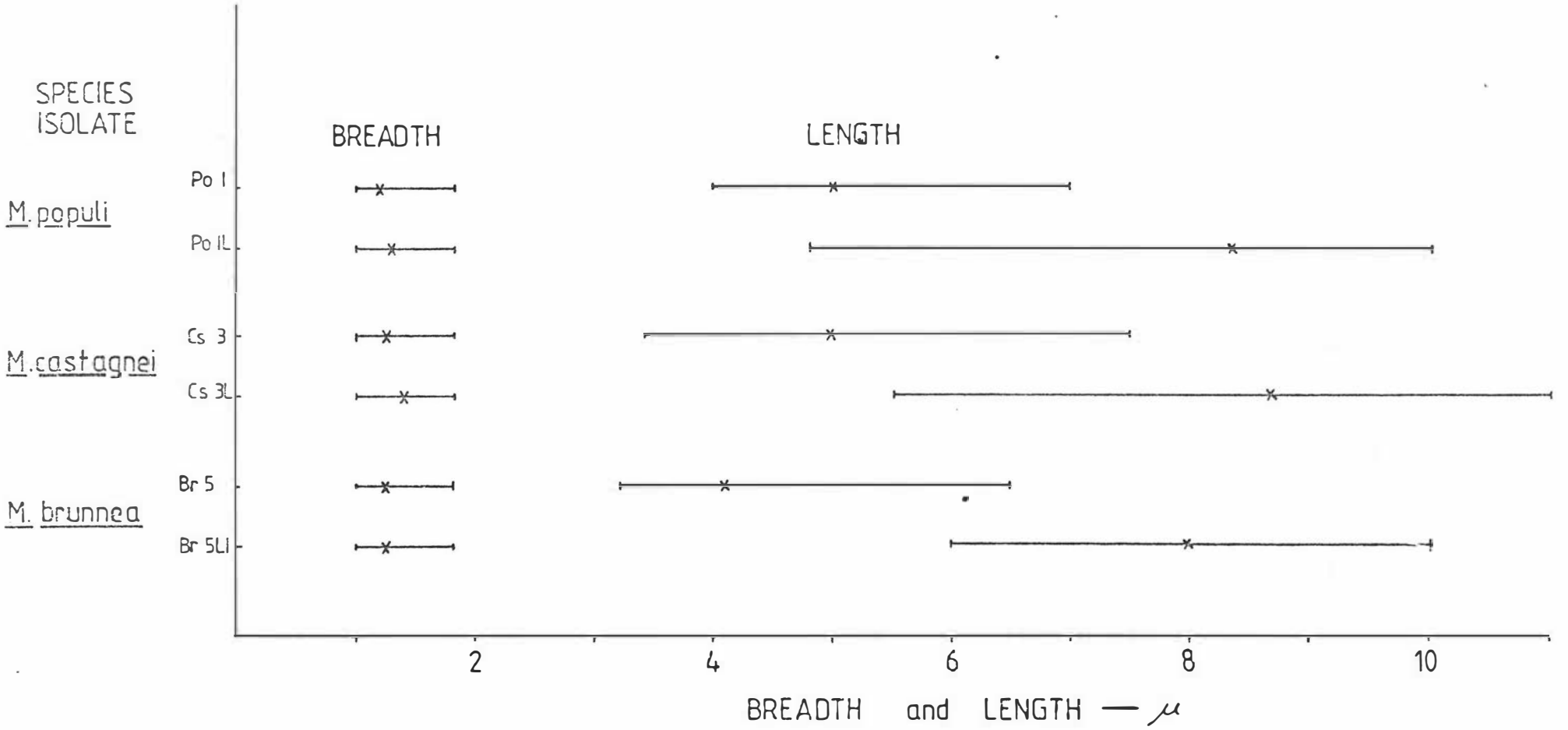


FIG 31: Range (⊢——⊣) and mean (—*—) microconidial dimensions of normal and 'Large conidium variants' of single isolates of Marssonina species on P. nigra cv Italica 'Aurea' following 12 days incubation at 20°C in darkness.

the microconidiophores, varied markedly in size and shape.

Ultrastructurally, microconidia from host tissue and 15%V8 were essentially similar and were bounded by a bilayered electron transparent wall and plasmalemma. A single large nucleus was present encircled by a bilayered nuclear membrane. The cytoplasm stained densly and contained numerous ribosomes, mitochondria and variously lipid bodies (Fig. 32).

DISCUSSION

The morphology and dimensions of microconidiophores and microconidia of *M. brunnea*, *M. castagnei* and *M. populi* from host tissue and 15%V8 agar were indistinguishable and therefore of no value in species delimitation. The dimensions of microconidia were essentially in agreement with those reported by Gremmen (1965a) and Pirozynski (1974).

As was the case with conidia, microconidia of the 'large conidium variants' were significantly larger ($P>0.05$) than microconidia formed by normal parental forms, further indicating the morphological stability of such isolates.

Microconidia are assumed to function as spermatia in fertilization (Castellani & Freccero, 1968), their relatively simple internal structure reflecting this function. That is, they have a large nucleus in proportion to their size, a relatively small amount of dense cytoplasm and a protective cell wall. Microconidia also contained ribosomes, mitochondria and lipid bodies and therefore possess some capacity for aerobic energy production.

G. EVALUATION OF PHYSIOLOGIC CHARACTERS AS SECOND ORDER TAXONOMIC CRITERIA

As previously stated, Snyder & Hansen (1954) believed that species should be delimited strictly on the basis of morphological features and that physiologic characters be used only at the sub-specific level. Preceeding studies demonstrated that species of *Marssonina* were readily delimited on the basis of conidial morphological features. However physiologic features such as symptom expression (Ellis & Everhart, 1889; Gremmen, 1964; 1965a) and host specificity (Klebahn, 1918; Rimpau, 1962; Gremmen, 1964; 1965a) have also been used to delimit species. Studies were conducted on these two physiologic factors to determine the extent of their expression and therefore possible value in delimitation of *formae speciales*.

1. SYMPTOM EXPRESSION

Symptom expression has been used in part by Ellis & Everhart (1889) and Gremmen (1964, 1965a) for separation of *M. brunnea* and *M. populi*. Black necrotic dots about 1 mm in diameter were considered typical of *M. brunnea* whereas tan or olive-brown, more or less roundish spots about 4-5 mm in diameter were characteristic of *M. populi*.

In the following studies symptoms expressed by poplars infected with *M. brunnea*, *M. castagnei* and *M. populi* were compared both on naturally infected field collections and laboratory inoculated leaf discs.

A. Symptom Expression on Field Collections

Symptoms exhibited by world-wide field collections of poplars (Appendix 1) infected with the three species of *Marssonina* were compared.

RESULTS

(i) *M. brunnea*

Symptoms were typically characterised by small (0.5-1.0mm diam.), black, angular punctiform spots which usually remained discrete but with heavy infections coalesced forming irregular necrotic patches (Fig. 33). On species of the Section *Aigeiros (P. nigra, P. deltoides, P.x euramericana)* both leaf surfaces were equally infected. In contrast, on many species of the Section *Tacamahaca (P. trichocarpa, P. maximowiczii, P. koreana x P. trichocarpa* and *P. candicans)* infection was largely confined to the abaxial leaf surface, the veins often being heavily infected (Fig. 34). On

P. alba small (1-2 mm diam.) black punctiform spots were formed on both leaf surfaces (Fig. 35). On species of the sub-section *Trepidae (P. tremula, P. tremuloides, P. grandidentata)*, infection levels were often higher on the abaxial leaf surface where irregular necrotic patches, covered with orange masses of conidia were formed (Fig. 36).

Leaves of *P. fremontii* and *P. fremontii* x *P. nigra* cv. 'Sempervirens' exhibited two distinct symptom patterns. On maturing leaves and during early stages of infection, amphigenous spots, typical of *M. brunnea* were formed (Fig. 37). However, on the adaxial surface of long-infected mature leaves, large (5-7 mm diam.), circular, tan blotches, dotted with white punctiform acervuli were formed (Fig. 37), these symptoms were more typical of *M. populi* and *M. castagnei* than of *M. brunnea*.

During late Autumn, on a wide range of host species, two distinct symptoms were exhibited by leaves infected with the microconidial state. Symptoms were either identical to those induced by the conidial stage, being small (0.5-1.0 mm diam.), punctiform spots (Fig. 38), or large (2-5 up to 10 mm diam.), epiphyllous blotches (Fig. 38). Adjacent blotches coalesced forming extensive necrotic areas dotted with numerous amber blisters enclosing microconidia.

On herbaceous shoots lesions were initially small, black, later elongating (0.5-10 cm) with the central portions becoming protuberant and turning white (Fig. 39). Young lesions were often encircled by a halo of red-pigmented tissue. Older lesions became roughly fissured and occasionally girdled small stems causing dieback. On 1-2 year old wood, black, circular (3-5 mm diam.), slightly protuberant lesions with white centres were formed (Fig. 39).

(ii) *M. castagnei*

This species was confined to *P. alba*. Symptoms were mostly epiphyllous and initially characterised by dark brown, circular lesions (0.5-3.0 mm diam.), often with a single central white acervulus. As lesions enlarged they either remained discrete forming (3.0-6.0 mm diam.) circular lesions dotted with punctiform whitish acervuli or coalesced forming irregular, dark brown or tan blotches which often covered the entire leaf surface. Such necrotic patches were dotted with whitish acervuli (Fig. 40). Diffuse dark patches were often observed on the abaxial surface of heavily infected leaves.

Identical symptoms were exhibited by leaves infected with the conidial and microconidial states.

(iii) *M. populi*

Symptoms were mainly epiphyllous and varied according to host species and disease severity. Three basic symptom types were observed namely, the spot, the blotch and the dendritic thread pattern.

The spot symptom consisted of small (0.5-2.0 mm diam.) circular to ancular, black lesions dotted with 1-3 acervuli. As infection developed adjacent necrotic lesions coalesced forming irregular necrotic patches (Fig. 41).

The blotch symptom consisted of chestnut brown to black, circular blotches up to 10 mm in diameter and dotted with whitish punctiform acervuli. Occasionally blotches were irregularly shaped and 'ink-drop' like. With heavy infection levels adjacent blotches coalesced forming large irregular, necrotic patches dotted with numerous whitish, punctiform acervuli (Fig. 42). This symptom type was exhibited by infections of *P. balsamifera*, *P. berolinensis*, *P. nigra* and *P.* x *euramericana*.

The dendritic symptom type was expressed by *P. tremuloides* and *P. angustifolia* and consisted of large circular dendritic, chestnut brown blotches (Fig. 43). Abundant amber masses of conidia accumulated on the surface of radiating dendrites. This symptom was very similar to those exhibited by roses infected with *Diplocarpon rosae*. Occasionally dendritic threads and dendritic blotches were formed (Fig. 44).

It should be noted that on occasions all three symptom types were exhibited on the one host species.

Symptoms exhibited by leaves infected with the conidial and microconidial states of *M. populi* were identical.

B. Symptom Expression on Leaf Discs

Symptoms expressed by poplars infected with the three *Marssonina* species were compared in two experiments.

Experiment one: Isolates of each *Marssonina* species were compared for uniformity of symptom expression within species on a single clone.

Experiment two: Symptoms expressed by several poplar clones infected with single isolates of each species were compared.

Experiment one:

The adaxial surface of leaf discs (2.5 cm diam.) of *P. nigra* cv. Italica 'Aurea' (mature leaves) inserted in 2% water agar (Spiers, 1978) were inoculated (5000 conidia/disc) with isolates of each *Marssonina* species (Table 34) and incubated at 20°C under 10 hour white light photoperiod. Symptom expression was recorded following 10 and 20 days incubation.

RESULTS

Symptoms induced by isolates within each species were essentially similar. Symptom expression enabled *M. populi* to be readily distinguished from *M. brunnea* and *M. castagnei*. Typically symptoms exhibited by leaf discs inoculated with *M. brunnea* and *M. castagnei* were small (1.0mm), discrete, punctiform black spots whereas symptoms induced by *M. populi* were circular, black, 'ink drop' like blotches (Fig. 45).

Experiment two:

Leaf discs (2.5 cm diam.) punched from mature leaves of 9 poplar clones were inoculated as described in Series I with a single isolate of each species. Poplar clones inoculated were:

(i)	*P. nigra cv.* Italica 'Aurea'	*(nigra)*
(ii)	*P. nigra cv.* Y83/66	*(nigra)*
(iii)	*P. nigra cv.* Vert de Garonne	*(nigra)*
(iv)	*P.* x *euramericana cv.* Robusta	*(deltoides* x *nigra)*
(v)	*P.* x *euramericana cv.* Bellini	*(deltoides* x *nigra)*
(vi)	*P.* x *euramericana cv.* Schiavone	*(deltoides* x *nigra)*
(vii)	*P. simonii*	*(balsam poplar)*
(viii)	*P. alba* NZ old clone	
(ix)	*P. alba* Morocco x *P. nigra cv.* Sempervirens cv. Mareg 2 *(alba* x *nigra)*.	

Individual treatments were replicated eight times and symptoms were recorded following 10 and 20 days incubation.

TABLE 34: Isolates of *Marssonina* used to inoculate poplars for laboratory experiments on comparative symptom expression.

Species and Isolate	Host Species	Origin
M. brunnea		
Br 5	*P.* x *euramericana cv.* I-214	Palmerston North, NZ
Br 10	*P. deltoides*	Illinois, USA
Br 13	*P. deltoides*	Iowa, USA
Br 15	*P. deltoides*	Colorado, USA
Br 16	*P.* x *euramericana* cv. Robusta	Surrey, England
Br 18	*P.* x *euramericana*	Nancy, France
Br 20	*P.* x *euramericana cv.* I-214	Ankara, Turkey
Br 24	*P. tremuloides*	Minnesota, USA
M. castagnei		
Cs 2	*P. alba*	Dublin, Ireland
Cs 3	*P. alba*	Ankara, Turkey
M. populi		
Po 1	*P. canadensis*	Herts., England
Po 2	*P. nigra cv.* Italica	Milton, England
Po 3	*P. nigra cv.* Italica	Kew, England
Po 4	*P. nigra*	Dublin, Ireland
Po 5	*P.* x *berolinensis*	Munchen, Germany
Po 7	*P. nigra*	Zurich, Switzerland

RESULTS

(i) *M. brunnea* and *M. castagnei*

Similar symptoms were exhibited by all poplar clones infected with *M. brunnea* and *M. castagnei* and at first consisted of black, circular to angular, punctiform spots (0.5-1.0-2.0 mm diam.). Usually spots remained discrete with a single central acervulus but with heavy infections coalesced forming irregular, necrotic patches with abundant acervuli (Fig. 46).

(ii) *M. populi*

Both the spot and dendritic symptom were exhibited by clones infected with *M. populi*, but were rarely expressed simultaneously by the same host.

(a) Spot Symptom

This symptom was formed by *P. simonii*, *P.* x *euramericana* cv. Robusta and *P.* x *euramericana* cv. Schiavone and consisted of irregularly shaped spots with faintly dendritic margins, on the surface of which several acervuli were formed. Adjacent spots tended to coalesce forming necrotic areas dotted with whitish punctiform acervuli (Fig. 47).

(b) Dendritic Symptom

On *P. nigra* cv. Italica 'Aurea' dendritic symptoms consisted of 'ink-drop' like spots with radial dendritic threads whereas on *P.* x *euramericana* cv. Bellini and *P. nigra* cv. Y83/66 they were in the form of black, often branched threads (Fig. 48). In both cases numerous conidia accumulated on the surface of the dendrites.

DISCUSSION

Although under field conditions *M. castagnei* has been reported only on *P. alba* (Cellerino, 1979), in the laboratory inoculations all nine clones were infected. On leaf discs of *P. alba* symptoms of *M. castagnei* and *M. brunnea* remained identical whereas in field collections they were initially similar (spots), but in the case of *M. castagnei* enlarged to form blotches. Thus in the field large blotches on *P. alba* are indicative of infection by *M. castagnei*. Discrete spots however, could be attributed to either species. It should be noted that *M. populi* is non-pathogenic to *P. alba*.

In the field, confusion is likely to arise in identifying the causal species on *P. nigra* and *P. nigra* x *P. deltoides* since they are susceptible to both *M. populi* and *M. brunnea*. Although the discrete punctiform spots of *M. brunnea* superficially resembled those of *M. populi* they may be differentiated by such subtle differences as outline and number of acervuli per spot. In *M. brunnea* typically a single acervulus is produced with the spot margin entire whereas in *M. populi* there are in excess of three acervuli, the periphery of spots being undulate to lobate. Further, spots of *M. populi* tended to coalesce forming necrotic patches dotted with whitish acervuli. Since only *M. populi* induces the dendritic symptom, expression of this symptom type indicates *M. populi* as the pathogen.

In summary, Ellis & Everhart (1889) and Gremmen (1964, 1965a) were correct in observing differences in symptoms induced by *M. brunnea* and *M. populi*. However in view of the variable expression of this factor they were not justified in adopting it as a criterion for species delimitation. However, to a person who has specialized in *Marssonina* diseases of poplars symptoms may assist in species identification since, in general:

(i) large (>5 mm diam.), circular to irregular blotches dotted with whitish punctiform acervuli on *P. alba* are typical of *M. castagnei*,

(ii) small (1-2 mm diam.), discrete, circular to angular, amphigenous, punctiform black spots are typical of *M. brunnea*,

(iii) large (>5 mm), discrete, circular blotches or extensive necrotic patches, dotted with acervuli on species other than *P. alba* are typical of *M. populi*. Further the dendritic spot and thread symptoms are specific to *M. populi*.

Finally, it must be emphasised that identifications based on symptom expression can only be tentative pending examination of conidia.

2. HOST SPECIFICITY

Species of *Marssonina* have been partly delimited on the basis of host specificity, as follows:

M. castagnei - specific to clones of the Section *Leuce* particularly *P. alba* (Klebahn, 1918; Rimpau, 1962; Gremmen, 1964; 1965a; Pirozynski,

1974; Cellerino, 1979);

M. tremulae (= M. brunnea) - specific to *P. tremula* (Klebahn, 1918; Rimpau, 1962; Magnani, 1966; and Cellerino, 1979);

M. brunnea - pathogenic to all poplar species, excepting those of the Section *Leuce* (Cellerino, 1979);

M. populi - pathogenic to species of the Sections *Aigeiros* and *Tacamahaca* (Cellerino, 1979).

Furthermore, Thompson (1937) and Boyer (1961) within their single species, *M. populi* recognised forms specifically pathogenic to *P. deltoides*, *P. alba* and *P. tremuloides*.

In the absence of previous definitive studies on host specificity, extensive inoculation experiments were conducted, primarily to obtain detailed information on the relative pathogenicity and host ranges of the three *Marssonina* species, and secondly, to determine whether differentiation of *formae speciales* was possible. A brief account of the taxonomy of poplar species is outlined in Appendix 10.

Materials and Methods

Host specificity was determined by examination of field collections, and by laboratory inoculation experiments.

A. Field Collections

Host specificity of the three *Marssonina* species was determined by examination of 280 field collections (Appendix 1), previously identified on the basis of conidium morphology. More detailed observations of the host range of *M. brunnea* were possible because of its presence in New Zealand and the range of poplar species grown at the Aokautere Science Centre Research Nursery.

B. Laboratory Inoculations

The host range and relative pathogenicity were compared of single isolates of *M. brunnea* from *P.x euramericana* cv. Robusta (indicated as *M. brunnea*/Rob.), *M. populi* from *P. nigra* cv. Italica and *M. castagnei* from *P. alba*. All three isolates were received from Dublin, Ireland. An American isolate (Minnesota) of *M. brunnea* from *P. tremuloides* (indicated as *M. brunnea*/trem) was included in the inoculations in view of previous

reports of isolates of *M. brunnea* from *P. tremuloides* and *P. tremula* being specific to these hosts (Rimpau, 1962; Cellerino, 1979).

Using the agar leaf-disc technique (Spiers, 1978), 306 poplar clones (Table 35) were inoculated with the above four isolates. The leaf discs (2.5 cm diam.) were from mature leaves and were inoculated on both surfaces with 25,000 conidia/disc. All treatments were replicated twice. Following inoculation the petri dishes were incubated at 20°C under natural light. Using a binocular microscope infection levels were assessed after 10 and 20 days by subjective assessment (0-3) based on the number and size of lesions formed per cm^2 leaf area (Table 36).

RESULTS

A. Field Collections

(i) *M. populi* Poplars from the Sections *Aigeiros (P. nigra, P.* x *euramericana, P. angustifolia); Tacamahaca (P. trichocarpa, P. berolinensis, P. balsamifera, P. simonii);* and a single specimen of *P. tremuloides* (Section *Leuce*) were infected with *M. populi*. *P. nigra* was by far the most commonly infected species.

(ii) *M. castagnei* All infections of *M. castagnei* were observed on a single species of the Section *Leuce*, namely *P. alba*

(iii) *M. brunnea* At the Aokautere Science Centre Research Nursery infection of *M. brunnea* was observed on species from all four Sections *(Leuce, Aigeiros, Tacamahaca, Leucoides)* and intersectional hybrids namely, *Leuce* x *Aigeiros, Leuce* x *Tacamahaca* and *Aigeiros* x *Tacamahaca*. The only species not infected were those of sub-section *Trepidae*, namely, *P. tremula* and *P. tremuloides*. However in overseas field collections infections of *M. brunnea* were commonly observed on these species.

B. Laboratory Inoculations

Infection levels following 10 and 20 days incubation were essentially similar and accordingly only those following 20 days incubation are presented in Table 37. The data are further summarised in Table 38 and the comparative susceptibility of the various sections of the genus to the four isolates is depicted graphically in Fig. 49.

TABLE 35; Poplar clones and species screened in the laboratory for susceptibility to *Marssonina* species.

SECTION LEUCE		
A. *ALBIDAE*		
P. alba L		cv. NZ old clone
"		cv. I40/57
"		cv. I42/57
"		cv. I49/51
"		cv. I59/1
"		cv. B02
P. alba L var. hickeliana Dode		cv. PE 90
" L var. pyramidalis Bunge		
New Zealand Selections		71/3/2
		72/8/3
		73/76/8
		73/77/5
		77/64/1
B. *TREPIDAE*		
P. pseudograndidentata		
P. tremula L		cv. FRI
" L		cv. Nelson
" L		cv. Wok.
P. tremuloides	ex Wiscon.	
"		clone 2
"		clone 6
"		clone 8
"		clone 17
"		clone 19
C. *ALBIDAE* x *TREPIDAE*		
P. canescens B		
P. alba x *P. glandulosa*		
		cv. K63-109
		cv. K63-110
		cv. K65-22-4
		cv. K66-20-1
SECTION AIGEIROS		
A. *AMERICAN BLACK POPLARS*		
P. deltoides		cv. AGr 21-6
"		cv. AGr 28-8
"		cv. AGr 61-58
"		cv. AGr 68-1
"		cv. ANU 28-8
"		cv. ANU 60/110
"		cv. ANU 60/129
"		cv. ANU 60/135
"		cv. ANU 60/166
"		cv. ANU 60/103

TABLE 35 Continued: Poplar clones and species screened in the laboratory for susceptibility to *Marssonina* species.

Section Aigeiros		
A.	*P. deltoides*	cv. ANU 60/125
	"	cv. ANU 60/146
	"	cv. APC 67/1-3
	"	cv. APC 67/5-1
	"	cv. APC 67/5-2
	"	cv. APC 67/5-6
	"	cv. APC 67/5-7
	"	cv. APC 67/5-13
	"	cv. APC 67/7-1
	"	cv. APC 67/7-2
	"	cv. APC 67/7-12
	"	cv. APC 67/10-3
	"	cv. APC 67/12-4
	"	cv. APC 67/16-8
	"	cv. APC 67/24-2
	"	cv. APC 67/28-6
	"	cv. APC 67/28-11
	"	cv. APC 67/31-1
	"	cv. APC 67/40-5
	"	cv. APC 67/47-2
	"	cv. APC 67/51-5
	"	cv. APC 67/65-14
	"	cv. APC 67/119A-8
	"	cv. APC 67/123A-8
	P. deltoides Marsh spp. *Angulata*	
	"	cv. Carolinensis
	"	cv. Chautagne
	"	cv. G3
	"	cv. G48
	"	cv. Mississippi R.B.
	P. deltoides	cv. Harvard
	"	cv. I63/51
	"	cv. I69/55
	"	cv. I70/51
	"	cv. I72/51SP
	"	cv. I74/51SP
	P. deltoides Marsh spp. Monilifera Henry cv. Frimley	
	P. deltoides	cv. Inta 14/71
	"	cv. Inta 16/69
	"	cv. Inta 39/71
	"	cv. Inta 66/71
	"	cv. Inta 71/67
	"	cv. Inta 79/71
	"	cv. Inta 91/71
	"	cv. Inta 158/69
	"	cv. Inta 341/69
	"	cv. Inta 372/69
	"	cv. NE 245
	"	cv. NL 1454
	"	cv. NL 1660
	"	cv. NL 2180
	"	cv. NL 2243

TABLE 35 Continued: Poplar clones and species screened in the laboratory for susceptibility to *Marssonina* species.

Section Aigeiros		
A.	*P. deltoides*	cv. NL 2433
	"	cv. NL 2515
	"	cv. Stoneville 62
	"	cv. Stoneville 66
	"	cv. Stoneville 70
	"	cv. Stoneville 71
	"	cv. Stoneville 81
	"	cv. Stoneville 92
	"	73/54/16
	"	73/62/15
	"	73/70/24
	"	74/136/63
	"	74/192/1
	"	75/4/9
	"	75/7/14
	"	75/10/16
	"	75/12/5
	"	75/32/3
	"	75/38/3
	"	75/43/2
	"	75/54/16
	"	75/105/27
	"	75/113/40
	"	75/142/25
	"	75/143/2
	"	75/114
	P. fremontii Wats.	cv. ANU 61/48
B.	*EURASIAN BLACK POPLARS*	
	P. nigra L	cv. Caudina
	P. nigra L	cv. Blanc de Garonne
	"	cv. CE9
	"	cv. TR 42/80
	"	cv. Italica
	"	cv. Italica aurea
	"	cv. Italica F
	"	cv. LP1
	"	cv. MC-18
	"	cv. MC-20
	"	cv. PG 14
	"	cv. PG 22
	"	cv. Poznan 7
	"	cv. Poznan 9
	"	cv. R103
	"	cv. Sempervirens
	"	cv. Thevestina
	"	cv. TR 56/32
	"	cv. TR 56/52
	"	cv. TR 56/72
	"	cv. TR 56/75
	"	cv. TR 62/27
	"	cv. TR 62/49

TABLE 35 Continued: Poplar clones and species screened in the laboratory for susceptibility to *Marssonina* species.

B.	*P. nigra* L	TR 62/52
	"	TR 62/57
	"	TR 62/127
	"	TR 62/140
	"	TR 62/149
	"	TR 62/154
	"	TR 62/191
	"	Vert de Garonne
	"	Y83/66
	"	Y95/67
	"	Granaracci
	P. x *euramericana* (Dode) Guinier	cv. (*deltoides* x *nigra*)
	"	cv. ANU 65-1 "
	"	cv. ANU 65-5 "
	"	cv. ANU 65-14
	"	cv. ANU 65-19 "
	"	cv. ANU 65-24 "
	"	cv. ANU 65-70 "
	"	cv. ANU 66/8 (*fremontii* x *ns*)
	"	cv. ANU 66/9 "
	"	cv. Altichiero (*d* x *n*)
	"	cv. Bellini "
	"	cv. B L Costanzo "
	"	cv. Boccalari "
	"	cv. Carpaccio "
	"	cv. Cima "
	"	cv. Eco 28 "
	"	cv. Eugenei PU *regen.* x *nI*
	"	cv. Eugenei UL "
	"	cv. Fierolo (*d* x *n*)
	"	cv. Flevo "
	"	cv. Fogolino "
	"	cv. Gelrica HA "
	"	cv. Giorgione "
	"	cv. Guardi "
	"	cv. Guariento "
	"	cv. Harff "
	"	cv. I30 "
	"	cv. I65 (Gwydyr) "
	"	cv. I74D "
	"	cv I78 "
	"	cv. I154 "
	"	cv. I214 "
	"	cv. I455 "
	"	cv. I488 "
	"	cv. I45/51 "
	"	cv. I92/40 "
	"	cv. laevigata "
	"	cv. Leipzig "
	"	cv. Longhi "
	"	cv. Marilandica F "
	"	cv. NL 925 "
	"	cv. NL 1070 "
	"	cv. NL 1601 "
	"	cv. NL 1602 "

TABLE 35 Continued: Poplar clones and species screened in the laboratory for susceptibility to *Marssonina* species.

B. *P.* x *euramericana*	cv. NL 1603	(*d* x *n*)
"	cv. NL 1605	"
"	cv. NL 1610	"
"	cv. NL 1775	"
"	cv. NL 2170	"
"	cv. NL 2171	"
"	cv. NL 2193	"
"	cv. NL 2194	"
"	cv. NL 2195	"
"	cv. NL 2196	"
"	cv. NL 2197	"
"	cv. NL 2200	"
"	cv. NL 2202	"
"	cv. NL 2205	"
"	cv. NL 2206	"
"	cv. NL 2207	"
"	cv. NL 2217	"
"	cv. NL 2220	"
"	cv. NL 2223	"
"	cv. NL 2226	"
"	cv. OP66	"
"	cv. Pacher	"
"	cv. Regenerata 360	(*nigra* x *serotina*)
"	cv. Robusta PH	(*d* x *n*)
"	cv. Robusta Zeeland	"
"	cv. Rubra	"
"	cv. San Martino	"
"	cv. Schiavone	"
"	Serotina du Poitu	(*regen.* x *serotina*)
"	cv. Tiepolo	(*d* x *n*)
"	cv. Triplo	"
"	cv. Veneziano	"
SECTION TACAMAHACA		
P. maximowiczii Henry		
"	cv. Kew	
"	cv. OJP M106	
"	cv. OJP M108	
"	cv. OJP M1011	
"	cv. OJP M1012	
"	cv. OJP M1020	
"	cv. OJP MA3	
"	cv. OJP MC-22	
P. trichocarpa Torr. & Gray	cv. CF	
"	cv. LA 99	
"	cv. V235	
"	cv. S617-16	
"	cv. S617-41	
"	cv. S617-88	
"	74/150/1 Seedling	ex California
"	74/150/5	" "
"	75/150/23	" "
"	75/150/31	" "

TABLE 35 Continued: Poplar clones and species screened in the laboratory for susceptibility to *Marssonina* species.

P. yunnanensis Dode			
"		76/200/5	Seedling ex. China
"		76/200/7	" "
P. candicans Ait.			
P. ciliata			
P. simonii			
P. simonii var. fastigiata			
P. szechuanica tibetica			
P. androscoggin			
P. koreana x *P. trichocarpa*			
SECTION AIGEIROS X TACAMAHACA			
(a) *Interamericana*	(*deltoides* x *trichocarpa*)		
"		cv. NL 1616	(*d* x *t*)
"		cv. NL 1623	"
"		cv. NL 1626	"
"		cv. NL 1647	"
"		cv. NL 1656	"
"		cv. NL 1785	"
"		cv. NL 2228	"
"		cv. NL 2233	"
"		cv. S909-1	"
"		cv. S909-10	"
"		cv. S909-12	"
"		cv. S910-2	"
"		cv. S910-5	"
"		cv. S910-8	"
"		cv. S910-10	"
"		cv. 69042-1	"
"		cv. 69042-3	"
"		cv. 69042-4	"
"		cv. 69042-5	"
"		cv. 69042-6	"
"		cv. 69043-1	"
"		cv. 69043-2	"
"		cv. 69043-3	"
"		cv. 69043-4	"
"		cv. 69044-1	"
"		cv. 69044-2	"
(b) Others		ANU 70-2	(*d* x *y*)
		I83/58	(*d* x *maxi.*)
		K62-9	(*n* x *maxi.*
		K63-100	(*Koreana* x *n*)
		K63-125	"
		K63-130	"
		K63-129	(*Koreana* x *nI*)
		K63-131	"
		Laurel S2 (*d* x *taca*)	x laurif.
		Oxford	(*maxi.* x *berol.*)

TABLE 35 Continued: Poplar clones and species screened in the laboratory for susceptibility to *Marssonina* species.

(b) Others	Rochester (*maxi.* x *nP*)
	NZ 5001 (*d* x *y*)
	NZ 5004 (*d* x *y*)
SECTION LEUCOIDES	*P. lasiocarpa* Oliv.
SECTION LEUCE X AIGEIROS	
P. deltoides x *P. alba* Maktar	cv. Delmak
" "	cv. Delmak 13
" "	cv. Delmak 16
" "	cv. Delmak 18
" "	cv. Delmak 19
" "	cv. Delmak 20
" "	cv. Delmak 22
" "	cv. Delmak 26
" "	var. pyramidalis
" "	cv. Delbo U
" "	cv. Delbo 9
P. alba 'Morocco' x *P. nigra*	cv. Sempervirens cv. Mareg 2
SECTION LEUCE X TACAMAHACA	
P. alba 'Morocco' x *P. yunnanensis*	cv. Mayu 1

KEY

d	*P. deltoides*
n	*P. nigra*
ns	*P. nigra cv.*'Sempervirens'
nI	*P. nigra cv.* 'Italica'
regen.	*P. regenerata*
t	*P. trichocarpa*
y	*P. yunnanensis*
maxi.	*P. maximowiczii*
taca.	*P. tacamahaca*
berol.	*P. berolinensis*
nP	*P. nigra* 'Planteriensis'

TABLE 36; Disease rating scale and susceptibility classification for assessing poplars for resistance to *Marssonina* species in laboratory inoculations.

Disease Rating	Lesions per cm^2/Leaf Area	Infection Level	Susceptibility Classification
0	0	nil	highly resistant
1	1-10	light	resistant
2	>10<25	medium	susceptible
3	>25	heavy	very susceptible

Within the Section *Leuce*, species of the subsection *Albidae* (*P. alba*) and *Albidae* x *Trepidae* (*P. alba* x *P. tremula*) were susceptible to all *Marssonina* species, except *M. populi*. However species of the subsection *Trepidae* (*P. tremula*, *P. tremuloides*) were only susceptible to *M. brunnea*/trem.

Within the Section *Aigeiros*, *P. deltoides* was susceptible only to isolates of *M. brunnea*/Rob. The single representative of *P. fremontii* was heavily infected by all four isolates. All cultivars of the European black poplar (*P. nigra*) were highly susceptible to infection by *M. brunnea*/Rob, and *M. populi* and were resistant to *M. castagnei* and *M. brunnea*/trem. As would be expected, cultivars of *P.*x *euramericana* (*P. deltoides* x *P. nigra*) were generally very susceptible to *M. brunnea*/Rob. and resistant to *M. populi*. No infections of *P.* x *euramericana* by *M. castagnei* or *M. brunnea*/trem were observed.

Within the Section *Tacamahaca* cultivars of *P. maximowiczii*, *P. trichocarpa*, *P. yunnanensis* and *P. simonii* were infected by all four isolates. In most instances, apart from *M. brunnea*/Rob only light infections were established. However seedlings of *P. trichocarpa* from California were heavily infected by all four isolates.

Only *M. brunnea*/Rob infected *P. lasiocarpa* the single representative of the small Section *Leucoides*.

Cultivars derived from intersectional crosses varied in resistance to the four isolates depending on the resistance of the component parent species. For instance, clones of *P.* x *interamericana* (*P. deltoides* x *P. trichocarpa*) were highly susceptible to the isolate of *M. brunnea* from *P. robusta* and highly resistant to the isolate of *M. brunnea* from *P. tremuloides*, *M. castagnei* and *M. populi*. *P.* x *Mareg* 2 of *P. alba* x *P. nigra cv.* 'Sempervirens' parentage, was highly susceptible to all species except *M. populi*.

DISCUSSION

The agar leaf-disc technique (Spiers, 1978) proved invaluable in the above studies for comparing the relative host ranges and pathogenicities of *Marssonina* species under identical conditions. Previously this technique

TABLE 37: Relative susceptibility of poplars to *Marssonina* species following inoculation of both leaf surfaces with 25,000 conidia/disc and 20 days incubation at 20°C under natural light.

Host species, clone or cultivar	*M. brunnea* AD	*M. brunnea* AB	*f.sp. trepidae* AD	*f.sp. trepidae* AB	*M. populi* AD	*M. populi* AB	*M. castagnei* AD	*M. castagnei* AB
LEUCE								
(a) *ALBIDAE*								
P. alba L. cv. NZ old clone	1	V	1	V	0	0	3	V
" cv. I40/57	0	0	0	0	0	0	1	V
" cv. I42/57	0	0	0	0	0	0	1	V
" cv. I49/51	0	0	0	0	0	0	1	V
" cv. I59/1	0	0	0	0	0	0	1	0
" cv. BO_2	1	0	0	0	0	0	1	V
P. alba var. hickeliana	1	0	1	V	0	0	3	V
" var. pyramidalis	0	0	0	0	0	0	1	V
71/3/2	1	V	0	0	0	0	3	V
72/8/3	1	V	1	V	0	0	3	V
73/76/8	1	V	1	V	0	0	2	V
73/77/5	1	V	1	V	0	0	2	V
77/64/1	1	V	0	0	0	0	2	V
(b) *TREPIDAE*								
P. pseudograndidentata	0	0	1	2	0	0	0	0
P. tremula cv. FRI	0	0	1	2	0	0	0	0
P. tremula cv. Christchurch	0	0	1	2	0	0	0	0
P. tremula cv. Wok	0	0	1	2	0	0	0	0
P. tremuloides CL 2	0	0	1	3	0	0	0	0
" CL 6	0	0	1	3	0	0	0	0
" CL 8	0	0	1	3	0	0	0	0
" CL 17	0	0	1	2	0	0	0	0
" CL 19	0	0	1	3	0	0	0	0
ALBIDAE x *TREPIDAE*								
P. canescens B	1	0	1	V	0	0	1	V
P. alba x *P. glandulosa*	0	0	1	2	0	0	3	V
K63-109	1	1	1	1	0	0	1	V
K63-110	0	0	1	0	0	0	1	V
K65-22-4	1	V	2	V	0	0	3	V
K66-20-1	1	V	1	V	0	0	3	3
MEAN LEUCE	0.4	0.4	0.7	1.2	0	0	1.3	0.8
AIGEIROS								
P. deltoides cv. AGr 21-6	2	2	0	0	0	0	0	0
" cv. AGr 28-8	2	2	0	0	0	0	0	0
" cv. AGr 61-58	1	1	0	0	0	0	0	0
" cv. AGr 68-1	2	2	0	0	0	0	0	0

CONTINUED

TABLE 37 Continued: Relative susceptibility of poplars to *Marssonina* species following inoculation of both leaf surfaces with 25,000 conidia/disc and 20 days incubation at 20°C under natural light

Host species, clone or cultivar	*M. brunnea* AD	*M. brunnea* AB	*f.sp. trepidae* AD	*f.sp. trepidae* AB	*M. populi* AD	*M. populi* AB	*M. castagnei* AD	*M. castagnei* AB
AIGEIROS Continued								
P. deltoides cv. ANU 28-8	3	3	0	0	0	0	0	0
" cv. ANU 60/110	1	1	0	0	0	0	0	0
" cv. ANU 60/129	1	1	0	0	0	0	0	0
" cv. ANU 60/135	2	3	0	0	0	0	0	0
" cv. ANU 60/166	2	2	0	0	0	0	0	0
" cv. ANU 60/103	2	2	0	0	0	0	0	0
" cv. ANU 60/125	3	3	0	0	0	0	0	0
" cv. ANU 60/146	1	0	0	0	0	0	0	0
" cv. APC 67/1-3	2	2	0	0	0	0	0	0
" cv. APC 67/5-1	3	3	0	0	0	0	0	0
" cv. APC 67/5-2	3	3	0	0	0	0	0	0
" cv. APC 67/5-6	3	3	0	0	0	0	0	0
" cv. APC 67/5-7	3	3	0	0	0	0	0	0
" cv. APC 67/5-13	3	3	0	0	0	0	0	0
" cv. APC 67/7-1	3	3	0	0	0	0	0	0
" cv. APC 67/7-2	3	3	0	0	0	0	0	0
" cv. APC 67/7-12	3	3	0	0	0	0	0	0
" cv. APC 67/10-3	3	3	0	0	0	0	0	0
" cv. APC 67/12-4	3	3	0	0	0	0	0	0
" cv. APC 67/16-8	3	3	0	0	0	0	0	0
" cv. APC 67/24-2	3	3	0	0	0	0	0	0
" cv. APC 67/28-6	3	3	0	0	0	0	0	0
" cv. APC 67/28-11	1	1	0	0	0	0	0	0
" cv. APC 66/31-1	3	3	0	0	0	0	0	0
" cv. APC 67/40-5	2	2	0	0	0	0	0	0
" cv. APC 67/47-2	2	2	0	0	0	0	0	0
" cv. APC 67/51-5	3	3	0	0	0	0	0	0
" cv. 67/65-14	2	2	0	0	0	0	0	0
" cv. APC 67/119A-8	1	1	0	0	0	0	0	0
" cv. APC 67/123A-8	3	3	0	0	0	0	0	0
P. deltoides spp. Angulata cv. Carolinensis	1	1	0	0	0	0	0	0
" cv. Chautagne	3	3	0	0	0	0	0	0
" cv. G3	1	1	0	0	0	0	0	0
" cv. G48	2	2	0	0	0	0	0	0
" cv. Mississippi RB	1	1	0	0	0	0	0	0
P. deltoides cv. Harvard	3	3	0	0	0	0	0	0
" cv. I63/51	2	3	0	0	0	0	0	0
" cv. I69/55	3	3	0	0	0	0	0	0
" cv. I70/51	1	1	0	0	0	0	0	0
" cv. I72/51	V	V	0	0	0	0	0	0
" cv. I74/51 SP	2	1	0	0	0	0	0	0
P. deltoides spp. Monilifera cv. Primley	3	3	0	0	0	0	0	0

CONTINUED

TABLE 37 Continued: Relative susceptibility of poplars to *Marssonina* species following inoculation on both leaf surfaces with 25,000 conidia/disc and incubation 20 days, 20°C under natural light

Host species, clone or cultivar		*M. brunnea* AD	*M. brunnea* AB	*f.sp. trepidae* AD	*f.sp. trepidae* AB	*M. populi* AD	*M. populi* AB	*M. castagnei* AD	*M. castagnei* AB
AIGEIROS Continued									
P. deltoides	cv. Inta 14/71	3	3	0	0	0	0	0	0
"	cv. Inta 16/69	3	3	0	0	0	0	0	0
"	cv. Inta 39/71	1	1	0	0	0	0	0	0
"	cv. Inta 66/71	2	1	0	0	0	0	0	0
"	cv. Inta 71/67	1	1	0	0	0	0	0	0
"	cv. Inta 79/71	1	1	0	0	0	0	0	0
"	cv. Inta 91/71	1	1	0	0	0	0	0	0
"	cv. Inta 158/69	1	1	0	0	0	0	0	0
"	cv. Inta 341/69	1	1	0	0	0	0	0	0
"	cv. Inta 372/69	2	2	0	0	0	0	0	0
"	cv. NL 1454	2	2	0	0	0	0	0	0
"	cv. NL 1650	V	V	0	0	0	0	0	0
"	cv. NL 2180	3	3	0	0	0	0	0	0
"	cv. NL 2243	3	3	0	0	0	0	0	0
"	cv. NL 2433	3	3	0	0	0	0	0	0
"	cv. NL 2515	3	3	0	0	0	0	0	0
"	cv. Stoneville 62	3	3	0	0	0	0	0	0
"	cv. Stoneville 66	3	3	0	0	0	0	0	0
"	cv. Stoneville 70	3	3	0	0	0	0	0	0
"	cv. Stoneville 71	2	2	0	0	0	0	0	0
"	cv. Stoneville 81	3	3	0	0	0	0	0	0
"	cv. Stoneville 92	2	2	0	0	0	0	0	0
"	73/54/16	3	3	0	0	0	0	0	0
"	73/62/15	3	3	0	0	0	0	0	0
"	73/70/24	3	3	0	0	0	0	0	0
"	74/139/63	3	3	0	0	0	0	0	0
"	74/192/1	1	1	0	0	0	0	0	0
"	75/4/9	3	3	0	0	0	0	0	0
"	75/7/14	3	3	0	0	0	0	0	0
"	75/10/16	1	1	0	0	0	0	0	0
"	75/12/5	2	2	0	0	0	0	0	0
"	75/32/3	3	3	0	0	0	0	0	0
"	75/38/3	2	2	0	0	0	0	0	0
"	75/43/2	3	3	0	0	0	0	0	0
"	75/54/16	3	3	0	0	0	0	0	0
"	75/105/27	3	3	0	0	0	0	0	0
"	75/113/40	3	3	0	0	0	0	0	0
"	75/142/25	3	3	0	0	0	0	0	0
"	75/143/2	3	3	0	0	0	0	0	0
"	75/114	3	3	0	0	0	0	0	0
P. fremontii	cv. 61/48	3	3	2	2	3	3	1	1
MEAN DELTOIDES		2.3	2.3	0.02	0.03	0.03	0.03	0.01	0.01

CONTINUED

TABLE 37 Continued: Relative susceptibility of poplars to *Marssonina* species following inoculation on both leaf surfaces with 25,000 conidia/disc and incubation 20 days, 20°C under natural light

Host species, clone or cultivar	*M. brunnea* AD	*M. brunnea* AB	*f.sp. trepidae* AD	*f.sp. trepidae* AB	*M. populi* AD	*M. populi* AB	*M. castagnei* AD	*M. castagnei* AB
AIGEIROS Continued								
P. nigra cv. Caudina	3	3	1	1	3	3	0	0
P. nigra cv. Blanc de Garonne	3	3	1	1	3	3	1	1
" cv. CE9	3	3	2	3	3	3	2	2
" cv. IR 42/80	3	3	2	2	3	3	2	2
" cv. Italica	3	3	1	1	3	3	1	1
" cv. Italica aurea	3	3	1	1	3	3	2	2
" cv. Italica F	3	3	1	1	3	3	1	1
" cv. LP1	3	3	2	2	3	3	1	1
" cv. MC-18	3	3	1	1	3	3	1	1
" cv. MC 20	3	3	1	1	3	3	1	1
" cv. PG 14	3	3	2	2	3	3	2	2
" cv. PG 22	3	3	1	1	3	3	1	1
" cv. Poznan 7	3	3	1	1	3	3	2	2
" cv, Poznan 9	3	3	1	1	3	3	2	2
" cv. R103	3	3	1	1	3	3	1	1
" cv. Sempervirens	3	3	1	1	3	3	1	1
" cv. Thevestina	3	3	1	1	3	3	2	2
" cv. TR 56/32	3	3	1	1	3	3	1	1
" cv. TR 56/52	3	3	1	1	3	3	1	1
" cv. TR 56/72	3	3	1	1	3	3	1	1
" cv. TR 56/75	3	3	1	1	3	3	1	2
" cv. TR 62/27	3	3	0	0	2	3	2	2
" cv. TR 62/49	3	3	1	1	3	3	1	1
" cv. TR 62/52	3	3	1	1	3	3	1	1
" cv. TR 62/57	3	3	1	1	3	3	1	1
" cv. TR 62/127	3	3	1	1	3	3	1	1
" cv. TR 62/140	3	3	1	1	3	3	1	1
" cv. TR 62/149	3	3	1	1	3	3	1	1
" cv. TR 62/154	3	3	1	1	3	3	1	1
" cv. TR 62/191	3	3	1	1	3	3	0	1
" cv. Vert de Garonne	3	3	1	1	3	3	2	2
" cv. Y83/66	3	3	0	0	2	2	1	1
" cv. Y95/67	3	3	1	1	3	3	1	1
" cv. Granaracci	3	3	1	1	3	3	1	2
MEAN P. NIGRA	3.0	3.0	1.1	1.1	3.0	3.0	1.2	1.3
P. x *euramericana* cv. ANU 65-1	3	3	0	0	0	0	0	0
" cv. ANU 65-5	3	3	0	0	0	0	0	0
" cv. ANU 65-14	3	3	0	0	0	0	0	0
" cv. ANU 65-19	3	3	0	0	0	0	0	0
" cv. ANU 65-24	3	3	0	0	0	0	0	0

CONTINUED

TABLE 37 Continued: Relative susceptibility of poplars to *Marssonina* species following inoculation on both leaf surfaces with 25,000 conidia/disc and incubation 20 days, 20°C under natural light

Host species, clone or cultivar	*M. brunnea* AD	*M. brunnea* AB	*f.sp.trepidae* AD	*f.sp.trepidae* AB	*M. populi* AD	*M. populi* AB	*M. castagnei* AD	*M. castagnei* AB
AIGEIROS Continued								
P. x euramericana cv. ANU 65-70	3	3	0	0	0	0	0	0
" cv. ANU 66/8	3	3	1	1	3	3	0	0
" cv. ANU 66/9	3	3	1	1	3	3	0	0
" cv. Altichiero	3	3	0	0	2	2	0	0
" cv. Bellini	3	3	0	0	2	2	0	0
" cv. B L Costanzo	3	3	0	0	2	2	0	0
" cv. Boccalari	3	3	0	0	1	1	0	0
" cv. Carpaccio	3	3	0	0	1	1	0	0
" cv. Cima	2	2	0	0	0	0	0	0
" cv. Eco 28	3	3	0	0	0	0	0	0
" cv. Eugenei PU	3	3	0	0	2	2	0	0
" cv. Eugenei UL	3	3	0	0	2	2	0	0
" cv. Fierolo	3	3	0	0	0	0	0	0
" cv. Flevo	3	3	0	0	0	0	0	0
" cv. Fogolino	3	3	0	0	0	0	0	0
" cv. Gelrica HA	3	3	0	0	2	2	0	0
" cv. Giorgione	3	3	0	0	0	0	0	0
" cv. Guardi	3	3	0	0	0	0	0	0
" cv. Guariento	3	3	0	0	1	1	0	0
" cv. Harff	3	3	0	0	1	1	0	0
" cv. I30	3	3	0	0	1	1	0	0
" cv. I65	3	3	0	0	0	0	0	0
" cv. I74D	3	3	0	0	0	0	0	0
" cv. I78	3	3	0	0	1	0	0	0
" cv. I154	3	3	0	0	0	0	0	0
" cv. I214	3	3	0	0	0	0	0	0
" cv. I455	3	3	0	0	2	2	0	0
" cv. I488	3	3	0	0	2	2	0	0
" cv. I45/51	3	3	0	0	0	0	0	0
" cv. I92/40	3	3	0	0	0	0	0	0
" cv. laevigiata	3	3	0	0	2	2	0	0
" cv. Leipzig	3	3	0	0	0	0	0	0
" cv. Longhi	3	3	0	0	0	0	0	0
" cv. Marilandica F	3	3	0	0	0	0	0	0
" cv. NL 925	3	3	0	0	0	0	0	0
" cv. NL 1070	2	2	0	0	0	0	0	0
" cv. NL 1601	3	3	0	0	0	0	0	0
" cv. NL 1602	3	3	0	0	0	0	0	0
" cv. NL 1603	3	3	0	0	0	0	0	0
" cv. NL 1605	3	3	0	0	1	0	0	0
" cv. NL 1610	3	3	0	0	2	2	0	0
" cv. NL 1775	3	3	0	0	0	0	0	0

CONTINUED

TABLE 37 Continued: Relative susceptibility of poplars to *Marssonina* species following inoculation on both leaf surfaces with 25,000 conidia/disc and incubation 20 days, 20°C under natural light

Host species, clone or cultivar	*M. brunnea* AD	AB	*f.sp. trepidae* AD	AB	*M. populi* AD	AB	*M. castagnei* AD	AB
AIGEIROS Continued								
P. x euramericana cv. NL 2170	3	3	0	0	0	0	0	0
" cv. NL 2171	3	3	0	0	0	0	0	0
" cv. NL 2193	3	3	0	0	0	0	0	0
" cv. NL 2194	3	3	0	0	0	0	0	0
" cv. NL 2195	3	3	0	0	1	1	0	0
" cv. NL 2196	3	3	0	0	1	1	0	0
" cv. NL 2197	3	3	0	0	1	1	0	0
" cv. NL 2200	3	3	0	0	0	0	0	0
" cv. NL 2202	3	3	0	0	1	1	0	0
" cv. NL 2205	3	3	0	0	1	1	0	0
" cv. NL 2206	3	3	0	0	0	0	0	0
" cv. NL 2207	3	3	0	0	0	0	0	0
" cv. NL 2217	3	3	0	0	0	0	0	0
" cv. NL 2220	3	3	0	0	1	1	0	0
" cv. NL 2223	3	3	0	0	0	0	0	0
" cv. NL 2226	3	3	0	0	0	0	0	0
" cv. OP66	3	3	0	0	2	2	0	0
" cv. Pacher	3	3	0	0	1	1	0	0
" cv. Regenerata 360	3	3	0	0	2	2	0	0
" cv. Robusta PH	3	3	0	0	2	2	0	0
" cv. Robusta Zeeland	3	3	0	0	2	2	0	0
" cv. Rubra	3	3	0	0	1	1	0	0
" cv. San Martino	3	3	0	0	0	0	0	0
" cv. Schiavone	3	3	0	0	0	0	0	0
" cv. Serotina du Poitu	3	3	0	0	2	2	0	0
" cv. Tiepolo	3	3	0	0	0	0	0	0
" cv. Triplo	3	3	0	0	0	0	0	0
" cv. Veneziano	3	3	0	0	0	0	0	0
MEAN P. x EURAMERICANA	3.0	3.0	0.03	0.03	0.7	0.6	0	0
TACAMAHACA								
P. maximowiczii cv. Kew	1	V	0	0	0	0	0	0
" cv. OJP M106	2	V	1	1	1	1	0	0
" cv. OJP M108	2	2	1	1	0	0	1	1
" cv. OJP M1011	2	3	1	1	1	1	1	1
" cv. OJP M1012	1	V	1	1	0	0	0	0
" cv. OJP M1020	1	2	1	1	1	1	1	1
" cv. OJP MA3	1	2	0	0	0	0	0	0
" cv. OJP MC-22	1	2	0	0	0	0	0	0

CONTINUED

TABLE 37 Continued: Relative susceptibility of poplars to *Marssonina* species following inoculation on both leaf surfaces with 25,000 conidia/disc and incubation 20 days, 20°C under natural light

Host species, clone or cultivar	*M. brunnea* AD	*M. brunnea* AB	*f.sp. trepidae* AD	*f.sp. trepidae* AB	*M. populi* AD	*M. populi* AB	*M. castagnei* AD	*M. castagnei* AB
TACAMAHACA Continued								
P. trichocarpa cv. CF	2	2	0	0	0	0	0	0
" cv. LA 99	2	2	0	0	0	0	0	0
" cv. V235	3	2	0	0	0	0	0	0
" cv. S617-16	3	2	0	0	0	0	0	0
" cv. S617-41	3	2	0	0	0	0	1	1
" cv. S617-88	3	3	0	0	0	0	0	0
" 74/150/1	3	3	3	3	1	1	3	2
" 74/150/5	3	3	1	1	1	1	2	1
" 75/150/23	3	3	2	3	1	1	2	2
" 75/150/31	3	3	2	2	1	1	2	2
P. yunnanensis	0	0	0	0	0	0	0	0
" 76/200/5	3	3	2	2	1	1	1	1
" 76/200/7	3	3	2	2	1	1	1	1
P. candicans	1	3	0	0	0	0	0	0
P. ciliata	0	0	0	0	0	0	0	0
P. simonii	3	3	2	3	2	2	2	3
P. simonii var. fastigatata	3	3	1	2	2	2	1	2
P. szechuanica tibetica	2	3	0	0	0	0	0	0
P. androscoggin	1	3	0	0	0	0	0	0
P. koreana x *P. trichocarpa*	1	2	1	1	0	0	0	0
MEAN TACAMAHACA	2.0	2.2	0.7	0.8	0.5	0.5	0.7	0.7
AIGEIROS x TACAMAHACA								
P.xInteramericana cv. NL 1616	3	3	1	1	0	0	1	1
" cv. NL 1623	3	3	1	0	0	0	1	1
" cv. NL 1626	3	3	0	0	0	0	0	0
" cv. NL 1647	2	2	1	1	0	0	1	0
" cv. NL 1656	3	3	1	1	0	0	1	1
" cv. NL 1785	3	2	1	1	0	0	1	1
" cv. NL 2228	3	3	0	0	0	0	0	0
" cv. NL 2233	3	3	0	0	0	0	0	0
" cv. S909-1	3	V	0	0	0	0	0	0
" cv. S909-10	3	3	1	1	0	0	1	1
" cv. S909-12	3	1	0	0	0	0	0	0
" cv. S910-2	3	2	0	0	0	0	0	0
" cv. S910-5	3	3	0	0	0	0	0	0
" cv. S910-8	3	2	1	0	0	0	0	0
" cv. S910-10	3	2	0	0	0	0	1	0
" cv. 69042-1	3	3	0	0	0	0	0	0
" cv. 69042-3	3	3	0	0	0	0	0	0
" cv. 69042-4	2	2	0	0	0	0	1	1

TABLE 37 Continued: Relative susceptibility of poplars to *Marssonina* species following inoculation on both leaf surfaces with 25,000 conidia/disc and incubation 20 days, 20°C under natural light

Host species, clone or cultivar	*M. brunnea* AD	*M. brunnea* AB	*f.sp. trepidae* AD	*f.sp. trepidae* AB	*M. populi* AD	*M. populi* AB	*M. castagnei* AD	*M. castagnei* AB
AIGEIROS x TACAMAHACA Continued								
P. x *Interamericana* cv. 69042-5	3	2	0	0	0	0	0	0
" cv. 69042-6	2	2	0	0	0	0	0	0
" cv. 69043-1	2	1	0	0	0	0	0	0
" cv. 69043-2	3	3	2	1	1	0	1	0
" cv. 69043-3	2	3	0	0	0	0	1	0
" cv. 69043-4	3	1	1	1	0	0	0	0
" cv. 69044-1	3	3	0	0	0	0	0	0
" cv. 69044-2	3	1	0	0	0	0	0	0
Others cv. ANU 70-2	1	2	1	1	0	0	1	1
cv. I83/58	2	3	0	0	0	0	0	0
K62-9	1	1	0	1	0	0	1	1
K63-100	1	1	0	0	0	0	2	3
K63-125	2	3	2	3	2	3	0	0
K63-130	3	3	2	3	1	1	0	0
K63-129	2	3	2	3	1	2	0	0
K63-131	0	0	0	0	0	0	0	0
Laurel S2	3	3	0	0	0	0	0	0
Oxford	2	2	1	1	0	0	1	1
Rochester	1	2	0	0	0	0	0	0
NZ 5001	3	3	1	1	3	3	1	1
NZ 5004	3	3	1	1	3	3	1	1
LEUCOIDES								
Plasiocarpa	1	1	0	0	0	0	0	0
LEUCE x AIGEIROS								
P. delt. x *P. alba* cv. Delmak 13	2	3	1	1	0	0	2	3
" " cv. Delmak 16	3	3	1	2	0	0	1	2
" " cv. Delmak 18	2	3	2	3	0	0	2	3
" " cv. Delmak 19	2	3	2	3	0	0	2	3
" " cv. Delmak 20	3	3	1	1	0	0	3	3
" " cv. Delmak 22	2	3	1	1	0	0	2	3
" " cv. Delmak 26	2	3	1	1	0	0	2	3
*P. delt.*x *P. alba* pyramidalis cv. Delbo U	2	2	1	1	0	0	2	3
" " cv. Delbo 9	2	3	1	2	0	0	2	3
P. alba x *P. nigra* cv. Mareg 2	3	3	2	2	0	0	3	3
LEUCE x TACAMAHACA								
P. alba x *P. yunnanensis* cv. Mayu 1	2	2	0	0	0	0	3	3

TABLE 38: Relative susceptibility of poplars to *Marssonina* species following inoculation on both leaf surfaces with 25000 conidia/disc and 20 days incubation at 20°C under natural light.

Name of Section or Poplar Species	*M. brunnea f.sp. brunnea*		*M. brunnea f.sp. tremulae*		*M. populi*		*M. castagnei*	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
LEUCE								
a. *Albidae*	0.6[1]	0.5	0.4	0.4	0.0	0.0	1.8	1.0
b. *Trepidae*	0.0	0.0	1.0	2.4	0.0	0.0	0.0	0.0
c. *Albidae x Trepidae*	0.7	0.5	1.2	1.0	0.0	0.0	2.0	1.3
Mean Leuce	0.4	0.4	0.9	1.2	0.0	0.0	1.3	0.8
AIGEIROS								
a. *P. deltoides*	2.3	2.3	0.02	0.02	0.03	0.03	0.01	0.01
b. *P. nigra*	3.0	3.0	1.1	1.1	3.0	3.0	1.2	1.3
c. *P. x euramericana*	3.0	3.0	0.03	0.03	0.7	0.6	0.0	0.0
Mean Aigeiros	2.8	2.8	0.4	0.4	1.2	1.2	0.4	0.4
TACAMAHACA								
a. *P. maximowiczii*	1.4	1.7	0.6	0.6	0.4	0.4	0.4	0.4
b. *P. trichocarpa*	2.8	2.5	0.8	0.9	0.4	0.4	1.0	0.8
c. Others	1.7	2.3	0.8	1.0	0.7	0.8	0.6	0.9
Mean Tacamahaca	2.0	2.2	0.7	0.8	0.5	0.5	0.7	0.7
AIGEIROS X TACAMAHACA								
a. *Interamericana*	2.8	2.3	0.4	0.3	0.04	0.0	0.4	0.2
b. Others	1.8	2.2	0.8	0.9	0.8	0.9	0.5	0.6
Mean Aigeiros x Tacamahaca	2.3	2.3	0.6	0.6	0.4	0.4	0.4	0.4
LEUCOIDES								
Mean Leucoides	1.0	1.0	0	0	0	0	0	0
LEUCE X AIGEIROS								
Mean	2.3	2.9	1.3	1.7	0.0	0.0	2.1	2.9
LEUCE X TACAMAHACA								
Mean	2.0	2.0	0.0	0.0	0.0	0.0	3.0	3.0
TOTAL	25.4	26.3	8.5	10.4	6.1	5.8	13.1	12.5
Overall mean	1.8	1.9	0.6	0.7	0.4	0.4	0.9	0.9

[1]0 No infection
1 1-10 lesions cm² leaf area = light infection
2 >10<25 lesions cm² leaf area = medium infection
3 >25 lesions cm² leaf area = heavy infection

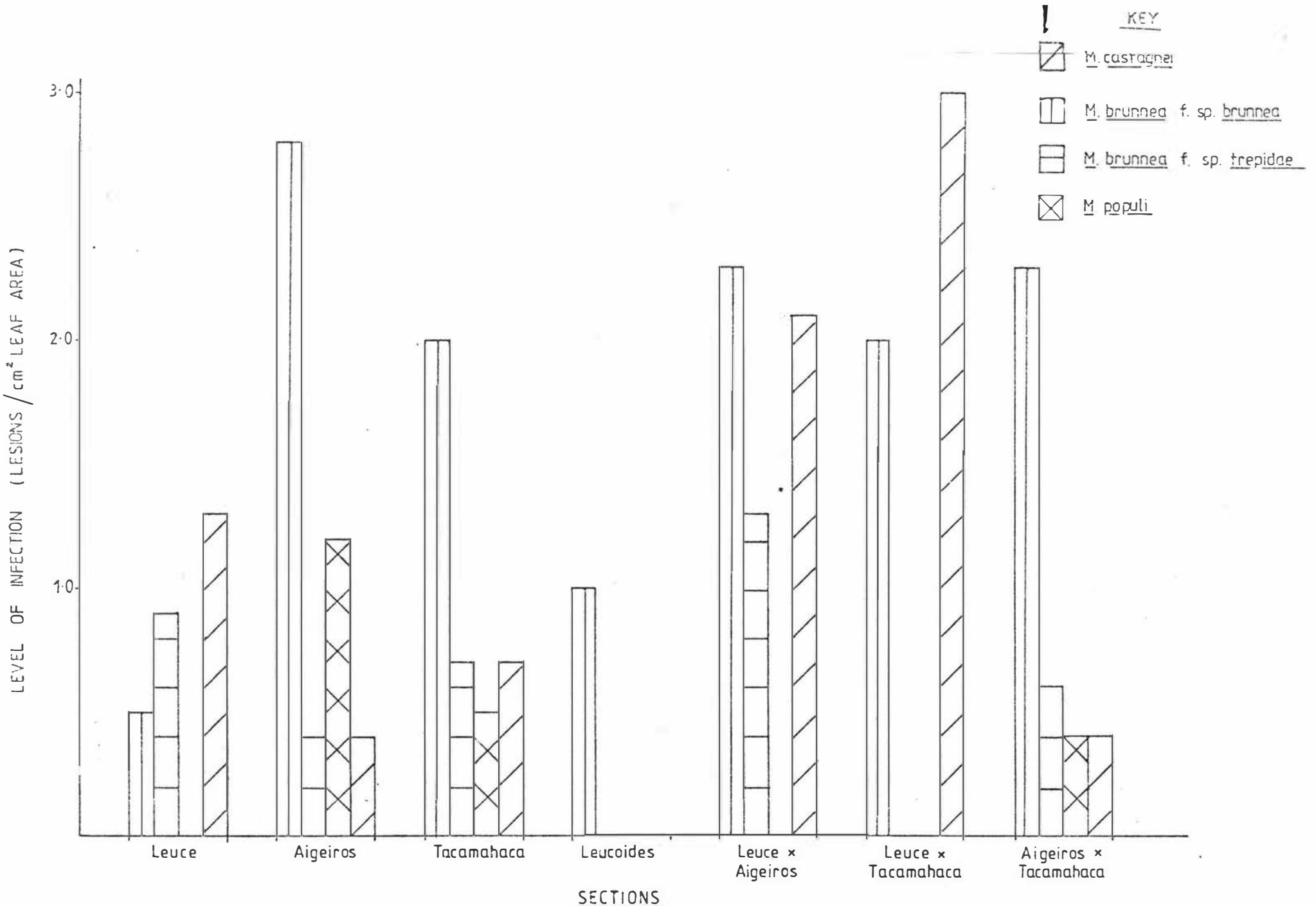


FIG 49: Comparative susceptibility of sections and intersectional crosses to infection by Marssonina species following inoculation on the adaxial leaf surface with 25 000 conidia and incubation for 20 days at 20 °C under natural light.

was used at the Aokautere Science Centre to evaluate resistance of poplar clones to infection by *M. brunnea*, and in most instances laboratory predictions were confirmed by field observations.

Marssonina brunnea was the most pathogenic species, seriously attacking species of the two largest and most commercially important Sections of the genus, *Aigeiros* x *Tacamahaca*. The pathogenicity and wide host range of this species no doubt accounts for its widespread distribution and serious impact on poplars throughout Europe, Asia and America. By contrast, in view of their limited pathogenicities and the relative commercial unimportance of their specific hosts, *M. populi*, *M. castagnei* and *M. brunnea* from *P. tremuloides/P. tremula* are not regarded as serious pathogens (Cellerino, 1979).

Although examination of field collections revealed that *P. alba* (Section *Leuce*) was the only species infected by *M. castagnei*, in laboratory inoculations species of the Sections *Aigeiros*, *Tacamahaca* and intersectional crosses, *Aigeiros* x *Tacamahaca*, *Leuce* x *Aigeiros* and *Leuce* x *Tacamahaca* were also susceptible. Within the Section *Aigeiros* in no instance were cultivars of the two most important species, *P. deltoides* and *P.* x *euramericana* infected. Apart from the report of Magnani (1966), who obtained weak infections on senescent leaves of *P. nigra*, *P. simonii*, *P.* x *berolinensis*, *P. balsamifera* and *P. yunnanensis*, this is the only record of *M. castagnei* on species other than *P. alba* (Section *Leuce*). Clearly, *M. castagnei* is not specifically pathogenic to *P. alba*, as previously reported (Klebahn, 1918; Rimpau, 1962; Gremmen, 1964; Pirozynski, 1974).

In accordance with the observations of Rimpau (1962), Gremmen (1964, 1965a), O'Riordain & Kavanagh (1965) and Cellerino (1979), *M. populi* proved pathogenic only to species of the Sections *Aigeiros*, *Tacamahaca* and *Aigeiros* x *Tacamahaca*. The report of *M. populi* on a species of the Section *Leuce (P. tremuloides)* by Pirozynski (1974) was confirmed by examination of his cited herbarium material. Since in leaf disc inoculations *M. populi* failed to infect species of the Section *Leuce* records of *M. populi* on species of this section must be regarded as atypical.

M. brunnea has previously been reported pathogenic to cultivars of the Sections *Aigeiros*, *Tacamahaca* and *Aigeiros* x *Tacamahaca* (Castellani & Cellerino, 1969; Cellerino, 1979), but not to cultivars of the Section

Leuce. However, in the present study both field collections and inoculation experiments established *M. brunnea* was pathogenic to *P. alba* and *P. canescens (P. alba* x *P. tremula)*, both members of the Section *Leuce*.

Previously, because of their host specificity, isolates of *Marssonina* from *P. tremula* and *P. tremuloides* have been regarded as distinct species, *M. tremulae* (Rimpau, 1962; Cellerino, 1979). In the present study isolates from these two hosts were morphologically identical to *M. brunnea* from clones within the Sections *Aigeiros, Tacamahaca* and *Leuce*. Accordingly *M. tremulae* is synonymous with *M. brunnea* and on the basis of priority this specific binomial has precedence. In the current study it was established that the isolate from *P.* x *euramericana* cv. Robusta was pathogenic to *P. deltoides* and *P. deltoides* x *P. nigra* but was non pathogenic to species of the subsection *Trepidae (P. tremula, P. tremuloides)*. Conversely the isolate from *P. tremuloides* was non pathogenic to *P. deltoides* x *P. nigra*. Accordingly, each should be recognised as a *forma specialis*. It is proposed that the form from *P. tremula* and *P. tremuloides* be designated *M. brunnea* f. sp. *trepidae*, and the form from other hosts be *M. brunnea* f. sp. *brunnea*. It should be pointed out that *M. brunnea* f. sp. *trepidae* was pathogenic to hosts other than *P. deltoides* and *P. nigra* x *P. deltoides*, but in all instances weakly so. This in no way invalidates the case for recognition of *formae speciales*.

H. SUMMARY

The preceeding studies established that features of conidium morphology, both on host material and agar justified recognition of three species of *Marssonina* pathogenic to the genus *Populus*. The following is a summary statement of the morphology of each species as expressed on host tissue and under the following cultural conditions:

15%V8, pH 6.5 and incubation at 20°C under 12 hour white light photoperiod.

The summary also includes symptoms and host range of each species.

I *Marssonina populi* (Lib.) Magn.

Conidia Hyaline broadly obovoid to pyriform, usually curved, divided by a single septum into smaller basal cell bearing a flat scar and a larger rounded apical cell, eguttulate or irregularly guttulate; measuring 12.0-20.0-30.0 x 4.0-6.5-11.0u; TL:S Ratio 3.40; LS:B Ratio 0.90-1.00; Septation 29.0%.

Symptom Expression (conidial and microconidial states). Principally epiphyllous, either as large circular discrete to diffuse tan blotches, extensive necrotic patches, or as coarsely dendritic 'ink-drop like' blotches or dendritic threads; often dotted with whitish punctiform acervuli.

Host Range Section *Aigeiros:* *P. nigra* and *P. fremontii* - very susceptible; *P.* x *euramericana* - variable in susceptibility; Section *Tacamahaca:* *P. maximowiczii* - resistant, *P. trichocarpa* - variable in susceptibility, *P. yunnanensis* - resistant, *P. simonii* - susceptible; Sections *Aigeiros* x *Tacamahaca:* *P.* x *interamericana* - resistant.

II *Marssonina castagnei* (Desm. & Mont.) Magn,

Conidia Hyaline, obovoid to broadly obovoid, straight or slightly curved, divided off centre by a single septum into a smaller basal cell and a larger rounded cell; eguttulate or irregularly guttulate; measuring 12.0-18.0-30.0 x 4.0-5.8-10.0u; TL:S Ratio 2.60-2.70; LS:B Ratio 1.10-1.30; Septation 37-39.0%.

Symptom Expression (conidial and microconidial states). Principally epiphyllous, discrete punctiform, dark brown spots (1-2 mm diam.) coalescing into irregular, chestnut brown blotches covering much of the leaf surface and dotted with whitish punctiform acervuli.

Host Range Principally on *P. alba* under field conditions although in the laboratory the following species were susceptible:

Section *Leuce: P. alba, P. alba* x *P. tremula;* Section *Aigeiros: P. fremontii, P. nigra;* Section *Tacamahaca: P. maximowiczii, P. trichocarpa, P. yunnanensis, P. simonii;* Sections *Aigeiros* x *Tacamahaca: P.* x *interamericana;* Sections *Leuce* x *Aigeiros: P. deltoides* x *P. alba, P. nigra* x *P. alba;* Sections *Leuce* x *Tacamahaca: P. alba* x *P. yunnanensis.*

III *Marssonina brunnea* (Ell. & Ev.) Magn.

Conidia Hyaline, narrowly obovoid to obovoid, straight to slightly curved, divided unequally by a septum into a very much smaller basal cell and a larger rounded cell; eguttulate or irregularly guttulate; measuring 10.0-15.0-23.0 x 3.5-4.1-7.0u; TL:S Ratio 3.00-3.10; LS:B Ratio 1.10; Septation 32.0%.

Symptom Expression Mainly amphigenous or hypophyllous depending on host species, typically forming punctiform black angular spots usually 1mm in diam. but occasionally confluent forming necrotic patches. Symptoms exhibited by the microconidial state often circular blotches up to 5mm diam., similar to those of *M. populi.*

Host Range

(i) *M. brunnea* f. sp. *brunnea* Species from all sections of the genus excepting species of the subsection *Trepidae,* namely *P. tremula* and *P. tremuloides.*

(ii) *M. brunnea* f. sp. *trepidae* Principally pathogenic to species of the Section *Leuce,* subsection *Trepidae,* namely *P. tremula* and *P. tremuloides* although in the laboratory the following species were also susceptible: Section *Aigeiros: P. fremontii, P. nigra;* Section *Tacamahaca: P. maximowiczii, P. trichocarpa, P. yunnanensis, P. simonii,* Sections *Aigeiros* x *Tacamahaca: P.*x *interamericana;* Sections *Leuce* x *Aigeiros: P. deltoides* x *P. alba, P. alba* x *P. nigra.*

SUGGESTED KEY TO MARSSONINA SPECIES PATHOGENIC TO POPLARS

A_1 Conidia generally straight to slightly curved, never pyriform.

B_1 Conidia narrowly obovoid; length 13-15-17u[a]; breadth 3.5-4.5-5.5u. Septum position 27-32-36% from conidium base ..... *M. brunnea.*

B_2 Conidia broadly obovoid; length 15-19-24u; breadth 5-6.5-9u. Septum position 35-40-45% from conidium base ..... *M. castagnei.*

A_2 Conidia generally strongly curved to pyriform.

Conidia broadly obovoid; length 16-19.5-24u; breadth 5-6.7-9u. Septum position 24-28-33% from conidium base ..... *M. populi*

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[a] 13-15-17u range of means, computed following examination of field collections (Appendix 2).

13 = smallest mean per individual collection
15 = overall mean of all collections
17 = largest mean per individual collection.

M. brunnea f. sp. *brunnea* and *M. brunnea* f. sp. *trepidae* may be identified, as follows:

A_1 Pathogenic to *P. deltoides* and *P.*x *euramericana* but non-pathogenic to *P. tremula* or *P. tremuloides*. ..... *M. brunnea* f. sp. *brunnea*

A_2 Pathogenic to *P. tremula* and/or *P. tremuloides* but non-pathogenic to *P. deltoides* or *P.* x *euramericana*. ..... *M. brunnea* f. sp. *trepidae*.

CHAPTER 2

CONIDIOGENESIS OF MARSSONINA SPECIES

In 1953 S.J. Hughes produced an experimental classification of the Hyphomycetes based on an understanding of conidiogenesis. This scheme sought to establish evolutionary relationships by precisely determining developmental patterns during conidiogenesis and was thought to provide a much more natural foundation for classification since it was assumed the mode of conidiogenesis was unaffected by varying nutritional or environmental factors. The Hughes (1953) system of classification of the Hyphomycetes has become widely accepted, with some modification (Tubaki, 1958; 1963; Subramanian, 1962; Barron, 1968).

The Coelomycetes (Sphaeropsidales & Melanconiales) have not been extensively incorporated into the several accounts of Deuteromycete classification based on developmental criteria. Hughes (1953) suggested that annellides typical of Section III were probably common in these groups, and further suggested that when the range of developmental types in the Sphaeropsidales and the Melanconiales became known they could be incorporated with the Hyphomycetes for the purposes of classification. Subsequent work has shown Hughes to be correct. Conidia are formed from definite conidiogenous cells in ways comparable to those described for the Hyphomycetes (Sutton, 1973). In the Coelomycetes, species with conidia and conidiophores typical of Sections 1A, II, III, IV, V, and VII (Hughes, 1953) have been found. Typically genera show annellidic (Section III) or phialidic (Section IV) ontogeny (Sutton, 1971).

Sutton (1980) recently outlined a suprageneric classification scheme for the Deuteromycotina which included both the Hyphomycetes and Coelomycetes. In this scheme conidiogenesis was accorded prime importance in distinguishing major taxonomic categories, the subdivision Deuteromycotina being divided into 2 classes depending on whether conidia were formed blastically (Blastodeuteromycetes) or thallically (Thallodeuteromycetes). These classes were further subdivided into subclasses according to whether conidia were produced holoblastically or enteroblastically; Hollo - Entero - thallomycetidae; Hollo - Entero - blastomycetidae). Three of these subclasses consisted of a single order, namely Thallales, Enterothallales and Blastales respectively. The subclass Enteroblastomycetidae comprised two orders, the Phialidales (phialidic) and the Tretales (tretic). Each of the orders Thallales, Blastales and Phialidales were further divided into 4 suborders

according to the type of conidiomata present, namely pycnidia (Thallo-pycnidiineae etc.); pycnothyria (Thallo-pycnothyriineae etc.); separate conidiophores (Thallo-hyphineae etc.) and stromata, a broad term including acervuli, sporodochia, synnemata, loculate and cupulate conidiomata (Thallostromatineae etc.). The order Tretales comprised the single suborder Tretohyphineae. Sutton (1980) stressed that his scheme had the advantages of enabling the mode of conidium ontogeny and the type of conidiomatal structure to be deduced from the suborder epithet, and further, eliminated unnatural distinctions perpetuated by previous systems. A further advantage was that his scheme applied to all the Deuteromycotina, not just the Hyphomycetes or the Coelomycetes.

Clearly before genera can be classified in the above scheme (Sutton, 1980) their mode of conidiogenesis must first be determined. Sutton (1980) reported the genus *Marssonina* to be annellidic and was therefore classified in his subclass Holoblastomycetidae, order Blastales, suborder Blasto-stromatineae. However, Sutton concluded that in view of the wide variation in conidial morphology and structure of conidiomata exhibited by the 150 taxa described in *Marssonina* it was probable that the genus would be fragmented into smaller generic units, particularly if differences in conidiogenesis were correlated with morphological distinctions. Relatively little is known concerning the conidium ontogeny of *Marssonina* species.

The possibility of currently accepted species of *Marssonina* exhibiting different modes of conidiogenesis is indicated by the claim by Pirozynski (1974) that conidium and microconidium ontogeny of *M. brunnea, M. populi* and *M. castagnei* are phialidic and the report by Moline & Pollack (1976) that *M. panattoniana* (lettuce) is annellidic.

It must be emphasised that the claim by Pirozynski (1974) that *Marssonina* species pathogenic to poplars were phialidic was based on light microscopy and therefore open to misinterpretation. In the present study the precise mode of *M. brunnea, M. castagnei* and *M. populi* was determined by transmission electron microscopy.

MATERIALS AND METHODS

A. CONIDIUM ONTOGENY

Conidium ontogeny of *M. populi, M. brunnea* and *M. castagnei* was determined in 15%V8 agar cultures, and in acervuli formed on leaf discs following 10 days

incubation at 20°C under a 12 h NUV light photoperiod. The species of poplar inoculated were:

(i) *M. brunnea* - *P. frem.* x *P. nigra* Sempervirens cv. ANU 61/48, *P.* x *euramericana* cv. Robusta, *P.* x *euramericana* cv. I-154, *P. candicans*, *P. nigra* cv. Vert de Garonne and *P. alba* x *P. nigra* Sempervirens cv. Mareg 2.

(ii) *M. populi* - *P.* x *euramericana* cv. Robusta, *P. nigra* cv. Italica, *P. nigra* cv. Vert de Garonne

(iii) *M. castagnei* - *P. alba* cv. NZ old clone and *P. alba* x *P. nigra* Sempervirens cv. Mareg 2.

Agar blocks (2mm square) from sporulating cultures and small triangular leaf pieces with acervuli were fixed in 3% glutaraldehyde + 2% formaldehyde in 0.1M phosphate buffer and prepared for scanning and transmission electron microscopy as detailed in Appendix 8.

B. MICROCONIDIUM ONTOGENY

Microconidium ontogeny of *M. brunnea*, *M. populi* and *M. castagnei* was determined in 15%V8 agar cultures following 10 days dark incubation at 20°C, and in acervuli from naturally infected leaves of *P.* x *euramericana* cv. NL 2194 (*M. brunnea*), *P. nigra* cv. Italica (*M. populi*), and *P. alba* (*M. castagnei*). Material from both substrates was prepared for scanning and transmission electron microscopy as outlined for studies of conidium ontogeny (Appendix 8).

RESULTS

A. CONIDIUM ONTOGENY

In agar and on host material in all instances conidium ontogeny of *M. brunnea*, *M. populi* and *M. castagnei* was annellidic. Conidiophores were obovoid to ampulliform and conidiogenesis was initiated by apical extension of the conidiophore. The conidiophore and conidium initial wall were inconspicuously bilayered and continuous, the first conidium thus being formed holoblastically. Outgrowth of the conidiophore wall continued until the conidium initial was fully formed. Soon after the basal conidiophore nucleus divided and a single daughter nucleus migrated into the newly formed conidium initial. The conidium initial was then delimited from the conidiophore following centripetal invagination of the plasmalemma and

formation of a bilayered septum. The delimiting septum was perforate with associated Woronin bodies. Prior to conidium secession the single central nucleus within the conidium initial divided and one of the daughter nuclei migrated to the base of the conidium initial. Soon after a perforate septum formed within the conidium initial by centripetal invagination of the plasmalemma dividing it unequally. Woronin bodies were not observed adjacent to conidial septa.

Prior to conidium secession the septal pore in the conidium delimiting septum was plugged by electron dense material which entered the septal pore from the conidiophore. Plug material remained embedded in the base of the conidium following secession. The septal pore of the underlying expanding conidium initial appeared to close by further wall growth since septal plugs were not observed in the apices of second formed conidium initials. Within the conidium further wall material was also deposited inside the septal plug.

Conidium secession was achieved by central splitting of the bilayered septum starting at the triangular pockets adjacent to the periclinal walls. The conidium was released by circumscissle rupture of the periclinal wall adjacent to the septum. This process was probably assisted by pressure from the extending apex of the underlying conidium initial. The broken edge of the outer conidium wall and the conidiophore formed a conspicuous basal frill on the conidium apex and corresponding annular scars on the apex of the conidiophore.

The distal exposed wall of the next formed conidium initial derived from the lower half of the previous conidium delimiting septum thickened, extended and blew out through the conidiophore apex. The second formed conidium initial was thus formed enteroblastically. Organelles entered blowing out conidial initials and the process continued until the conidium initial reached full size. The basal conidiophore nucleus then divided and one of the daughter nuclei entered the conidium initial. The conidium delimiting septum then formed by centripetal invagination of the plasmalemma at a slightly higher level than the previously formed septum. Prior to septum plugging and secession the nucleus within the conidium initial divided and one of the daughter nuclei migrated to the conidium base. The conidium initial was then divided by formation of a conidial septum. The conidium delimiting septum was plugged and conidium secession occurred as

for the first formed conidium.

Successive conidial initials were formed (as described above) by extension of the conidiophore wall enteroblastically from inside the base of the last formed annular scar. Walls of successive conidia originated at successively higher loci forming annellations on the conidiophore apex. The steps involved in conidium ontogeny are depicted in Figs. 50-60, and interpreted in Figs. 61, 62.

B. MICROCONIDIUM ONTOGENY

In agar and on host material in all instances microconidium ontogeny was annellidic. Microconidiophores were cylinderical to ampulliform with a single basal nucleus. The primary microconidium arose holoblastically as a protrusion or elongation of the microconidiophore apex. When the microconidium initial was fully formed the basal nucleus divided and a daughter nucleus migrated into a microconidium initial. The microconidium was then delimited from the microconidiophore by formation of a septum. Septum formation occurred by centripetal invagination of the plasmalemma. Median sections through delimiting septa revealed septal pores and Woronin bodies. Two modes of septation were observed. In the most common type a single bilayered septum formed which later split centrally releasing the microconidium. The second type of septation occurred only in field infected material of *M. brunnea*. With this mode of septation the basal wall of the microconidium and the wall forming the apex of the next formed microconidium were single layered and appeared to have formed independently, enclosing a pool of cytoplasm which was subsequently lost on secession. Immediately prior to secession the septal pore of the delimiting septum was plugged and on release of the microconidium the plug remained embedded in the base of the microconidium. Further wall material was deposited on the inner wall of the microconidium opposite the septal plug. Microconidium release in both septal types was achieved by circumscissle rupture of the periclinal wall at the same level as the septum. As a result of microconidium secession large primary secession scars were formed surrounding the microconidiophore apex and a corresponding basal frill was formed on the base of the seceded microconidium. The size of the basal frill and the primary annular scar were determined by the point of succession and thickness of the periclinal wall.

Following microconidium secession the distal exposed wall of the microconidiophore derived from the lower half of the bilayered septum which previously delimited the first formed conidium expanded and began 'blowing out' through the microconidiophore apex. Organelles entered 'blowing out' microconidium initials and the process continued until the microconidium initial had fully expanded. Nuclear division at the basal microconidiophore nucleus then occurred and one daughter nucleus migrated into the fully expanded microconidium initial. The second formed microconidium was then delimited by septum formation and the process of secession repeated as for release of the first formed microconidium. Successive microconidial initials were formed enteroblastically by extension of the microconidiophore wall from inside of the base of the last formed annular scar. Walls of succeeding microconidial initials originated at successively higher levels, resulting in a series of annular scars surrounding the apex of the microconidiophore. At most, three annular scars were observed. The steps involved in microconidium ontogeny are depicted in Figs. 63-71, and interpreted in Fig. 72. The two modes of septum formation are shown in Figs. 73, 74.

Microconidia were also formed holoblastically from conidia of *M. brunnea*, *M. populi* and *M. castagnei* in the same manner as for microconidium production from microconidiophores. These steps are summarised diagrammatically in Fig. 75. The microconidia as formed were either seceded directly at the conidium wall or at some distance away on the newly formed microconidiophore. Although only formation of the first microconidium was observed it is assumed that successive microconidium formation occurred as described for microconidia from microconidiophores.

DISCUSSION

The present studies established that conidium and microconidium ontogeny of all isolates of *M. brunnea*, *M. populi* and *M. castagnei* was annellidic both in culture and host tissue. Kendrick (1971) defined annellidic conidium ontogeny as:

> "A special form of holoblastic conidium ontogeny in which the growth of successive, very short, percurrent, vegetative proliferations of a conidiogenous cell is terminated by the development of a conidiogenous locus which gives rise to a single conidium. The conidiogenous cell usually becomes longer as it produces a basipetal sequence of conidia. What remains of each vegetative proliferation after the resulting conidium has seceded may be termed an annellation,

> and the conidiogenous cell itself may be described as annellated or termed annellide. This method of producing a plurality of conidia from a single conidiogenous cell may be exemplified by the *Spilocaea* state of *Venturia inaequalis, Doratomyces stemonitis, Scopulariopsis brevicaulis, Sporidesmium atrum, Triposporium elegans*".

Kendrick (1971) defined a phialide as:

> "A conidiogenous cell in which at least the first conidium initial is produced within an apical extension of the cell, but is liberated sooner or later by rupture or dissolution of the upper wall of the parent cell. Thereafter, from a fixed conidiogenous locus, a basipetal succession of enteroblastic conidia is produced, each clad in a newly-laid-down wall to which the wall of the conidiogenous cell does not contribute. Any phialide wall distal to the conidiogenous locus is the collarete. The length of the phialide does not change during the production of a succession of conidia, though some phialides undergo intermittent vegetative proliferation, either percurrent (as in *Catenularia*) or sympodial (as in *Codinaea*) between conidiogenous episodes".

The essential points of difference inherent in these definitions is that in annellides the first conidium is formed holoblastically and conidia are usually formed at successively higher levels on the conidiophore whereas in phialides the first conidium is formed enteroblastically and conidia are always formed at the same conidiogenous locus. Difficulties in distinguishing annellides from phialides arise if the first formed conidium is not observed or if conspicuous annellations are not formed on the annellophore. A further difficulty is that in some phialidic species formation of the first conidium may appear to be holoblastic, for example in *Aspergillus* spp. (Trinci *et al*, 1968; Hanlin, 1976). It is thus imperative that all observations made during conidiogenesis must be interpreted with great care.

A further criterion proposed by Carroll & Carroll (1974) for distinguishing the two modes of conidiogenesis is that conidium delimiting septa of phialidic species are always non-perforate and lack Woronin bodies, whereas annellidic species possess septal pores and Woronin bodies. This proposal was considered tenable by Jones (1977). Although septal pores have been reported in phialidic fungi (Zachariah & Fitz-James, 1967; Fletcher, 1971; Hammill, 1972 ab; Kahn & Aldrich, 1973; Hanlin, 1977) only once have Woronin bodies been observed associated with delimiting septa of phialides (Kahn & Aldrich, 1973). In this instance, (*Termitaria snyderi*) the delimiting septa eventually closed and the Woronin bodies disappeared. At no time have Woronin body like plugs characteristic of annellidic fungi, well illustrated by *Scopulariopsis* spp. (Cole & Aldrick, 1971; Hammill, 1971) been observed in phialidic conidium delimiting septa.

Conidiogenesis of *M. brunnea, M. populi* and *M. castagnei* was clearly annellidic in accordance with the above definitions of annellidic conidiogenesis by Kendrick (1971) and Carroll & Carroll (1974). That is, the first conidium was formed holoblastically and a new higher conidiogenous locus was formed with each percurrent vegetative proliferation through the annellophore apex. Furthermore, conidium and microconidium delimiting septa were perforate with associated Woronin bodies, and septal pore plugs were observed in seceded conidia.

In the present study the prominent primary annellation scars left by secession of the first formed conidium were visible by light microscopy and were probably mistaken for collaretes (a characteristic of phialides) by Pirozynski (1974) in his studies of *M. brunnea, M. castagnei* and *M. populi*. The corresponding basal fringe on the conidium base was also visible in the light microscope. The small size of conidiophores, their hyaline nature and the close spacing of successive annular scars on the conidiophore apex all contribute to making observations of conidiogenesis in this genus very difficult by light microscopy. Similar errors have been made previously. For example. Pirozynski & Morgan-Jones (1968) considered conidiogenous cells of *Cryptosporiopsis turgida* to be phialides on the basis of light microscope studies whereas by electron microscopy Sutton & Sandhu (1969) showed them to be annellides. Such misinterpretations led Hammill (1971) to speculate that many fungi considered to be phialidic by light microscopy would in fact be shown by electron microscopy to be annellidic. Clearly, if accurate conclusions as to the mode of conidium ontogeny are to be reached it is imperative that wall relationships during conidiogenesis are studied by transmission electron microscopy.

The present studies have established that *Marssonina* species pathogenic to poplars are annellidic and thus conform to Sutton's (1980) definition of this genus. Implicit in his definition is that conidiogenesis of the type species of the genus namely, *M. fragariae* (Lib.) Kleb. is also annellidic. To the authors knowledge detailed evidence showing that *M. fragariae* is in fact annellidic is lacking.

CHAPTER 3

TAXONOMY OF DREPANOPEZIZA SPECIES

The genus *Drepanopeziza* is small, homogenous, and worldwide in distribution. It is a member of the inoperculate Discomycetes belonging to the order Helotiales of the family Dermateaceae (Dennis, 1960).

In recent years *Drepanopeziza* species pathogenic to poplars have been studied by Rimpau (1962) and Gremmen (1965 ab) and the following *Drepanopeziza* = *Marssonina* connections have been established:

D. populorum (Desm.) v. Hohn. = *M. populi* (Lib.) Magn.
D. populi - albae (Kleb.) Nannf. = *M. castagnei* (Desm. & Mont.) Magn.
D. tremulae Rimpau = *M. tremulae* (Lib.) Kleb.
D. punctiformis Gremmen = *M. brunnea* (Ell. & Ev.) Magn.

The following is a historical account of the above *Drepanopeziza* species, plus a statement of their original descriptions.

1. *DREPANOPEZIZA POPULORUM* (Desm.) v. Hohn. Ann. Mycol. 15, (1917).
Synonyms (after Rimpau, 1962).

Trochila populorum Desm. - Bull. Soc. Bot. France 4, 858 (1857).
Pseudopeziza populorum (Desm.) Pot. - Ann. Mycol. 8, 80 (1910).
Pyrenopeziza greinichii Petr. - Ann. Mycol. 27, 405 (1929).

Trochila populorum was first described by Desmazières (1857) from *P. nigra* in France.

> "Maculis minutis, bruneis velgriseo - plumbeis dein albidis, irregulariter rotundalis, demum confluentibus. Discus innatus, erumpens minutissimus, laxè subgregarius, humidus planus cinereus, siccus concavus brunneus. Ascis clavatis, sporidiis octonis, ellipsoideis; sporulis 2, hyalinis, globosis."

Translation

> "Minute spots, brown or pearly gray, then white, irregularly rounded, later coalescing. Apothecia disc shaped, minute and immersed, later erumpent, scattered or grouped; when moist, flat, ash grey; when dry, concave, brown. Asci club shaped with eight hyaline, ellipsoidal to spherical biguttulate ascospores".

Desmazières (1857) also established the connection between *T. populorum* and *M. populi*. *T. populorum* Desm. was renamed *Drepanopeziza populorum* by von Höhnel (1917) the current epithet thus being *D. populorum* (Desm.) v. Hohn.

2. *DREPANOPEZIZA POPULI - ALBAE* (Kleb.) Nannf. - Nov. Act. Reg. Soc. Sci. Upsal. Ser. 4, 8, 170 (1932).

Synonyms (after Rimpau, 1962).

Trochila populorum (Desm.) Edgert. - Mycologia 2, 169 (1910).

Pseudopeziza populi - albae Kleb.-Haupt-u. Nebenfruchtf. Ascomyc. p. 344 (1918).

This species was originally described as *Trochila populorum* by Edgerton (1910) from overwintered leaves of *P. alba* in the United States.

> "The apothecia are at first somewhat globose, but as they grow older they generally become more or less flattened and concave at the top. The upper portion of the apothecium is forced out of the leaf during its growth, so that at maturity it projects some little distance from the surface of the leaf. The outer layer of the apothecium is composed of a pseudo-parenchymatous tissue of a dark-brown colour. Inside of this, there is a more delicate layer of hyaline cells. The outer layer entirely surrounds the developing asci and paraphyses and is not broken apart at the top until the ascospores are nearly mature. In size, the apothecia are about 90-140 x 100-190u. The asci are clavate, 12-14 x 60-80u with the ascus wall thickened at the apex. This thickened apex is ruptured when the spores are shot out. The spores are hyaline, one-celled, 12-16 x 5-7.2u, almost always containing two large guttulae, one at each end of the spore. The paraphyses are very abundant, 80-100, long, narrow, septate, and somewhat broadened at the apex".

Edgerton (1910) was unable to provide absolute proof of the connection between *T. populorum* and *M. castagnei*. However this was later established by Klebahn (1918) who named the perfect state *Pseudopeziza populi-albae* Kleb. Nannfeldt (1932) transferred this species to the genus *Drepanopeziza* naming it *D. populi-albae* (Kleb.) Nannf., the current epithet.

Thompson (1937) examined *exsiccati* specimens of *D. populorum* (Desm.) v. Hohn. and *D. populi-albae* (Kleb.) Nannf., and concluded that in view of their close morphological similarity recognition of only one species *D. populorum* (Desm.) v. Hohn., was warranted.

Rimpau (1962) examined fresh and herbarium specimens of *D. populorum* (but not the type specimen) and fresh specimens of *D. populi-albae* and concluded that because of differences in dimensions of apothecia, asci and ascospores the two species were distinct. Rimpau (1962) made no reference to Thompson (1937), which suggests that he was unaware of his studies.

3. *DREPANOPEZIZA TREMULAE* Rimpau

Rimpau (1962) erected an additional species *D. tremulae* as the perfect stage of *M. tremulae*.

> "Status ascophorus: Apothecia substrato immersa, plerumque epiphylla, raro hypophylla, leniter aggregata, 180-210u latitudine et 140-160u altitudine. Pseudostroma parce evoluta. Excipulum 70-80u crassitudine cellulis 6-10u magnitudine compositum; cellulae stratis extremis crasse tunicatae, umbrinae, polyedricae, stratis interioribus clarae, tenuiter tunicatae, isodiametricae. Margo ad 25u crassitudine et compositus 3-5 stratis cellularum tenuiter tunicatarum, elongatarum, Basaliter pseudostroma parce evolutum cellulis rotundis, brunneis, plerumque 1-2 stratis compositum in cellulas emortuas substratae penetrat. Hypothecium 8-10u crassitudine cellulis hyalinis, tenuiter tunicatis, distincte polyedricis compositum. Asci 50-60 x 8-12u magnitudine claviformes vel anguste ellipsoidei, tenuiter tunicati, poro Jodo coerulescente. Paraphyses numerosae, hyalinae, filiformes, apice ad 6u crassitudine ascis eminent. Sporae octanae ascis, unicellulatae, hyalinae ellipsoideae, ellipsoideae, univel biseriatae 8-9 x 1.8-2.0u magnitudine".

Translation:

> "Apothecia immersed in substrate, mostly epiphyllous, rarely hypophyllous, slightly clustered, 180-210u wide by 140-160u high. Pseudostroma moderately developed. Excipulum 70-80u thick, composed of cells measuring 6-10u, cells of outermost layer thickly tunicate and umber; inner layers clear, thin-walled and isodiametrical; margin up to 25u thick, composed of 3-5 layers of thin walled elongated cells. The base of the pseudostroma moderately developed with almost circular, brown cells, mainly 1-2 layers, penetrating the dead cells of the substrate. Hypothecium composed of flat-sided, rope like, hyaline cells 8-10u thick. Asci 50-60 x 8-12u, narrowly ellipsoid, thin walled, becoming sky blue when iodine stained. Paraphyses numerous, hyaline, thread-like with thickened apex (6u). Asci producing eight, one celled, hyaline, ellipsoid spores uniseriately or biseriately arrayed, 8-9 x 1.8-2.0u in size".

Criteria used by Rimpau (1962) for differentiating apothecia of *D. tremulae* from those of *D. populorum* and *D. populi-albae* were shape (conical v hemispherical), height, and smaller asci and ascospores (Table 39). In addition the inner excipulum of *D. tremulae* was considered to be more strongly developed.

TABLE 39: Dimensions of apothecia, asci and ascospores of *Drepanopeziza* species cited by Rimpau (1962)

Morphological Feature	*Drepanopeziza populi-albae*	*Drepanopeziza populorum*	*Drepanopeziza tremulae*
APOTHECIA			
Shape	hemispherical	hemispherical	conical
Diameter (u)	110-180	200-350	180-210
Height (u)	80-110	not stated	140-160
ASCI			
Length (u)	not stated	75-90	50-60
Breadth (u)	not stated	12-17	8-12
ASCOSPORES			
Length (u)	10-12	10-18	8-9
Breadth (u)	not stated	4-5	1.8-2.0

Gremmen (1965a) subsequently studied *Drepanopeziza* species pathogenic to poplars in some detail, but it appears he was unaware of the erection of *D. tremulae* Rimpau. He examined fresh material of *D. populorum* (Desm.) v. Hohn, and *D. populi-albae* (Kleb.) Nannf., and although concurring with Thompson (1937) he maintained *D. populorum* and *D. populi-albae* as separate species, primarily because conidia of their respective *Marssonina* states (*M. populi* and *M. castagnei*) were morphologically and physiologically distinct.

4. *DREPANOPEZIZA PUNCTIFORMIS* Gremmen

Gremmen (1965a) erected an additional species *D. punctiformis* as the perfect stage of *M. brunnea* (Gremmen, 1965b).

> "Status ascophorus: Apothecia 100-200u magna. Excipulum textura globulosa, 10-14u crassum. Asci 90-115 x 11-14u matura; 50-70 x 8-11u in herbario, claviformes. Paraphyses hyalinae, filiformes".
>
> Typus: In herbario J. Gremmen, 9.x.1961, in foliis *Populi euroamericana* 'Serotina', Meppel, Batava, (Gremmen 1811).

Translation:

> "Apothecia 100-200u. Excipulum of globular tissue, 10-14u thick. Asci 90-115 x 11-14u when mature; 50-70 x 8-11u in herbarium, clubshaped. Paraphyses hyaline, thread like".
>
> Type: In J. Gremmen's herbarium, 9/10/1961, on leaves of *Populus euramericana* 'Serotina', Meppel, Holland (Gremmen 1811).

Although apothecia of *D. punctiformis* were morphologically similar to those of *D. populorum* and *D. populi-albae* (Table 40), Gremmen (1965a) justified the erection of *D. punctiformis* on the following grounds:

(i) "The type of leaf spot was distinct from other poplar inhabiting *Marssonina* species,

(ii) the measurements of the macroconidia are significantly smaller than in the other species mentioned,

(iii) the maturity of the apothecia and the time of ascospore discharge in spring appeared to be much earlier than in *D. populorum*,

(iv) conspicuous conidial pustules may occur on the leaf petiole and on the green, one-year old twigs of various poplars, but without any deformation of these stems".

Comparing dimensions of apothecia, asci and ascospores of the various *Drepanopeziza* species cited by Rimpau (1962) and Gremmen (1965a) (Tables 39, 40) it is apparent that even within the same species there are large discrepancies in dimensions reported by these authors. Furthermore, in both works only the extremes of dimensions have been cited making valid comparisons difficult.

In view of the fact that Rimpau (1962) regarded *M. brunnea* as being synonymous with *M. tremulae*, and since Gremmen (1965a) independently described *D. punctiformis* as the perfect state of *M. brunnea*, it follows that *D. tremulae* and *D. punctiformis* may be synonymous. Indeed, since previous taxonomic studies of *Marssonina* species (Chap. 1) established that *M. brunnea* and *M. tremulae* were synonymous, with *M. brunnea* having priority, it follows that *D. tremulae* and *D. punctiformis* must also be synonymous. However, from the original descriptions of *D. tremulae* (Rimpau, 1962) and *D. punctiformis* (Gremmen, 1965a) the synonymy of these species appears doubtful. Currently, *D. punctiformis* is widely accepted as the perfect state of *M. brunnea* (Castellani & Freccero, 1968; Byrom & Burdekin, 1970; Pinon & Poissonnier, 1975; Cellerino, 1979). Dimensions of apothecia, asci and ascospores of *D. punctiformis* cited by these authors are listed in Table 41. It would seem *M. tremulae* and *D. tremulae* have been largely overlooked, being only recognised recently by Cellerino (1979).

In view of the lack of agreement concerning the morphology and therefore taxonomy of *Drepanopeziza* species, particularly *D. tremulae* and *D. punctiformis*, all four species were reappraised by examination of type and herbarium specimens.

Materials and Methods

(a) Type Material

Type material of three of the four *Drepanopeziza* species was examined. Type specimens of *D. populorum* were obtained from both the Musée National D'Histoire Naturelle, Paris and the Jardin Botanique National de Belgique. Difficulties were experienced in obtainirg type specimens of *D. punctiformis* Gremmen and *D. tremulae* Rimpau. Correspondence with Gremmen established that he could not remember where he had lodged his type material of *D. punctiformis!* Fortunately, a type specimen was included with other specimens obtained from the Royal Botanic Gardens, Kew. As regards *D. tremulae*, correspondence with Dr. Horak (Director,

TABLE 40: Dimensions of apothecia, asci and ascospores of *Drepanopeziza* species cited by Gremmen (1965a)

Morphological Feature	*Drepanopeziza populi-albae*	*Drepanopeziza populorum*	*Drepanopeziza punctiformis*
APOTHECIA			
Diameter (u)	240-320	130-260	100-200
Excipulum (u)	12-18	12-18	10-14
ASCI			
Length (u)	80-100 (50-80)	80-100 (50-70)	90-115 (50-70)
Breadth (u)	13.5-14.0(8-12)	13-14 (9-13)	11-14 (8-11)
ASCOSPORES			
Length (u)	14.5-18.0(7-12)	10-16	10-14
Breadth (u)	7-9 (4-7)	5-9	3-7

( ) herbarium material

TABLE 41: Dimensions of apothecia, asci and ascospores of *D. punctiformis* cited by various authorities

Morphological Feature	AUTHORITY				
	Gremmen (1965a)	Castellani & Freccero (1968)	Byrom & Burdekin (1970)	Pinon & Poissonnier (1975)	Cellerino (1979)
APOTHECIA					
Diameter (u)	100-200	100-200	1000	80-100	100-200
Height (u)	not stated	70-100	not stated	60-80	70-100
ASCI					
Length (u)	90-115	not stated	45-65-84	not stated	90-115
Breadth (u)	11-14	not stated	9-12-17	not stated	11-14
ASCOSPORES					
Length (u)	10-14	9-11	6-13-17	9-11	9-14
Breadth (u)	3-7	3-5	4-5-7	3-5	3-7

ETH Zurich) established that type material of this species was not included with other specimens deposited by Rimpau and was probably lost. Fortunately, two syntypes of *D. tremulae* (so designated by Rimpau, 1962) were obtained from the Botanisches Garten and Museum, Berlin-Dahlem.

Despite extensive overseas enquiries the type specimen of *D. populi-albae* was not located.

(b) Other Herbarium Material

Herbarium specimens alleged to be *D. populorum (Trochila populorum, Pyrenopeziza greinichii)* were received from: Rijksherbarium, Leiden the Netherlands; Jardin Botanique National de Belgique; Botanischer Garten und Botanisches Museum, Berlin-Dahlem; ETH Zurich; The Herbarium, Royal Botanic Gardens, Kew, England; the Farlow Herbarium and Reference Library, Harvard University, Massachusetts, USA. Specimens alleged to be *D. punctiformis* fixed in glutaraldehyde were received from J. Pinon, Nancy, France. Leaves of *P. deltoides* infected with the macro and microconidial states of *M. brunnea* were received from T.E. Hinds, Colorado, USA. In the laboratory these leaves were later induced to form the perfect state which was tentatively identified as *D. punctiformis*.

The type and herbarium material was examined by light, scanning electron, and transmission electron microscopy.

Light Microscope Studies

Apothecia were mounted in lactophenol and stained with 0.5% acid fuchsin. A cover-slip was added and the slides left for 15 minutes to allow dried apothecia to rehydrate and soften. Apothecia were then squashed by gentle tapping on the coverslip. For each specimen the length and breadth dimensions of at least 50 asci and 60 ascospores were measured using a Leitz optical micrometer (500x).

Scanning Electron Microscope Studies

Small fragments of leaf material bearing apothecia were vacuum dried, glued to metal stubs and coated with approximately 150^{0}A of gold and examined with a Cwikscan 100 field emission scanning electron microscope.

Transmission Electron Microscope Studies

Where possible up to five apothecia were removed on small triangular portions of leaf tissue and prepared for transmission electron microscope

studies. The material was first rehydrated by standing on moist blotting paper in a petri dish for several hours. Specimens were then placed in 3% glutaraldehyde and 2% formaldehyde in 0.1m phosphate buffer and prepared for electron microscopy, as outlined in Appendix 8.

RESULTS

With one exception type and alleged specimens of *D. populorum* and *D. punctiforms* were in each instance morphologically similar. The single exception was a specimen alleged to be *Trochila populorum* received from Farlow Herbarium. In view of the morphological similarity with syntypes of *D. tremulae* and the common host *(P. tremula)* this specimen was identified as *D. tremulae*. The following morphological descriptions apply to both type and herbarium specimens of each *Drepanopeziza* species, in view of their close morphological similarity.

Morphological Description of Drepanopeziza spp.

1. *DREPANOPEZIZA POPULORUM* (Desm.) V. Hohn.

Apothecia - Partly immersed in host tissue, scattered or grouped in circular clusters on both sides of leaves (Fig. 76). Circular in outline, 75 - (140-180) - 300u diam. x 120-180u deep (Figs. 77, 78). In vertical section either conical, rectangular, concave disk-like, or flat and distorted (Fig. 79). Amber brown when moist, drying black. External Morphology (Fig. 80), either lumpy or fused vertical, finger-like processes on sides and top. Ectal excipulum (Figs. 81, 82, 83), strongly developed, 25-40u thick, consisting of an outer layer of irregular elongate cells arrayed in a dark-pigmented matrix. Inner layers 3-4 rows of globular, regularly arranged cells, bounded on the inside by paraphyses. Medullary excipulum (Fig. 84), large (5-7u diam.), angular, globose to subglobose cells, interspersed with smaller cells (3-4u diam.). Hypothecium (Fig. 84), small cells (3-4u diam.) globose to subglobose, regularly arrayed around the base of asci. Epithecium (Fig. 85), irregular cells loosely embedded in a dark matrix. Asci (Fig. 86), cylindrical to clavate, tapering from a broad apex to the stalk with a claw like foot, measuring 50.0-80.7-130.0 x 9.0-13.6-20.0u, apex thickened (2.5-3.5u), 8 spored. Ascospores (Fig. 86), irregularly uniseriate or biseriate, unicellular, ellipsoidal, smooth walled, hyaline with two conspicuous polar bodies, 10.0-14.0-20.0 x 3.5-5.1-8.0u, L:B ratio 2.74. Paraphyses filiform, septate, longer than asci,

terminal cell clavate, commonly enlarged, 3.5-5.0u.

Specimens examined -

Type *P. nigra* L, France, 1857 Col. Desmazières (Sub. *Trochila populorum* Desm.) ex Musée National D'Histoire Naturelle Paris; Co-type as above ex Jardin Botanique National de Belgique; *P. nigra* L, Konigstein, Elbe, Saxonia, Germany 14/1899 col. W. Krieger, Rabenhorst-Pazschke, Fungi Europaei et extraeuropaei, no. 4267 (sub. *Trochila populorum* Desm.), ex. Rijksherbarium, Leiden, Netherlands; as above ex Jardin Botanique National de Belgique; *P. canadensis* Moench, Baumschulen Tamsel, Brandenburg, Deutschland 20/5/1924, col. P. Vogel (Sub. *Pyrenopeziza greinichii* Petr.) Syndow, Mycoth. Germ. Nr. 2536 ex Rijksherbarium, Leiden, Netherlands; *P. nigra* L, Heimbach Siegen, Westfalen, Deutschland, 1/5/1938 Col. A. Ludwig, Herb. A. Ludwig ex Botanischer Garten, Berlin-Dahlem; *P. canadensis* Moench, Baumschulen, Tamsel, Brandenburg, Deutschland, 25/5/1932, Col. P. Vogel, Herb. A. Ludwig ex Botanischer Garten, Berlin-Dahlem; *P. nigra* L, Siegen, Westfalen, Deutschland, 20/5/1938, col. Ipse Herb. A. Ludwig ex Botanischer Garten, Berlin-Dahlem; *P. nigra* L, Ferndorf, Siegen, Westfalen, Deutschland 26/5/1938 col. Ipse, Herb. A. Ludwig ex Botanischer Garten, Berlin-Dahlem; *P. nigra* L, Kreis Lebus Brandenburg, Deutschland, 10/5/1941, col. Fahrendorff (sub. *Trochila populorum*) Herb. Fahrendorff ex Botanischer Garten, Berlin-Dahlem; *P. nigra* L, Zurich, Affoltern, Wehntaler - Str, 7/5/1961, col. R.H. Rimpau, ETH No. 4548 ex ETH Zurich; *P. nigra* spp. pyramidalis (Roz.) Celak., Gesammelt Limmatufer Fisherweg Hardturm, Zurich, 6/5/1961, col. R.H. Rimpau ex ETH Zurich; *P. nigra* L, Sihlholzli Str., Zurich, 5/5/1961, Col. R.H. Rimpau ex ETH Zurich. *P. tacamahaca* x *P. trichocarpa* cv. 37, Monastereum Co. Kildare, Ireland, Autumn 1965, Col. F. O'Riordain, National Museum of Ireland ex The Herbarium, Kew.

2. *DREPANOPEZIZA TREMULAE* Rimpau

Apothecia partly immersed in host tissue, scattered or grouped in circular clusters, amphigenous, circular in outline, 75-(110-150)-200 diam. x 90-140u deep (Fig. 87). In vertical section conical to barrel shape or concave disc-like (Fig. 88). Amber brown when moist, drying black. External morphology (Fig. 89), lumpy on sides and top. Ectal excipulum (Fig. 90), 15-20u thick, consisting of 2-3 layers of large (5-7u) globular to irregular cells embedded in a dark matrix, bounded on the inside by 2-4 layers of closely packed elongate to globular cells and

paraphyses. Medullary excipulum (Fig. 91), cells angular, globose to subglobose (5-7u diam.), interspersed with smaller cells (3-4u). Hypothecium (Fig. 91), cells irregular (3-4u diam.), closely packed around base of asci. Epithecium cells globular to irregular, loosely arrayed in a darkly stained matrix. Asci (Fig. 92), clavate, narrowly to broadly cylinderical, tapering from the broad apex to the stalk with a claw-like foot, 45.0-63.6-120.0 x 8.0-12.1-20.0u, apex thickened 2.5-3.0u, 8 spored. Ascospores (Figs. 92, 94), irregularly uniseriate or biseriate, unicellular, ellipsoidal, smooth walled, hyaline with two conspicuous polar bodies, 9.0-12.0-17.0 x 3.0-4.4-7.0u, L:B ratio 2.73. Paraphyses (Fig. 93), filiform, septate, longer than asci, terminal cell clavate enlarged 2.5-3.5u.

Specimens examined -

Syntype *P. tremula* L. Wald Finkenkrug, Osthavelland, Deutschland 24/4/1937, Col. W. Kirschstein (sub. *Trochila populorum* Desm.) Herb. W. Kirschstein ex Botanischer Garten, Berlin - Dahlem; Syntype *P. tremula* L. Alt Landsberg, krs. Niederbarnim, Prov. Brandenburg, Deutschland, 11/10/1940 Col. Fahrendorff (sub. *Drepanopeziza populorum* (Desm.) v. Hohn), Herb. Dr. A. Ludwig ex Botanischer Garten, Berlin-Dahlem; *P. tremula* L, Niederdonau bei Wien, Osterreich, April Col. - sub (*Trochila populorum* Desm.) Herb. Fr. v. Hohnel no. 1241 ex Farlow Herb, Harvard University.

3. *DREPANOPEZIZA PUNCTIFORMIS* Gremmen

Apothecia partly immersed in host tissue, scattered or grouped in circular clusters, amphigenous, circular in outline, 75-(100-150)-200 diam. by 100-140u deep (Figs. 95, 96). In section, conical to barrel shape or concave disc-like (Fig. 97). Amber brown when moist, drying black. External morphology (Fig. 98), lumpy on sides and top. Ectal excipulum (Fig. 99), varied in structure, either 20-30u thick and composed of 2-3 rows of globular to irregular cells bounded on the inside by several layers of elongated paraphyses-like cells, or 15-20u thick composed of 2-3 outer rows of globular to irregular cells bounded on the inside by 3-4 rows of smaller (3-4u diam.) closely packed, elongate to globular cells. Medullary excipulum cells angular to globose to subglobose (5-7u diam.), interspersed with smaller cells (3-4u diam.). Hypothecium cells globose to subglobose (3-4u diam.) and regularly arrayed around the base of asci. Epithecium (Fig. 100), cells globular to irregularly shaped, loosely arrayed

in a dark stained matrix. Asci (Fig. 101), clavate, narrowly to broadly cylindrical, tapering from the broad apex to the stalk with a claw-like foot, 45.0-73.6-140.0 x 8.0-11.2-20.0u, apex thickened 2.5-3.0u, 8 spored. Ascospores (Figs. 101, 102), irregularly uniseriate or biseriate, unicellular, ellipsoidal, smooth walled, hyaline with two conspicuous polar bodies, uninucleate with numerous ribosomes and mitochondria, 8.0-11.9-16.0 x 3.0-4.4-7.0u, L:B ratio 2.70. Paraphyses filiform, septate longer than asci, terminal cell clavate and enlarged (2.5-3.5u).

Specimens examined -

Type - *P. canadensis* cv. Marilandica, Oost Flevoland, Netherlands, 21/5/1965 Col. J. Gremmen. Herb. J. Gremmen No. 2242 ex The Herbarium, Kew, England ; *P.* x *euramericana*, Nancy, France, Col. J. Pinon, 1970s ex J. Pinon; *P. deltoides*, Colorado, T.E. Hinds, 30/10/1979, induced to form apothecia in the laboratory.

The comparative mean overall dimensions of apothecia, asci and ascopsores of type specimens of *D. populorum*, *D. punctiformis* and syntypes of *D. tremulae* are presented in Table 42. The dimensions of the examined type and herbarium specimens of *D. populorum*, *D. tremulae* and *D. punctiformis* are presented in Tables 43, 44, 45 respectively. The range and mean overall dimensions of apothecia, asci and ascospores of the three species are presented in Table 46. To facilitate comparison of the three species, the data are depicted graphically in Fig. 103 (dimensions of asci) and Fig. 104 (dimensions of ascospores). It should be noted that asci of one specimen (*D. punctiformis*, ex *P. deltoides* USA) were abnormally long and as a consequence skewed mean overall ascus length of this species.

DISCUSSION

Examination of type and herbarium specimens of *D. populorum*, *D. tremulae* and *D. punctiformis* showed that all were morphologically similar. This fact confirms observations by Gremmen (1965a) that, "these *Drepanopeziza* species form a group of very similar units difficult to separate".

Light and electron microscope studies of vertical sections of apothecia showed that within and between species there were often large differences in structure of the various component tissues. In *D. populorum* the ectal and medullary excipulum were generally well developed. Whereas these tissues were poorly developed in *D. tremulae* and *D. punctiformis*.

The hypothecium and epithecium tissues of the three species were comparable. Furthermore in vertical section the shape of apothecia varied within species and was influenced by the maturity of the apothecium. In view of the basically similar structure of apothecia between species it was not possible to delimit any one species on structural features of the apothecium as was reported by Rimpau (1962).

Asci and ascospores of the three *Drepanopeziza* species were morphologically indistinguishable. Although asci and ascospores of *D. populorum* were significantly larger ($P>0.05$, t-test) than those of *D. tremulae* and *D. punctiformis*, species delimitation was not possible because of overlapping length and breadth dimensions. Previously Rimpau (1962) used dimensions of asci and ascospores to delimit *D. populorum*, *D. populi-albae* and *D. tremulae*.

Although specimens of *D. populi-albae* were not included in the present study, from the descriptions of this species by Edgerton (1910), Rimpau (1962), and Gremmen (1965a) it is apparent that apothecia of *D. populi-albae* closely resemble those of *D. populorum* *D. tremulae* and *D. punctiformis*. This is further confirmed by the decision of Thompson (1937) to unite *D. populi-albae* and *D. populorum*. Thus in spite of subtle differences in structure, apothecia of the four *Drepanopeziza* species are morphologically inseparable. Gremmen (1965a) acknowledged the close morphological similarity of *D. populi-albae*, *D. populorum* and *D. punctiformis* and accordingly used host specificity and morphological features exhibited by conidia of the respective imperfect states to delimit *Drepanopeziza* species pathogenic to poplars. This is a logical solution to a difficult taxonomic problem providing only morphological features of *Marssonina* species are used to delimit species. However the difficulty with identification of *Drepanopeziza* species is not a serious problem in view of the limited period during which this state is active. For positive identification ascospores could be inoculated onto leaf discs and the resultant *Marssonina* species identified.

Taxonomic studies of *Marssonina* species (Chapt. 1) showed that *M. brunnea* and *M. tremulae* were synonymous, with *M. brunnea* having priority over *M. tremulae*. It follows therefore that the perfect state of *M. brunnea (D. punctiformis)* and the perfect state of *M. tremulae (D. tremulae)* must also be synonymous, with *D. tremulae* having priority. Synonymy of these

species is further supported by the particularly close morphological similarity of apothecia, asci, and ascospores of *D. punctiformis* and *D. tremulae*. The following *Drepanopeziza-Marssonina* connections are thus recognised:

D. populi-albae (Kleb.) Nannf. = *M. castagnei* (Desm. & Mont.) Magn.
D. populorum (Desm.) V. Hohn. = *M. populi* (Lib.) Magn.
D. tremulae Rimpau = *M. brunnea* (Ell. & Ev.) Magn.

Connections between these perfect-imperfect states have been independently established:

(i) *D. populi-albae* = *M. castagnei* - Klebahn (1918), Rimpau (1962) and Gremmen (1965a).

(ii) *D. populorum* = *M. populi* - Desmazières (1857), Rimpau (1962), Gremmen (1965a), and O'Riordain & Kavanagh (1965).

(iii) *D. tremulae* = *M. brunnea* - Rimpau (1962), and sub. *D. punctiformis* - Gremmen (1965ab), Castellani & Freccero (1968) Byrom & Burdekin (1970), Pinon & Poissonnier (1975) and the present study.

TABLE 42: Dimensions of apothecia, asci, and ascospores of type specimens of *D. populorum*, *D. punctiformis* and *D. tremulae*.

Morphological Feature	*Drepanopeziza populorum*	*Drepanopeziza punctiformis*	*Drepanopeziza tremulae*
APOTHECIA			
Diameter	100 (125-150) 250	75(100-125) 200	75 (100-150) 200
ASCI			
Length (u)	50-74-100	50-70-90	50-66-120
Breadth (u)	9-13.7-20	8-11-16	8-11.8-16
L:B ratio	5.40	6.30	5.61
ASCOSPORES			
Length (u)	10-13.5-18	8-11-16	9-12.7-17
Breadth (u)	3-5-8	3-4-6.5	3.5-4.7-7.0
L:B ratio	2.66	2.80	2.71

TABLE 43: Dimensions of apothecia, asci and ascospores of type and herbarium specimens of *Drepanopeziza populorum*.

Specimen No.[a]	Apothecia Diameter (u)			Dimensions of Asci (u)				Asci L:B ratio	Dimensions of Ascospores (u)				Asco-spores L:B ratio
				Length		Breadth			Length		Breadth		
	Min.	Mean	Max.	Mean	Range	Mean	Range		Mean	Range	Mean	Range	
1 type	100	(125-150)	250	$75.0_{8.0}$[b]	(60-100)	$13.8_{2.4}$	(9-20)	5.43	$13.6_{1.6}$	(10-18)	$5.3_{0.4}$	(4-7)	2.56
2 "	100	(125-150)	225	$73.0_{12.0}$	(50-90)	$13.6_{1.6}$	(10-18)	5.36	$13.3_{1.3}$	(10-17)	$4.8_{0.6}$	(3-8)	2.77
3	100	(125-150)	250	$81.3_{9.3}$	(60-110)	$14.2_{1.5}$	(10-18)	5.72	$13.3_{1.5}$	(10-18)	$4.8_{0.8}$	(4-7)	2.77
4	100	(125-150)	250	$83.3_{12.0}$	(60-110)	$14.0_{2.3}$	(9-20)	5.72	$13.7_{1.4}$	(10-17)	$4.7_{0.5}$	(3-7)	2.91
5	75	(125-150)	250	$79.0_{11.6}$	(60-110)	$14.1_{1.9}$	(10-19)	5.60	$13.5_{0.9}$	(10-17)	$5.0_{0.7}$	(4-7)	2.70
6	75	(125-150)	250	$76.0_{10.0}$	(60-100)	$13.8_{2.0}$	(10-20)	5.51	$14.0_{1.7}$	(11-18)	$5.3_{0.4}$	(4-7)	2.64
7	75	(125-150)	250	$87.0_{12.0}$	(60-110)	$12.4_{1.3}$	(9-18)	7.01	$13.7_{1.6}$	(11-20)	$5.1_{0.5}$	(4-7)	2.68
8	75	(125-150)	250	$84.0_{9.0}$	(60-110)	$12.2_{1.5}$	(10-18)	6.90	$14.0_{1.0}$	(12-18)	$5.0_{0.5}$	(4-7)	2.80
9	75	(125-150)	250	$85.7_{14.0}$	(60-120)	$12.5_{2.0}$	(10-18)	6.85	$14.5_{2.1}$	(11-20)	$5.1_{1.0}$	(4-8)	2.84
10	75	(125-150)	250	$75.3_{10.0}$	(60-90)	$14.0_{2.1}$	(10-17)	5.38	$14.4_{1.5}$	(12-20)	$5.1_{0.6}$	(4-7)	2.82
11	75	(125-150)	250	$79.0_{10.0}$	(60-90)	$12.6_{1.6}$	(10-17)	6.27	$13.0_{2.0}$	(11-18)	$5.0_{0.4}$	(3-7)	2.60
12	75	(125-150)	250	$73.8_{6.1}$	(60-130)	$14.8_{2.0}$	(10-20)	4.98	$14.3_{1.2}$	(10-18)	$5.2_{0.4}$	(4-7)	2.75
13	75	(125-175)	250	$76.0_{6.5}$	(65-100)	$13.3_{1.7}$	(10-18)	5.71	$15.0_{1.9}$	(11-20)	$5.1_{0.6}$	(4-7)	2.94
14	75	(100-150)	250	$96.0_{16.0}$	(70-130)	$15.2_{1.2}$	(12-18)	6.31	$15.3_{1.3}$	(12-19)	$5.6_{0.6}$	(4-8)	2.73
15	75	(125-150)	250	$76.0_{6.5}$	(65-100)	$13.3_{1.7}$	(10-16)	5.71	$15.0_{1.9}$	(11-20)	$5.1_{0.6}$	(4-7)	2.94
16	75	(125-175)	300	$91.0_{12.0}$	(60-110)	$14.0_{2.3}$	(9-20)	6.50	$14.2_{1.3}$	(10-18)	$5.1_{0.5}$	(4-7)	2.78
MEAN	75	(125-150)	300	$80.7_{6.6}$	(50-130)	$13.6_{0.8}$	(9-20)	5.93	$14.0_{0.7}$	(10-20)	$5.1_{0.2}$	(3-8)	2.76

[a] For identification of isolates see overleaf

[b] Standard deviation

HERBARIUM SPECIMENS OF *DREPANOPEZIZA POPULORUM* (Desm.) V. Hohn. EXAMINED

Type -

1. *P. nigra* L, France 1857, Col. Desmazières (sub. *Trochila populorum* Desm.), ex Musée National D'Histoire Naturelle - Paris.
2. Co-type as above ex Jardin Botanique National De Belgique.
3. *P. nigra* L, Konigstein, Elbe, Saxonia, Germany, 14/1899, Col. W. Krieger, Rabenhorst - Pazschke, Fungi Europaei et extraeuropaei no. 4267 (sub. *Trochila populorum* Desm.) ex Rijksherbarium, Leiden - Netherlands.
4. As above ... ... ex. Jardin Botanique National De Belgique.
5. *P. canadensis* Moench., Baumschulen, Tamsel, Brandenburg, Deutschland, 20/5/1924, Col. P. Vogel (sub. *Pyrenopeziza greinichii* Petr.), Sydow, Mycoth. Germ. Nr. 2536, ex Rijksherbarium Leiden - Netherlands.
6. *P. nigra* L, Heimbach, Siegen, Westfalen, Deutschland, 1/5/1938, Col. A. Ludwig, Herb. A. Ludwig - ex Botanischer Garten und Botanisches Museum, Berlin-Dahlem.
7. *P. canadensis* Moench., Baumschulen, Tamsel, Brandenburg, Deutschland, 25/5/1932, Col. P. Vogel, Herb. A. Ludwig ex Botanischer Garten und Botanisches Museum, Berlin - Dahlem.

8 & 9. *P. nigra* L, Siegen, Westfalen, Deutschland, 20/5/1938, Col. Ipse, Herb. A. Ludwig ex Botanischer Garten und Botanisches Museum, Berlin - Dahlem.

10 & 11. *P. nigra* L, Ferndorf, Siegen, Westfalen, Deutschland, 26/5/1938, Col. Ipse. Herb. A. Ludwig ex Botanischer Garten und Botanisches Museum, Berlin - Dahlem.

12. *P. nigra* L, Kreis Lebus Brandenburg, Deutschland, 10/5/1941, Col. Fahrendorff (sub. *Trochila populorum*). Herb. Fahrendorff ex Botanischer Garten und Botanisches Museum, Berlin - Dahlem.
13. *P. nigra* L, Zurich - Affoltern, Wehntaler - Str., Zurich, 7/5/1961, Col. R.H. Rimpau, ETH, Nr. 4548 ex Eidgenossischen Technisches Hochschule, Zurich.
14. *P. nigra* spp. pyramidalis (Roz.) Celak, Gesammelt Limmat - Ufer Fischerweg, Hardturm, Zurich, 6/5/1961, Col. R.H. Rimpau, ex. ETH Zurich.
15. *P. nigra* L, Sihlholzli - Str., Zurich, 5/5/1961, Col. R.H. Rimpau, ex ETH Zurich.
16. *P. tacamahaca* x *P. trichocarpa* 37, Monastereum, Co. Kildare, Ireland, Autumn 1965, Col. F. O'Riordain, National Museum of Ireland, ex The Herbarium Royal Botanic Gardens Kew, England.

TABLE 44: Dimensions of apothecia, asci and ascospores of syntype and herbarium specimens of *Drepanopeziza tremulae*

Specimen No.[a]	Diameter of Apothecia (u) Min.	Mean	Max.	Dimensions of Asci (u) Length Mean	Range	Breadth Mean	Range	Asci L:B Ratio	Dimensions of Ascospores (u) Length Mean	Range	Breadth Mean	Range	Ascospores L:B Ratio
1 Syntype	75	(100-150)	200	$64.0_{10.0}$[b]	(50-90)	$12.1_{1.4}$	(9-16)	5.28	$13.2_{1.2}$	(10-17)	$4.7_{0.5}$	(3.5-6.5)	2.81
2 "	90	(125-150)	200	$68.9_{11.0}$	(50-120)	$11.6_{1.5}$	(8-16)	5.94	$12.3_{1.1}$	(9-17)	$4.7_{0.4}$	(3.5-7.0)	2.61
3	75	(100-125)	200	$60.0_{10.0}$	(45-90)	$12.8_{1.3}$	(9-20)	4.75	$11.1_{0.9}$	(9-17)	$4.1_{0.7}$	(3.0-6.5)	2.70
MEAN	75	(100-150)	200	$64.6_{4.1}$	(45-120)	$12.2_{0.6}$	(8-20)	5.32	$12.2_{1.0}$	(9-17)	$4.5_{0.3}$	(3.0-7.0)	2.71

[a] Specimens examined [b] Standard deviation

1. *P. tremula* L - Wald Finkenkrug, Osthavelland, Deutschland, 24/4/1937 Col. W. Kirschstein (sub. *Trochila populorum* Desm.) Herb. W. Kirschstein ex Botanischer Garten und Botanisches Museum, Berlin - Dahlem Syntype.

2. *P. tremula* L - Alt - Landsberg, Krs Niederbarnim, Prov. Brandenburg, Deutschland, 11/10/1940. Col. Fahrendorff (sub. *Drepanopeziza populorum* (Desm.) V. Hohn.) Herb. Dr. A. Ludwig ex Botanischer Garten und Botanisches Museum, Berlin - Dahlem Syntype.

3. *P. tremula* L - Niederdonau, bei Wien, Osterreich, April - Col. - (Sub. *Trochila populorum* Desm.). Herb. Fr. V. Hohnel no. 1241 ex Farlow Herbarium and Reference Library, Harvard University, Massachusetts USA.

TABLE 45: Dimensions of apothecia, asci and ascospores of type and herbarium specimens of *Drepanopeziza punctiformis*.

Specimen[a]	Diameter of Apothecia (u) Min.	Mean	Max.	Dimensions of Asci (u) Length Mean	Range	Breadth Mean	Range	Asci L:B Ratio	Dimensions of Ascospores (u) Length Mean	Range	Breadth Mean	Range	Ascospores L:B Ratio
1 Type	75	(100-150)	200	$70.0_{7.2}$[b]	(50-90)	$11.1_{1.5}$	(8-16)	6.30	$11.1_{1.2}$	(8-16)	$4.0_{0.3}$	(3.0-6.5)	2.80
2a	75	(100-125)	200	$68.0_{8.3}$	(50-90)	$12.0_{1.1}$	(9-16)	5.66	$12.3_{0.8}$	(9-16)	$4.7_{0.5}$	(3.5-6.5)	2.62
b	75	(100-140)	200	$64.0_{8.8}$	(45-120)	$11.6_{1.5}$	(9-20)	5.52	$12.8_{0.9}$	(9-16)	$4.6_{0.3}$	(3.5-7.0)	2.78
3	100	(125-150)	200	$97.2_{11.0}$	(65-140)	$11.0_{1.0}$	(9-14)	8.83	$11.0_{1.0}$[c]	(8-15)	$4.3_{0.5}$	(3.5-6.0)	2.56
MEAN	75	(100-150)	200	$74.8_{15.1}$	(45-140)	$11.4_{0.4}$	(8-20)	6.56	$11.8_{0.9}$	(8-16)	$4.4_{0.3}$	(3.0-7.0)	2.69

[a] Specimens examined [b] Standard deviation

1. *P. canadensis* cv. Marilandica, Oost Flevoland, Netherlands, 21/5/1965 Col. J. Gremmen, Herb. J. Gremmen no. 2242 ex The Herbarium, Royal Botanic Gardens, Kew, England - Type specimen.

2a + b *P.* x *euramericana*, Nancy, France, Col. J. Pinon.

3. *P. deltoides*, Colorado, USA, Col. T.E. Hinds, 30/10/1979, induced to form apothecia in the laboratory.

TABLE 46: Mean overall dimensions of apothecia, asci and ascospores of type and herbarium specimens of *D. populorum*, *D. tremulae* and *D. punctiformis* (mean & range)

DREPANOPEZIZA Species	Apothecial diam. (u) Min.	Mean	Max.	Asci (u) Length Mean	Range	Breadth Mean	Range	Asci L:B Ratio	Ascospores (u) Length Mean	Range	Breadth Mean	Range	Asco-spores L:B Ratio
D. populorum	75	(125-150)	300	$80.7_{6.6}$[a]	(50-130)	$13.6_{0.8}$	(9-20)	$5.93_{0.6}$	$14.0_{0.7}$	(10-20)	$5.1_{0.2}$	(3-8)	$2.76_{0.1}$
D. tremulae	75	(100-150)	200	$64.6_{4.1}$	(45-120)	$12.2_{0.6}$	(8-20)	$5.32_{0.6}$	$12.2_{1.0}$	(9-17)	$4.5_{0.3}$	(3-7)	$2.71_{0.1}$
D. punctiformis	75	(100-150)	200	$74.8_{15.1}$	(45-140)	$11.4_{0.4}$	(8-20)	$6.56_{1.5}$	$11.8_{0.9}$	(8-16)	$4.4_{0.3}$	(3-7)	$2.69_{0.1}$

[a] Standard deviation

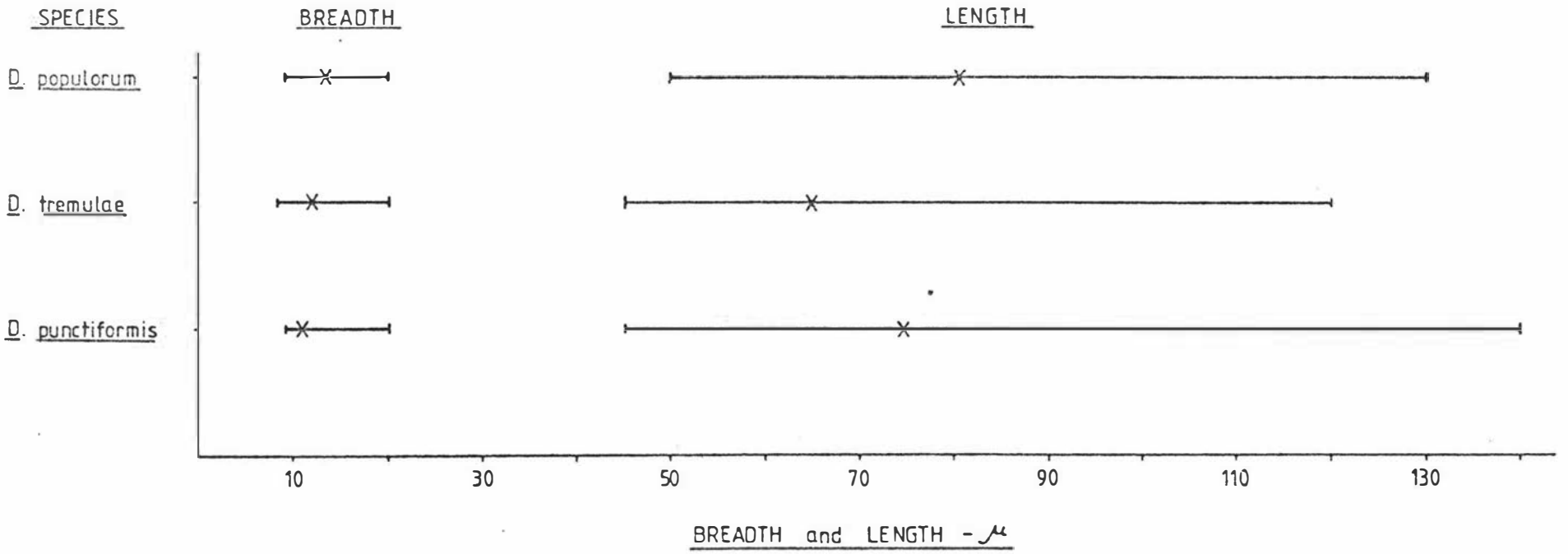

FIG. 103: Range (⊢—⊣) and mean (—×—) length and breadth dimensions of asci of Drepanopeziza species from field collections.

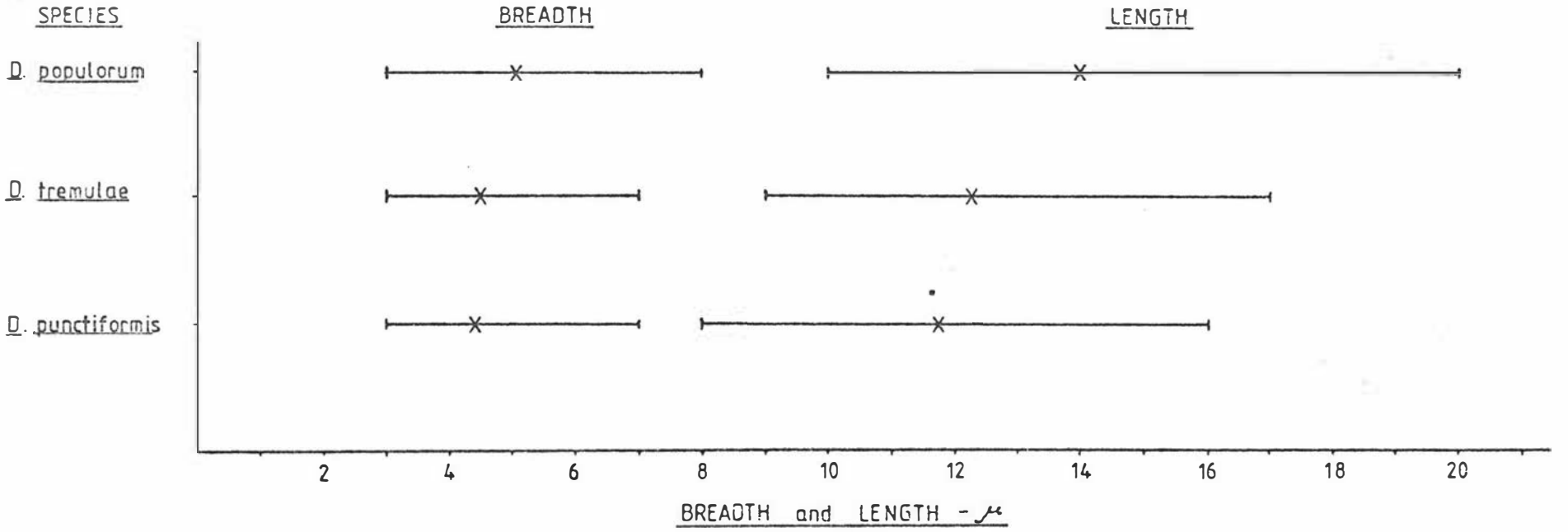

FIG. 104: Range (⟷) and mean (⟷X⟷) length and breadth dimensions of ascospores of Drepanopeziza species from field collections.

CHAPTER 4

LABORATORY INDUCTION OF APOTHECIA

Laboratory induction of apothecia by *Marssonina* species would facilitate comparative morphological studies of the respective perfect states and further, would enable confirmation of the connections between *Drepanopeziza* and *Marssonina* species. However the production of apothecia requires that the microconidial stage be first induced since microconidia of *Marssonina* species (pathogenic to poplars) are thought to function as spermatia (Castellani & Freccero, 1968). Although the role of microconidia in spermatization has not been proven their function as such is strongly suggested by their formation in autumn prior to leaf fall, and their inability to germinate (writers observations).

A INDUCTION OF MICROCONIDIA

The influence of substrate (host, agar), photoperiod and temperature on intensity of microconidium production was investigated using a single New Zealand isolate of *M. brunnea* (Br 5). Plates of PDA, PCA, 2%V8, 5%V8, 15%V8 agar, and leaf discs from young maturing and senescing leaves of *P.* x *euramericana* cv. 'Robusta' were inoculated and incubated for 12 days as follows:

1.	12°C	(a) 10h light/14h dark*	(b) constant dark
2.	16°C	(a) 10h light/14h dark	(b) constant dark
3.	16°C 10h/6°C 14h**	(a) 10h light/14h dark	(b) constant dark
4.	20°C	(a) 10h light/14h dark	(b) constant dark
5.	24°C	(a) 10h light/14h dark	(b) constant dark
6.	24°C 10h/10°C 14h	(a) 10h light/14h dark	(b) constant dark
7.	24°C 10h/6°C 14h	(a) 10h light/14h dark	(b) constant dark
8.	25-18°C room temp.	(a) natural light	(b) constant dark
9.	28°C	(a) 10h light/14h dark	(b) constant dark

* 10h light/14h dark - incubator programmed for 10 hours light followed by 14 hours dark.

** 16°C 10h/6°C 14h - incubator programmed to 16°C for 10 hours followed by a temperature change within an hour to 6°C for 14 hours.

Lighting was by cool-white, daylight fluorescent lamps. Intensity of microconidial production was subjectively assessed by microscopic examination (500X) of agar cultures and leaf discs by counting the approximate number of microconidia per field of view. Four levels of production were recognised:

0 = No microconidia present.
1 = Light production, up to 50 microconidia per field.
2 = Medium production, up to 300 microconidia per field.
3 = Heavy production, well in excess of 300 microconidia per field.

RESULTS

The intensity of microconidial production was influenced by substrate, temperature and photoperiod (Table 47). Irrespective of light regime optimum production occurred on 15%V8 agar and host tissue (maturing and senescing) at temperatures with diurnal fluctuations, namely 16/6°C, 24/6°C and 24/10°C. The negative effects of unfavourable temperatures (12, 16, 20, 24°C) were mitigated to some extent by dark incubation and senescence of leaf tissue.

Since the above results pertained to a single isolate of *M. brunnea* the following experiment was conducted to determine whether such conditions applied to other isolates of *M. brunnea*, and isolates of *M. populi* and *M. castagnei* (Table 48). Plates of 15%V8 and leaf discs from senescing leaves of *P. alba* Morocco x *P. nigra* Sempervirens cv. Mareg 2 *(M. castagnei)* and *P.* x *euramericana* cv. Robusta *(M. brunnea, M. populi)* were inoculated with conidia of the three *Marssonina* species and incubated for 12 days under a daily regime of:

(a) 10h at 24°C with light and 14h at 10°C in darkness,
(b) in constant darkness for 10h at 24°C and 14h at 10°C.

RESULTS

All isolates formed abundant microconidia (>300/field 500X) both on agar and leaf discs under the two light regimes.

TABLE 47: Influence of substrate, photoperiod and temperature on intensity of microconidium production of a New Zealand isolate of *M. brunnea* (Br 5).

Temperature °C	Culture Media										Host Tissue				Total per Temperature
	PDA		PCA		2%V8		5%V8		15%V8		Maturing		Senescing		
	L/D[c]	D	L/D	D	L/D	D	L/D	D	L/D	D	L/D	D	L/D	D	
12	1[d]	1	0	1	1	2	1	2	1	3	0	3	0	3	19
16	1	1	0	1	1	2	1	2	1	3	0	3	3	3	22
16/6[a]	1	1	0	1	1	1	2	2	3	3	3	3	3	3	27
20	0	1	0	1	1	1	1	2	0	3	0	3	3	3	19
24	0	0	0	0	0	1	0	1	0	1	0	1	0	2	6
24/6	1	3	1	1	1	1	2	2	3	3	3	3	3	3	30
24/10	1	3	1	1	1	1	2	2	3	3	3	3	3	3	30
25-18[b]	1	1	1	1	1	1	2	2	1	3	0	3	1	3	21
28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total per Light Regime	6	11	3	7	7	10	11	15	12	22	9	22	16	23	
Total per Substrate	17		10		17		26		34		31		39		

[a] 16/6 16°C for 10h then 6°C for 14h

[b] 25-18 room temperature

[c] L/D 10h white light then 14h dark

[d] intensity of microconidial production
0 no microconidia
1 <50 microconidia/field (500X)
2 >50<300 microconidia/field (500X)
3 >300 microconidia/field (500X)

TABLE 48: Isolates of *Marssonina* species screened for production of microconidia on 15%V8 agar and host tissue.

Isolate	Species	Host	Origin
Br 3	*M. brunnea*	*P. fremontii* cv. 61/48	Palmerston North, NZ
Br 5	"	*P.* x *euramericana* cv. I-214	Palmerston North, NZ
Br 6	"	*P. alba*	Palmerston North, NZ
Br 7	"	*P.* x *euramericana* cv. Flevo	Palmerston North, NZ
Br 10	"	*P. deltoides*	Illinois, USA
Br 12	"	*P. deltoides*	Illinois, USA
Br 13	"	*P. deltoides*	Iowa, USA
Br 14	"	*P. trichocarpa*	Minnesota, USA
Br 15	"	*P. deltoides*	Colorado, USA
Br 17	"	*P. Robusta*	Dublin, Ireland
Br 18	"	*P.* x *euramericana*	Nancy, France
Br 19	"	*P.* x *euramericana*	Hees, Holland
Br 20	"	*P. nigra*	Ankara, Turkey
Br 21	"	*P. tremuloides*	Colorado, USA
Br 23	"	*P. tremuloides*	Fairbanks, Alaska
Br 24	"	*P. tremuloides*	Minnesota, USA
Cs 2	*M. castagnei*	*P. alba*	Dublin, Ireland
Cs 3	"	*P. alba*	Ankara, Turkey
Po 1	*M. populi*	*P.* x *canadensis*	Herts., England
Po 3	"	*P. nigra* cv. 'Italica'	Kew, England
Po 4	"	*P. nigra* cv. 'Italica'	Dublin, Ireland
Po 5	"	*P.* x *berolinensis*	Munchen, Germany
Po 6	"	*P. nigra*	Zurich, Switzerland

B INDUCTION OF APOTHECIA

Induction of apothecia of *Drepanopeziza tremulae*, *D. populi-albae* and *D. populorum* was attempted on naturally infected leaves, leaf discs and agar.

(1) NATURALLY INFECTED LEAVES

Overseas collections of leaves infected with the microconidial states of *M. brunnea*, *M. populi* and *M. castagnei* (Table 49) were soaked in water and placed separately in sealed plastic containers with moist paper towels. The leaves were dark incubated at 8°C for 10 days, 5°C for 20 days, 2°C for 3 days, 5°C for 20 days, 2°C for 3 days, 5°C for 20 days, 8°C for 10 days, 12°C for 5 days, 5°C for 5 days and finally 8°C for 20 days. Leaves were examined for apothecia every 15 days.

RESULTS

Apothecia were formed only on leaves of *P. deltoides* received from Colorado. Following 60 days incubation the apothecia *(D. tremulae)* produced mature ascospores which proved pathogenic to leaf discs of *P.* x *euramericana* cv. Robusta. Ten days following inoculation the conidial state *(M. brunnea)* was formed on the leaf discs, thus confirming the connection between these two species.

(2) LEAF DISCS

In three experiments leaf discs were inoculated with:

(i) sixteen isolates of *M. brunnea* received from throughout the world (Table 50),

(ii) conidia of *M. brunnea* washed from leaves of *P. deltoides* from Colorado,

(iii) isolates of *M. castagnei* from Turkey and Ireland.

Experiment A

Leaf discs (2.5 cm diam.) of *P.* x *euramericana* cv. Robusta (40 discs) and *P. nigra* cv. Italica 'Aurea' (40 discs) inserted adaxial surface uppermost into wells in 2% water agar were inoculated with a one ml suspension of conidia (approx. 6000) prepared by blending 16 isolates of *M. brunnea* (Table 50). The leaf discs were dark incubated for 10h at 24°C and 14h at 10°C (15 days) to induce formation of microconidia. To induce

TABLE 49: Origin of naturally infected leaves used in laboratory experiments to induce apothecia

Species	Origin	Host	Collector	Date
M. brunnea	Palmerston North, NZ	*P.* x *euramericana*	Author	25/3/78-79
"	Dublin, Ireland	*P.* x *euramericana* cv. Robusta	F. O'Riordain	30/10/79
"	Colorado, USA	*P.* x *deltoides*	T.E. Hinds	30/10/79
M. castagnei	Dublin, Ireland	*P. alba*	F. O'Riordain	30/10/79
M. populi	" "	*P. nigra* cv. Italica	"	30/10/79
"	Surrey, England	*P. nigra* cv. Italica	A.G. Bailey	10/11/79

TABLE 50: Isolates of *M. brunnea* used in experiments to induce apothecia on leaf discs and agar.

Isolate	Host	Origin
Br 1	*P. yunnanensis*	Palmerston North, NZ
Br 3	*P. fremontii* cv. 61/48	Palmerston North, NZ
Br 6	*P. alba*	Palmerston North, NZ
Br 7	*P.* x *euramericana* cv. Flevo	Palmerston North, NZ
Br 8	*P.* x *euramericana* cv. NL 2194	Palmerston North, NZ
Br 10	*P. deltoides*	Illinois, USA
Br 11	*P. deltoides*	Illinois, USA
Br 12	*P. deltoides*	Illinois, USA
Br 13	*P. deltoides*	Iowa, USA
Br 14	*P. trichocarpa*	Minnesota, USA
Br 15	*P. deltoides*	Colorado, USA
Br 16	*P.* x *euramericana* cv. Robusta	Surrey, England
Br 17	*P.* x *euramericana* cv. Robusta	Dublin, Ireland
Br 18	*P.* x *euramericana*	Nancy, France
Br 19	*P.* x *euramericana*	Hees, Holland
Br 20	*P. nigra*	Ankara, Turkey

the production of apothecia the leaf discs were then removed from agar and held in sealed plastic containers lined with moistened paper towels. The containers were dark incubated, as for naturally infected leaves.

Experiment B

Leaf discs (2.5 cm diam.) of *P.* x *euramericana* cv. Robusta (40 discs) and *P. nigra* cv. Italica 'Aurea' (40 discs) were inoculated with a conidial suspension prepared from leaves of *P. deltoides* ex. Colorado. Compatible mating strains of *M. brunnea* must have been present on this material since apothecia had previously been induced on these leaves. The inoculated discs were incubated as in experiment A to firstly induce production of microconidia and then apothecia.

Experiment C

Forty leaf discs (2.5cm diam.) of *P. alba* 'Morocco' x *P. nigra* Sempervirens cv. Mareg 2 were each inoculated with a one ml conidial suspension (approx. 6000 conidia/ml) prepared by blending two isolates of *M. castagnei* (Ireland & Turkey). To induce microconidial and apothecial production the leaf discs were dark incubated as in the previous experiments.

RESULTS

Although microconidia were produced in abundance in each experiment, apothecia *(D. tremulae)* were formed only on leaf discs inoculated with conidia of *M. brunnea* from Colorado. Apothecia were first observed 40 days following inoculation and mature ascospores at 60 days. Apothecia were morphologically similar to those induced on naturally infected leaves.

On leaf discs inoculated with conidia of *M. brunnea* (experiments A and B) numerous spherical, raised cushion-like stromatal bodies developed. Stromata superficially resembled immature apothecia in shape and size measuring 150-200u in diameter x 150-250u in depth, and extended 80-100u above the cuticle (Fig. 105). However electron microscopy revealed that such bodies were structurally distinct being composed of closely packed, isodiametrical, oval to elongate thick-walled cells containing many lipid globules and plugged septal pores (Fig. 106), whereas in apothecia the component tissues (ectal, medullary excipulum, hypothecium) were quite distinct and variously developed. With further

development of the stromata, microconidia and conidia were formed within and on the surface of stromatal tissue, such conidia were pathogenic to leaf discs of *P.* x *euramericana* cv. Robusta.

(3) AGAR

Plates of 15%V8 and PC-10 were inoculated with isolates of *M. brunnea* and *M. castagnei* used in experiments A & B, and with isolates from leaves of *P. deltoides* from Colorado. Plates were then sealed with parafilm and dark-incubated (as previously) to induce production of microconidia and apothecia.

RESULTS

Although abundant microconidia were produced apothecia were not formed. However isolates of *M. brunnea* formed large, elevated black leathery stromatic bodies morphologically similar to those formed on leaf discs (experiments B & C).

C INFLUENCE OF TEMPERATURE ON DEVELOPMENT AND MORPHOLOGY OF APOTHECIA *(D. TREMULAE)*

Dried leaves of *P. deltoides* infected with *M. brunnea* ex. Colorado were rehydrated and placed on moist blotting paper in glass petri dishes which were sealed with parafilm and dark incubated at the following 25 temperatures:

1. (a) Constant 3°C.
   (b) 3°C 5 days/5°C 2 days*.
   (c) 3°C 5 days/8°C 2 days.
   (d) 3°C 5 days/12°C 2 days.
   (e) 3°C 5 days/16°C 2 days.

2. (a) Constant 5°C.
   (b) 5°C 5 days/3°C 2 days.
   (c) 5°C 5 days/8°C 2 days.
   (d) 5°C 5 days/12°C 2 days.
   (e) 5°C 5 days/16°C 2 days.

3. (a) Constant 8°C.
   (b) 8°C 5 days/3°C 2 days.
   (c) 8°C 5 days/5°C 2 days.
   (d) 8°C 5 days/12°C 2 days.
   (e) 8°C 5 days/16°C 2 days.

4. (a) Constant 12°C.
   (b) 12°C 5 days/3°C 2 days.
   (c) 12°C 5 days/5°C 2 days.
   (d) 12°C 5 days/8°C 2 days.
   (e) 12°C 5 days/16°C 2 days.

5. (a) Constant 16°C.
   (b) 16°C 5 days/3°C 2 days.
   (c) 16°C 5 days/5°C 2 days.
   (d) 16°C 5 days/8°C 2 days.
   (e) 16°C 5 days/12°C 2 days.

* X°C 5 days/Y°C 2 days.
Leaves were incubated for 5 days at X°C then transferred to Y°C for 2 days before transfer back to X°C. This cycle was repeated for the duration of the experiment.

All treatments were replicated twice. Leaves were examined for apothecia at 30, 40, 50, 65 and 80 days. Leaves with emerging apothecia were sectioned, mounted in oil and stained with toluidine blue. Developing apothecia were squashed in lactophenol and stained in 0.5% acid fuchsin. To determine the influence of incubation temperature on the dimensions of asci and ascospores, 60 of each from mature apothecia produced at 3°C, 5°C and 8°C were measured with a Leitz optical micrometer (500X).

RESULTS

Temperatures prevailing during the period of apothecial development clearly influenced the rate at which apothecia matured (Table 51). Apothecia were first observed at 40 days and mature ascospores were produced within 50 days. The most favourable temperatures for apothecial maturation were 5°C 5 days/8°C 2 days; 5°C 5 days/12°C 2 days; 5°C 5 days/16°C 2 days; 8°C constant; 8°C 5 days/5°C 2 days; 8°C 5 days/12°C 2 days and 8°C 5 days/16°C 2 days.

Apothecial development was cleistohymenial (Van Brunnelen, 1967) and began with the formation of an ascogonium surrounded by somatic hyphae (Fig. 107). The apothecial initial expanded rapidly, the outer tissues forming a distinctive dark brown, pseudoparenchymatous cortex (Fig. 107). Later, as the developing apothecium enlarged and induced a bulging of the outer epidermis a white mucilaginous cavity was delimited inside the apothecial ball (Fig. 108). By the time the epidermis had ruptured numerous clavate paraphyses and asci had developed from ascogenous hyphae at the base of the apothecium. At maturity each ascus contained 8 unicellular ellipsoidal, smooth walled, hyaline ascospores, uniseriately or biseriately arrayed.

Incubation temperature had no significant ($P>0.05$, t-test) effect on dimensions of asci and ascospores (Table 52). Asci measured 70.0-102.0-140.0 x 9.0-10.7-13.0u, and ascospores 8.5-10.8-14.0 x 3.6-4.3-5.5u.

DISCUSSION

In the laboratory apothecia of *D. tremulae* were formed both on naturally infected leaves and leaf discs. As far as the author is aware this is the only report of laboratory induction of apothecia of

Drepanopeziza species (pathogenic to poplars). Failure of the remaining isolates to form apothecia was attributed to an absence of compatible mating types rather than to unfavourable conditions of incubation. The inability of *M. brunnea* to form apothecia under field conditions in New Zealand is also attributed to incompatible mating types since prolific numbers of microconidia are formed prior to leaf-fall and the temperatures prevailing during winter should not have precluded apothecial development. It is unknown whether *Marssonina* species are heterothallic or homothallic.

As anticipated the rate of apothecial development in the laboratory was temperature dependent, optimum development occurring in the vicinity of 8°C. Field observations of *D. punctiformis* ≡ *D. tremulae* by Castellani & Freccero (1968) confirmed the significance of temperature since apothecia in southern Italy matured two months sooner than those in the colder north where 6 months was required.

The present studies have shown that the production of stromatal bodies on decaying leaves is a particularly well developed feature of *M. brunnea*. The fact that such bodies readily produce conidia in spring provides an additional means whereby the pathogen may overwinter. Accordingly one may speculate that the production of stromatal bodies by *M. brunnea* is a factor contributing to the success of this species as a pathogen of poplars.

TABLE 51: Influence of temperature on the development of apothecia of *D. tremulae* on naturally infected leaves.

Temperature	Days 30	40	50	65	80
3°C Constant	0	0	0	E	M
3°C/5°C	0	0	E	E	M
3°C/8°C	0	0	E	M	M
3°C/12°C	0	0	E	M	M
3°C/16°C	0	0	E	M	M
5°C Constant	0	E	E	E & M	M
5°c/3°C	0	E	E	E & M	M
5°C/8°C	0	E	M	M	M
5°C/12°C	0	E	M	M	M
5°C/16°C	0	E	M	M	M
8°C Constant	0	E	M	M	M
8°C/3°C	0	E	E	M	M
8°C/5°C	0	E	M	M	M
8°C/12°C	0	E	M	M	M
8°C/16°C	0	E	M	M	M
12°C Constant	0	0	0	0	0
12°C/3°C	0	0	0	0	0
12°C/5°C	0	0	0	0	0
12°C/8°C	0	0	0	0	0
12°C/16°C	0	0	0	0	0
16°C Constant	0	0	0	0	0
16°C/3°C	0	0	0	0	0
18°C/5°C	0	0	0	0	0
16°C/8°C	0	0	0	0	0
16°C/12°C	0	0	0	0	0

X°C/Y°C X°C = 5 days Y°C = 2 days

O = No apothecia
E = Apothecia emerging from leaves, asci developing
M = Apothecia fully emerged, asci fully formed with mature ascospores.

TABLE 52: Influence of temperature on dimensions of asci and ascospores of *D. tremulae*

Temperature	Asci					
	Length (u)			Breadth (u)		
	min.	mean	max.	min.	mean	max.
3°C	70.0	$101.4^{a}_{12.2}$	140.0	9.0	$10.5_{0.8}$	12.0
5°C	70.0	$100.3_{15.0}$	140.0	9.0	$10.8_{0.9}$	13.0
8°C	70.0	$105.3_{10.0}$	140.0	9.0	$10.8_{1.0}$	13.0
Temperature	Ascospores					
	Length (u)			Breadth (u)		
3°C	8.5	$10.9_{1.0}$	14.0	3.6	$4.3_{0.3}$	5.5
5°C	8.5	$10.9_{1.3}$	14.0	3.6	$4.3_{0.5}$	5.5
8°C	8.5	$10.5_{0.8}$	14.0	3.6	$4.1_{0.3}$	5.5

[a]Standard deviation

CHAPTER 5

SEED TRANSMISSION STUDIES

Previous taxonomic studies (Chapter 1) identified *M. brunnea* as the *Marssonina* species pathogenic to poplars in New Zealand. Circumstantial evidence associated with the outbreak of this pathogen amongst poplar seedlings strongly suggested seed as the source of primary inoculum. Although in recent years an increasing number of pathogens have been shown to be seed-borne, there are no confirmed reports of seed transmission of any *Marssonina* species (Neergaard, 1977).

A. LABORATORY SIMULATION OF SEED TRANSMISSION

Convincing evidence must be provided to substantiate a first assertion that a specific disease is in fact seed-borne. In effect the evidence must establish that:

(i) inoculum can transfer from the parent plant to seed of that plant - the PS transfer (Baker & Smith, 1966),

(ii) such inoculum (now associated with seed) is viable at the time of seed sowing and germination,

(iii) such inoculum can initiate seedling infection - the SP transfer (Baker & Smith, 1966).

Laboratory studies were conducted to investigate each of the above steps with regard to possible seed transmission of *M. brunnea*. Artificially inoculated seed capsules were used of necessity because mature seed bearing poplars infected with *M. brunnea* were not available at the time.

1. The PS Transfer

Depending on the nature of the association between inoculum and seed so two broad categories are recognised:

(A) Seed Contamination where free inoculum of the pathogen is present on the testa as superficial adherents, or where fragments of crop detritus bearing inoculum accompanies the seed;

(B) Seed Infection where the pathogen is established within the seed, usually as mycelium in the testa and/or embryo.

Knowledge as to the nature of the seed/pathogen association is important since the adoption of specific seed screening and seed treatment methods is determined by the nature of the relationship.

The following account of flowering, seed production and the manner in which poplar seed is harvested suggests that both seed infection and seed contamination by *M. brunnea* may occur.

Most poplar species are dioecious and first flower after 5-15 years. Flowers are wind pollinated and mature seed is shed two to three months after fertilization. Seed is formed within capsules and is embedded within fine cotton-like material. Individual capsules contain up to 32 seeds and are grouped (up to 30 capsules) around a central stalk forming a catkin. In the field dry capsules split releasing wind-borne cotton and emeshed seed. It is common practice to extract seed from air dried capsules with a vacuum cleaner which separates seed and capsular debris from the cotton. Final cleaning is by sieving to remove capsular fragments and dust. Seed may also be extracted by the air blast method where compressed air separates seed from capsules and cotton by passage through a series of sieves. Seed is normally sown immediately after extraction since viability is lost within two to three weeks. However, dried seed may be stored safely at sub-zero temperatures for several years (Schreiner, 1974).

Seed capsules on infected trees are likely to become infected by *M. brunnea* in view of close proximity to foliage for at least 3 months prior to seed release. Conceivably hyphae established in capsule walls could reach and penetrate developing seed, seed infection resulting.

As regards possible seed contamination, it can be postulated that formation of acervuli on the outer capsule wall and the manner in which seed is extracted would ensure seed contamination. As stated, a vacuum cleaner is used to separate seed and capsular debris from the cotton and in this process freed conidia could be concentrated and intimately mixed with seed. Either free conidia on the seed surface or mycelium established within seed need remain viable for only short periods since seed is normally

sown immediately after collection. Further, the requirement for long-term storage of seed at sub-zero temperatures would also favour longevity of inoculum, particularly mycelium established within seed (Neergaard, 1977).

(A) SEED INFECTION STUDIES

As stated, seed infection implies the pathogen is established within the seed, usually as mycelium in the testa and/or embryo. The following two experiments were conducted to determine whether infection of poplar seed was possible, and if so, the mode of PS transfer.

EXPERIMENT 1

Flowering branches of *P. deltoides* cv. Frimley were collected during August, placed in a peat/soil mix and subjected to a warm day (16°C), cold night (-2°C) regime for ten days to break dormancy and initiate root development (Wilkinson, 1975). The branches were then placed in a glass house (20°C) and the resultant flowers fertilized with pollen of *P. nigra* cv. Italica. One month later developing seed capsules were spray inoculated with *M. brunnea* (1000 conidia/ml), which resulted in the establishment of light infections.

To determine whether seed infection had occurred, two months following inoculation 20 ripened capsules were surface sterilized by immersion in 95% ethanol and flaming and both placental tissue and seed were transferred aseptically to plates of PDA (2 plates/capsule) and incubated at 20°C under natural light. Ten days later plates were examined for colonies of *M. brunnea*.

EXPERIMENT 2

During late November mature capsules of *P.* x *euramericana* cv. I214 and *P. deltoides* cv. NE 245 were collected from healthy trees. The catkin bearing branches were stood in water and spray inoculated with a conidial suspension of *M. brunnea* (100,000 conidia/ml). Ten days later the capsules were heavily infected. Thirty days following inoculation 10 capsules from each clone were surface sterilized (ethanol/flaming), the contents transferred to PDA (2 plates/capsule) and incubated at 20°C under natural light. Ten days later plates were examined for colonies.

Results from both experiments were negative in the sense that they failed to provide evidence of seed infection. That is, the contents of

heavily infected capsules (placental tissue and seeds) when plated to PDA in no instance yielded colonies of *M. brunnea*. These negative results strongly suggested that hyphae of *M. brunnea* had failed to penetrate placental tissue and/or developing seed, which was surprising in view of the ability of *M. brunnea* to deeply penetrate leaf tissues.

The structure of the seed capsule wall was examined and the extent of mycelial invasion determined in an attempt to account for the absence of seed infection. Small portions of infected capsule wall from the above experiments were fixed in 3% glutaraldehyde + 2% formaldehyde and prepared for electron microscopy (Appendix 8). Concurrently thick sections stained with 0.5% toluidine blue were examined by light microscopy.

Capsule walls were approximately 20 cells thick (400 u), with most cells thin walled (1.0 u). However walls of the innermost 2-3 layers of cells adjoining the capsule lumen were considerably thickened (2-5 u) (Fig. 109). Mycelium of *M. brunnea* was observed within cells of all layers, except the innermost 2-3 layers of thick walled cells (Figs. 110, 111). Profuse development of acervuli occurred on the outer capsule walls.

Results of the above experiments failed to provide evidence of seed infection. Hyphae were shown to be well established in seed capsule walls although no evidence was obtained to suggest that such hyphae penetrated the thickened innermost layers and entered placental tissue or developing seed.

(B) SEED CONTAMINATION STUDIES

The above seed infection studies demonstrated the high susceptibility of seed capsules to *M. brunnea* and the capacity for intense acervuli production on capsule walls. A profuse inoculum source in such close proximity to seed suggested the likelihood of seed contamination during harvest. The following experiment was conducted to determine whether in fact seed harvested from artificially infected capsules became contaminated with conidia (PS transfer).

Maturing catkins of *P.* x *euramericana* cv. I214 and *P. deltoides* cv. NE 245 were spray inoculated (100,000; 10,000; 1,000 conidia/ml) and incubated at 20°C under natural light. Ten days later catkins inoculated with 100,000 and 10,000 conidia/ml were heavily infected.

Catkins inoculated with 1,000 conidia/ml were lightly infected (Fig. 112). Twenty days following inoculation catkins were spread on the laboratory bench to dry and split, releasing seed. Seed was extracted from the cotton following normal practice using a Tellus vacuum cleaner. Seed and capsular debris were further separated by sieving and seed from the three treatments was stored with a desiccant (silica gel) at - 18°C.

The relative levels of seed contamination from the three inoculation series were assessed using the Bolley test and inoculation of leaf discs. The Bolley test (Bolley, 1902) has long been the standard method for demonstrating the presence of surface-borne conidia associated with seed but has had the limitation of not establishing whether such inoculum is viable. In the present study this limitation was overcome by using the resuspended pellet to inoculate poplar leaf discs inserted into 2% WA (Spiers, 1978).

Four seed samples (0.25g) from each series were shaken vigorously in 10 mls distilled water and centrifuged (5000 rpm, 2 mins.). The pellet was resuspended in one ml of supernatent and inoculated onto leaf discs (2) of *P.* x *euramericana* cv. Robusta (0.5 ml per 2.5 cm disc). Leaf discs were incubated at 20°C in natural light for 10 days.

Seed extracted from both lightly and heavily infected capsules were contaminated with conidia. In excess of 400 acervuli were formed on leaf discs inoculated with extracts from seed harvested from the two most heavily infected treatments (previously inoculated with 10,000 and 100,000 conidia/ml) whereas 30 acervuli (mean) were formed on leaf discs inoculated with extracts washed from seed harvested from lightly infected capsules (1000 conidia/ml).

The above results demonstrate that seed contamination may occur when seed is extracted from infected capsules confirming successful PS transfer.

The nature of the association between contaminating inoculum and the testa was investigated by scanning electron microscope examination of seed (*P. deltoides* cv. NE 245) harvested from heavily infected capsules. Air dried seed was glued to metal stubs, coated with gold and examined with a Cwikscan, 100 scanning electron microscope. The testa was

convoluted with prominent hairs. Conidia were loosely associated with the testa and lay both between and on top of convolutions in the testa (Fig. 113).

2. VIABILITY OF INOCULUM CONTAMINATING SEED

Inoculum of *M. brunnea* contaminating poplar seedlines could conveivably be in the form of free conidia, or conidia retained within acervuli on leaf and capsular fragments.

(A) VIABILITY OF FREE CONIDIA

Seed of *P. deltoides* cv. NE 245 was contaminated with a conidial suspension of *M. brunnea* (200,000 conidia/ml), dried by vacuum filtration, sealed in air tight bottles (silica gel) and stored at the following temperatures:

(i) room temperature (15-25°C),
(ii) constant 5°C,
(iii) constant - 18°C.

At 10 day intervals conidia were examined for viability by shaking seed (0.05 g) in water, centrifuging (4000 rpm, 2 mins.) and inoculation of leaf discs (2.5cm diam.) of *P.* x *euramericana* cv. Robusta with the resuspended pellet. Leaf discs were incubated at 20°C in natural light and examined for infection 8 days later. The formation of acervuli was the criterion indicating conidium viability.

With free conidia stored at room temperature, up until 100 days little loss in viability occurred, as evidenced by the large numbers of acervuli formed on leaf discs. Thereafter viability declined rapidly and by 120 days no acervuli were formed on inoculated leaf discs, indicating a total loss of conidium viability. In contrast, conidia stored at 5°C and -18°C showed little loss in viability following storage for 660 days.

As imported poplar seed sown in New Zealand is less than one month old (usually 10-14 days) it follows that disease outbreaks could arise from superficial conidia contaminating seed. Similarly, since conidium and seed viability is extended by storage at sub-zero temperatures, seedling infection could well arise from use of such seed.

(B) VIABILITY OF CONIDIA WITHIN ACERVULI

Lilley & Barnett (1951) and Neergaard (1977) hypothesised that the gelatinous matrix binding conidia within acervuli prolonged conidial viability. This hypothesis was tested with acervuli of *M. brunnea* on seed capsules, and on leaves. Air dried capsules and leaves were sealed separately in air tight bottles and stored as for free conidia above. At 10 day intervals conidium viability was tested by rehydrating dried leaf and capsular tissue on moist filter paper for several hours. The rehydrated material was then shaken vigorously in distilled water, centrifuged and used to inoculate leaf discs of *P.* x *euramericana* cv. Robusta.

The results were identical to those of the previous experiment in which free conidia were used. That is, conidia within acervuli stored at room temperature lost total viability after 120 days whereas conidia within acervuli stored at 5°C and -18°C showed no appreciable loss of viability following storage for 660 days. Conceivably, disease outbreaks in seedlings could arise from conidia released from acervuli on debris accompanying seed since conidia within acervuli would be viable when the seed was sown. The gelatinous matrix binding conidia of *M. brunnea* within acervuli did not prolong their viability, as has been hypothesized by Lilley & Barnett (1951) and Neergaard (1977).

3. THE SP TRANSFER

The above experiments did no more than demonstrate that poplar seed harvested from infected capsules following usual commercial practice were contaminated with viable conidia at the time of sowing. The following experiment was conducted to investigate whether seedling infection could, in fact, result from use of seed so contaminated. In so doing, the blotter test and the soil test were evaluated for suitability as techniques for demonstrating seedling infection.

A two gram seed sample of *P. deltoides* cv. NE 245 was immersed in a 10 ml conidial suspension of *M. brunnea* (100,000 conidia/ml) washed from a 10 day PDA culture. After thorough agitation the seed was vacuum dried and sown immediately as follows:

(i) 800 seeds were set out on blotters (25 seeds per blotter) in Copenhagen germinators and incubated at 20°C,

(ii) the remaining seed (approx. 3000 seeds) was scattered on the surface of sterilized soil and germinated under intermittent mist for three days, following normal nursery practice. Seed trays were then moved to a glasshouse bench and kept continuously moist. The temperature throughout was approximately 20°C.

Following 10 days incubation 800 seedlings from each test were examined for symptoms under a binocular microscope. In the soil test, the 800 seedlings were selected at random.

Results from the blotter and soil test were comparable and approximately 10% of the seedlings were infected with *M. brunnea*. In both tests 5 days after sowing symptoms were first evident as small (1mm), necrotic lesions on the cotyledons and hypocotyl. Five days later white masses of conidia accumulated on expanding lesions (Fig. 114). In many seedlings the cotyledons were infected at their apices close to the position previously occupied by the empty testa. Symptoms were not always obvious since acervuli sometimes formed on the abaxial surface of cotyledons. The blotter test proved more convenient since seedlings were easily examined for infection and seed germination was readily determined.

The above experiment demonstrated that seedling infections can arise from viable inoculum associated with seed at the time of sowing; that is, SP transfer.

The relationship between the inoculum load contaminating poplar seed and percentage seedling infection was investigated. Two gram seed samples of *P. deltoides* cv. NE 245 (approx. 4000 seed) were immersed separately into suspensions (10 ml) of *M. brunnea* containing 200,000, 100,000, 50,000, 25,000, 10,000, 5000, 2500, 1000 and 500 conidia/ml. After thorough agitation the seed was vacuum dried and sown as follows:

(i) eight hundred seeds were placed on blotters (25/blotter) and germinated in Copenhagen germinators at 20°C,

(ii) the remaining seed (approx. 3000) was sown on sterilized soil.

Following 10 days incubation 800 seedlings from soil and blotters (from each dilution level) were examined for disease expression. The experiment was conducted over three successive days.

Comparable results were obtained within inoculum levels on blotters and in soil. The results (Table 53) showed that trace levels of seedling infection resulted when seed was contaminated with low levels of inoculum (<1000 conidia/ml), with higher levels of contamination (>10,000 conidia/ml) there was a linear relationship between inoculum load and percentage seedling infection (Fig. 115).

From this experiment it was concluded that even when poplar seed is contaminated with low levels of inoculum there is some likelihood of seedling infection occurring.

DISCUSSION

Laboratory studies established that *M. brunnea* may be readily transmitted on poplar seed as free contaminating conidia, and that seedling infection can result from use of contaminated seed. A consideration of features associated with the harvesting of seed and raising of poplar seedlings in commercial practice indicates the probable significance of contaminated seed.

Seed capsules are borne in the crowns of mature trees and accordingly are exposed to infection. Laboratory studies showed that seed capsules were susceptible to infection and abundant conidia formed in acervuli on capsule walls. Seed contamination occurs during seed cleaning, in the course of which conidia are released from infected tissue and distributed amongst the seed. During the cleaning process seed, cotton and conidia are sucked from the dried capsules and the seed along with capsular fragments, dust and conidia collects in the base of the vacuum cleaner whereas the lighter cotton collects in the bag. Seed is then separated from associated debris by sieving, some fine dust and conidia remaining associated with the seed. The potential significance of such inoculum is highlighted by the fact that seed must be sown immediately after harvest and that contaminating conidia retains viability for at least three months.

Seed is sown thickly on the soil surface and germinated under intermittent mist in a glasshouse hot bed. Within 12 hours the emergent hypocotyl lifts the testa aloft and the cotyledons emerge, often retaining the testa at one or both of their apices. Twelve to fifteen days following

TABLE 53: Relationship between the level of seed contamination and percentage seedling infection level on blotters, and in soil.

Inoculum Level Conidia/ml	Theoretical Nos. Conidia/Seed [a]	% Seedling Infection Blotters	% Seedling Infection Soil
200,000	500	15.0	14.0
100,000	250	8.0	6.0
50,000	125	4.5	4.0
25,000	62.0	3.5	3.0
10,000	25.0	2.0	2.0
5,000	12.0	2.0	2.0
2,500	6.2	1.0	1.0
1,000	2.5	0.5	0.5
500	1.2	0.2	0.0

[a] Theoretical only, since it is assumed that all seeds will be equally contaminated with all of the inoculum.

Calculation - conidia/seed = $\frac{10\text{ml} \times \text{conc./ml}}{4000 \text{ (approx. seed in 2 gm)}}$.

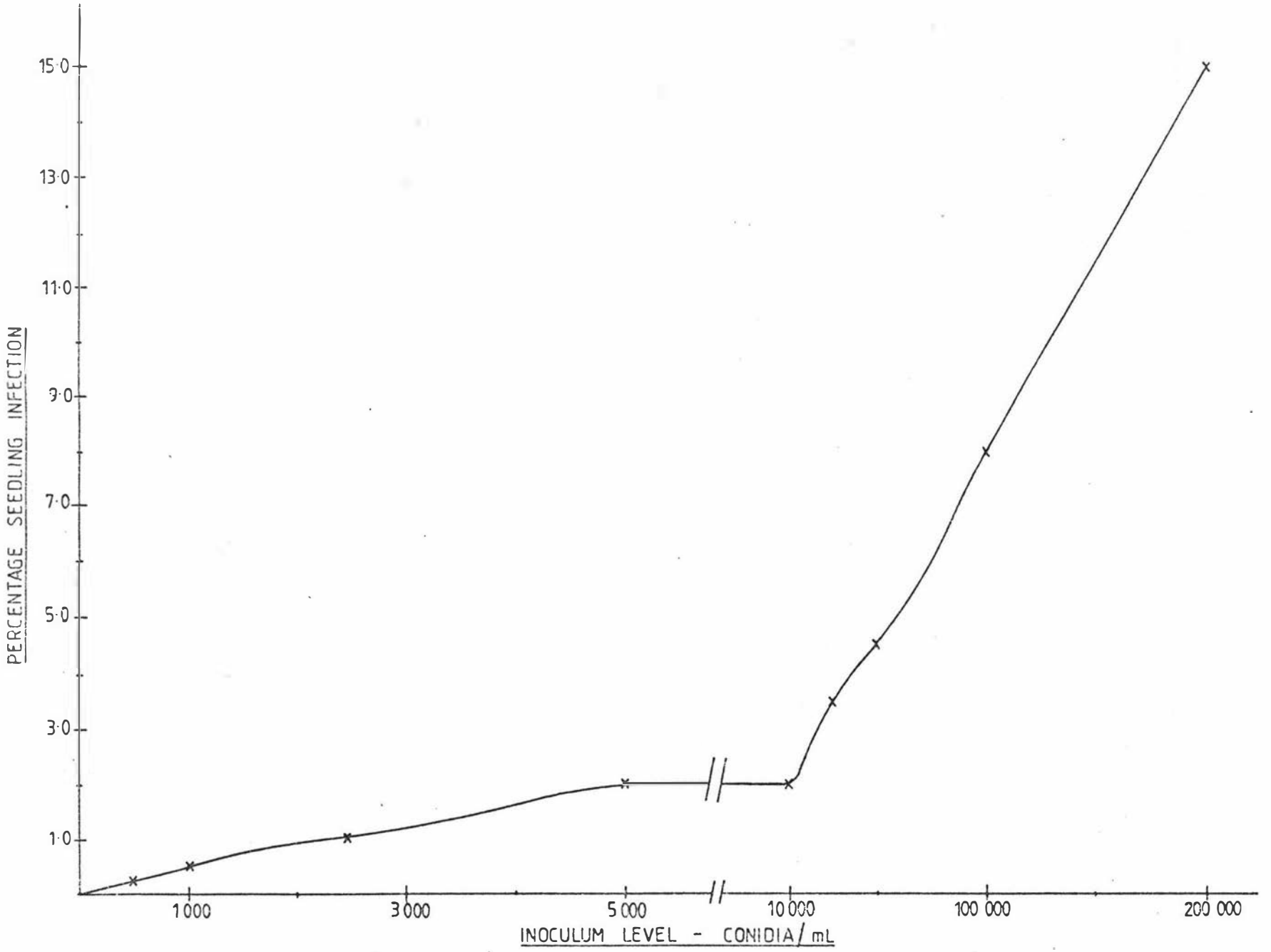

FIG 115: Relationship between inoculum level contaminating poplar seed and the percentage seedling infection level ten days following inoculation on blotters

sowing seedlings are removed from the mist chamber and pricked out to seedling trays (40 x 30 cm) at the rate of 60-70 seedlings/tray. The seedlings are grown on for at least two months in the glasshouse before being transferred to cold frames to harden off. The seedings are then planted outside in seedling beds.

The epigeal germination of poplar seed and the tenacity with which the cotyledons retain the testa ensures that inoculum is in close proximity to the infection court. Water droplets collecting at the cotyledon/testa interface facilitate transfer of conidia from the testa to seedling tissue. Further, the constant moisture and warm temperatures are conducive to infection of soft seedling tissue. The persistent high humidity favours conidium production and the close spacing of seedlings and overhead watering ensures local dispersal of inoculum by water splash and the initiation of secondary infection. These conditions all contribute to the likelihood of single conidia initiating primary infection foci.

B. EXTENT OF DISEASE TRANSMISSION IN IMPORTED SEEDLINES

Twenty-three collections of poplar capsules were imported from two seed orchards in Holland, one at Hees (15 collections, Table 54) and the other at Flevoland (8 collections, Table 55). In no instance were acervuli observed on the surface of capsules. Eight of the 23 collections were from individual mother trees which in 1975 yielded seedlings infected with *M. brunnea*.

Three collections were imported from the United States (Table 56), two of which were received both as cleaned seed and capsules. The seed capsules were distinctly lesioned with acervuli. The third collection was received as cleaned seed only. Seed was extracted from capsules imported from both countries by the vacuum cleaner method.

The inoculum level and viability of conidia contaminating all seedlines from Holland was determined. Seed (0.25g) was shaken in 10 ml distilled water and centrifuged (5000 rpm/2 min). The pellet was resuspended in one ml of the supernatent and:

(i) examined for conidia of *M. brunnea* using a haemocytometer,
(ii) inoculated (0.5ml) onto two leaf discs (2.5cm diam.) of *P.* x *euramericana* cv. Robusta.

TABLE 54: Poplar seedlines received from Hees, Holland.

Seedlot Number	Species, Descendence and/or Origin
6157	*P. deltoides*, S4-253, Grammont, Belgium
6158	*P. deltoides*, S4-404, Grammont, Belgium
6159	*P. deltoides*, F1 of S602-3, Grammont, Belgium
6160	*P. deltoides*, F1 of S626-31, Grammont, Belgium
6161	*P. deltoides*, F1 of S626-31, Grammont, Belgium
6162	*P. deltoides*, Ohio, (Collection 67-194 by US Poplar Council)
6163	*P. deltoides*, F1 of 1490 (Tegelen, Holland) Origin Michigan
6164	*P. deltoides*, F1 of S197-7, Grammont, Belgium
6165	*P. deltoides*, F1 cross of 1825 x 1454 *P. deltoides* Grammont x *P. deltoides* Missouri
6166	*P. deltoides*, F1 of S197-1, Grammont, Origin Ontario
6167	*P. deltoides*, F1 of 1850, Telegen, Holland Origin Michigan
6168	*P. deltoides*, F1 of 1473, Tegelen, Holland Origin Michigan
6169	*P. deltoides*, F1 of S336-1, Grammont, Origin Connecticut
6170	*P. deltoides*, F1 of S336-1, Grammont, Origin Connecticut
6171	*P. deltoides*, F1 of cross 193 x 1405

TABLE 55: Poplar seedlines received from Flevoland, Holland

Seedlot Number	Species, Descendance and/or Origin
6149	*P. deltoides*, Fl of S197-1, Grammont, Belgium
6150	*P. deltoides*, Fl of S197-10, Grammont, Belgium
6151	*P. deltoides*, Fl of S602-7, Grammont, Belgium
6152	*P. deltoides*, Fl of S602-7, Grammont, Belgium
6153	*P. deltoides*, Williamstown x *P. deltoides* S4-300 Grammont, Belgium
6154	*P. deltoides*, Fl of S626-31, Grammont, Belgium
6155	*P. deltoides*, Fl of S626-31, Grammont, Belgium
6156	*P. deltoides*, Fl of S628-10, Grammont, Belgium

TABLE 56: Poplar seedlines received from the United States of America

Seedlot Number	Species, Origin and State Received
78070	*P. deltoides*, Urbana, Illinois, cleaned seed
78070	*P. deltoides*, Urbana, Illinois, seed capsules
78072	*P. deltoides*, Urbana, Illinois, cleaned seed
78074	*P. deltoides*, Urbana, Illinois, cleaned seed
78074	*P. deltoides*, Urbana, Illinois, seed capsules

In addition seed capsule fragments (0.25g) were centrifuged and the relative inoculum level determined using a haemocytometer. Seed and capsular fragments from each seedline were examined on five occasions.

Only cleaned seed (not capsular debris) from the United States were examined as above.

Contaminated seedlines so identified were:

(i) sown on blotters (400 seeds, 25/blotter) and incubated in Copenhagen germinators,

(ii) sown on sterilized soil (approx. 4000 seeds) and germinated in a mist cabinet.

In both tests seedlings were examined for infection following 10 days incubation at 20°C.

The results (Table 57) show that 20 of the 23 Dutch seedlines were contaminated with viable conidia of *M. brunnea*. Sixteen seedlines were lightly contaminated and were estimated to have an inoculum load of approximately 2000 conidia/0.25g of seed (approx. 500 seeds). These results were confirmed by the leaf disc pathogenicity test. Three seedlines in particular (6150, 6153, 6156) were heavily contaminated as evidenced by microscopic examination (Fig. 116) and profuse development of acervuli on leaf discs. Consistent results were obtained between replications, especially with the three most heavily contaminated seedlines. With lightly contaminated seedlines conidia were not always observed microscopically, nor were acervuli invariably formed on leaf discs. For this reason figures in Table 57 have been rounded off to whole numbers.

Although acervuli were not observed on capsules of the Dutch seed collections the haemocytometer test showed that capsule walls were in fact contaminated with conidia of *M. brunnea*. Capsules of nine seedlines were more heavily contaminated than seed extracted from those capsules. Seed and capsules of the remaining seedlines were similarly contaminated that is, 2000 conidia/0.25g. It is possible that the presence of acervuli on the capsule walls may have been overlooked since many of the capsules had turned black as a result of sweating during transit.

TABLE 57: Extent of seed transmission in imported Dutch seedlines.

Seedline	Origin	[a] Presence of Conidia		Conidia on Seed		Conidia/Seed Capsule [2]Haemocytometer
		Seed	Capsules	[b] Haemocytometer	[c] Leaf-discs	
6149	Flevoland	+	+	2,000	2.0	2,000
6150	"	+	+	20,000	44.0	40,000
6151	"	+	+	<2,000	1.0	2,000
6152	"	+	+	2,000	4.0	2,000
6153	"	+	+	100,000	230.0	120,000
6154	"	+	+	6,000	8.0	20,000
6155	"	+	+	<2,000	1.0	2,000
6156	"	+	+	60,000	150.0	100,000
6157	Hees	+	+	2,000	2.0	2,000
6158	"	+	+	2,000	4.0	4,000
6159	"	+	+	2,000	2.0	2,000
6160	"	+	+	2,000	2.0	6,000
6161	"	+	+	<2,000	2.0	2,000
6162	"	+	+	<2,000	1.0	2,000
6163	"	+	+	<2,000	2.0	10,000
6164	"	+	+	2,000	2.0	2,000
6165	"	+	+	<2,000	1.0	2,000
6166	"	+	+	2,000	2.0	2,000
6167	"	+	+	2,000	3.0	6,000
6168	"	0	0	0	0.0	0.0
6169	"	+	+	4,000	5.0	18,000
6170	"	0	0	0	0.0	0.0
6171	"	0	0	0	0.0	0.0

[a] Bolley technique - microscopic examination + = conidia observed
0 = no conidia observed

[b] Estimated no. of conidia per ml of resusp. pellet washed from 0.25g seed/capsules
no = mean conidia/5 counting chambers x 2000 (dilution factor)

[c] Mean no. of acervuli on 10 leaf discs.

TABLE 58: Percentage seedling infection of contaminated seedlines

Seedline	Origin	% Seedling Infection Blotters	% Seedling Infection Soil
6149	Flevoland	0.25	0.00
6150	"	1.50	0.25
6151	"	0.00	0.00
6152	"	0.50	0.00
6153	"	5.00	3.00
6154	"	1.00	0.25
6155	"	0.00	0.00
6156	"	4.00	2.00
6157	Hees	1.00	0.50
6158	"	0.00	0.25
6159	"	0.00	0.00
6160	"	0.00	0.00
6161	"	0.00	0.00
6162	"	0.00	0.00
6163	"	0.50	0.00
6164	"	0.00	0.00
6165	"	0.00	0.00
6166	"	0.00	0.00
6167	"	0.50	0.00
6169	"	1.00	0.00
78070	Urbana	0.00	0.00
78074	Urbana	0.25	0.00

Seed capsules from the United States arrived in good condition and acervuli were evident on capsule walls (Fig. 117). Examination of American seedlines (haemocytometer) revealed contaminating conidia (<2000/0.25g of seed) only in those seedlines cleaned in New Zealand. Few acervuli (1-2) were formed on leaf discs inoculated with the resuspended pellet washed from these seedlines. In contrast, conidia and acervuli were not observed when cleaned seed from America was examined. This seed was cleaned in America using the air blast technique and was remarkably clean with only a few yeast cells and conidia of *Fusarium* species present. These fungi were commonly observed in Dutch seedlines (Bolley technique).

Contaminated Dutch and American seedlines germinated on blotters and sown in soil yielded only trace levels of seedling infection, with the exception of the three most heavily contaminated Dutch seedlines (Table 58). On blotters, 10 of 20 Dutch and 1 of 2 American seedlines produced infected seedlings. In soil, infected seedlings were observed in 6 of 20 Dutch seedlines whereas seedlings from the American seedlines were disease free. Symptoms were difficult to detect with the naked eye and consisted of small (1mm diam.), circular, necrotic lesions on the cotyledons and hypocotyl (Fig. 118). As lesions were occasionally formed on the undersurface of cotyledons infected seedlings were easily overlooked. Within 10 days abundant conidia were produced within acervuli, providing ample inoculum for secondary spread.

DISCUSSION

The results obtained from screening imported seedlines provides support for the hypothesis that *M. brunnea* was imported into New Zealand in 1975 as a contaminant of poplar seed. During the survey 20 of 23 Dutch seedlines were contaminated with *M. brunnea* and it is highly likely that seedlines imported during 1975 were similarly contaminated since seed was harvested from the same mother trees as in 1975. Further, 7 of the 8 seedlines which yielded infected seedlings in 1975 were again shown to be contaminated viable conidia of *M. brunnea*.

The dusting of seed with thiram and the regular spray schedule applied to poplar seedlings in the glasshouse probably suppressed the disease in 1975 since the first signs of infection were observed months later on seedlings transplanted outside in seedling beds.

Although acervuli were not observed on seed capsules imported from Holland, the haemocytometer test established that in some instances capsules were heavily contaminated with conidia. Although it is possible that the presence of acervuli may have been masked by the necrotic state of the capsules, consideration of how *M. brunnea* overwinters in mature trees suggests the presence of acervuli on the outer capsule walls may not be essential for seed contamination. *Marssonina* infection on poplars is cumulative and the pathogen overwinters between seasons in acervuli formed on young shoots and twigs (Castellani & Freccero, 1968; 1969; Pinon & Poissonnier 1975). In early spring numerous conidia are formed by these lesions and are readily rain dispersed, contaminating foliage and presumably seed capsules alike. Pinon & Poissonnier (1975) studied the build up of infection within trees in early spring and demonstrated the presence of high inoculum loads within trees shortly after flushing prior to symptom expression. It is therefore quite possible that in a seed orchard of mature *Marssonina* susceptible trees, such as those at Hees and Flevoland in Holland, high inoculum levels were present within mother trees on foliage and capsules, but without visible symptoms.

The level of seed contamination may be influenced also by the method of seed cleaning. Seed extracted in America by the air-blast method was free of conidial contamination whereas seed extracted from the same collections in New Zealand by the vacuum cleaner method was contaminated. With the air blast method, seed cotton and capsular debris are separated in one operation whereas with the vacuum cleaner method, seed and cotton are separated initially and thereafter the seed is cleaned by sieving thereby enabling free conidia to make contact with and contaminate seed.

Results from the seed health survey revealed the extent to which poplar seed were contaminated with the conidia of *M. brunnea*. Since imported poplar seed form the basis of an intensive breeding and selection programme intended to identify seedlings either resistant or immune to both *Marssonina* and *Melampsora* species it is important that this programme be continued.

Accordingly, it is imperative that effective seed treatment methods be found for controlling *Marssonina* and other potential pathogens.

C. FUNGICIDAL CONTROL OF SEEDBORNE CONTAMINATION

Marssonina contamination of poplar seed should be amenable to fungicidal control since the inoculum in the form of free conidia on the seed surface is readily accessible to the action of fungicides. Poplar seed may be treated by dusting or the slurry method prior to sowing. However before such treatments can be implemented effective fungicides must be identified. In the following experiments fungicides were screened initially for effectiveness in preventing the establishment of infection by *M. brunnea* on leaf discs. Effective compounds so identified were then tested further for prevention of seedling infection on blotters and in soil.

(A) FUNGICIDAL INHIBITION OF M. BRUNNEA ON LEAF-DISCS

Foliage of *P.* x *euramericana* cv. Robusta was sprayed separately during mid-November with 15 fungicides (Table 59). Immediately following spray application and ten days later mature leaves from individual treatments were picked and 16 leaf discs per treatment inserted adaxial surface uppermost into holes punched into plates of 2% water agar. Leaf discs (2.5 cm diam.) were then inoculated with *M. brunnea* (5000 conidia/disc) and incubated at 20°C under natural light, and the relative effectiveness of the fungicides in preventing the establishment of infection assessed by counting acervuli.

The results (Table 60) show that MBC, carbendazim, benomyl, captafol, dodine, chlorothalonil, thiophanate and triforine prevented infection by *M. brunnea* both immediately following application and 10 days later. Thiram was effective immediately following application but not 10 days later. The above compounds, with the exception of triforine (liquid formulation) were thus considered worthy of screening as seed treatment chemicals.

(B) SEED TREATMENT STUDIES ON BLOTTERS

Seed samples (1.0g approx. 2000 seed) of *P. deltoides* cv. NE.245 were contaminated by immersion in 10ml conidial suspensions of *M. brunnea* (200,000 conidia/ml), dried by vacuum filtration and dusted with the following fungicides: MBC, carbendazim, benomyl, captafol, dodine, thiophanate, thiram and chlorothalonil. Fungicides were applied at full strength (x), half strength (0.5x) and quarter strength (0.25x) of the

TABLE 59 - Fungicides used to inoculate leaves of *P.* x *euramericana* cv. Robusta and their application rates

Trade Name and % active ingredient	Common or Chemical name	Application Rate (% formulated product)
Derosal 60%	MBC, methyl 2-benzimidalzole carbamate	1.0%
Benlate 50%	benomyl, methyl 1-(butyl-carbamoyl)-2-benzimidazole carbamate	1.0%
Bavistin 50%	carbendazim	1.0%
None 50%	BAS 33172 benzimidazole	2.0%
Cercobin 75%	thiophanate	1.0%
Bayleton 25%	triadimefon	1.0%
Bayleton* 250	triadimefon	0.025%
Saprol* 20%	triforine	0.5%
Difolatan 50%	captafol	1.5%
Melprex 26%	dodine, n-dodecylguanidine acetate	1.5%
Dacanil 75%	chlorothalonil	1.5%
Demosan 65%	chloroneb	1.5%
Thiram 80%	thiram, bis (dimethylthio-carbamoyl) disulfide	2.0%
Rovral 50%	isopropyl carbamoyl	1.0%
Ronilan 50%	vinclozolin	1.0%
Sumisclex 50%	procymidone	1.0%

* liquid formulation

TABLE 60: Effects of fungicide treatment with time on the level of infection recorded on leaf discs of *P.* x *euramericana* cv. Robusta

Fungicide Treatment	Time	
	0 days	10 days
MBC	0.0[a]	0
carbendazim	0	0
benomyl	0	0
captafol	0	0
dodine	0	0
thiophanate	0	0
chlorothalonil	0	0
triforine	0	0
thiram	0	$297_{22.0}$
triadimefon 250	$55^{b}_{8.7}$	$143_{16.5}$
triadimefon WP	$58_{10.0}$	$183_{19.7}$
B 33172	$132_{15.8}$	$304_{20.0}$
Sumisclex	$295_{24.8}$	$309_{27.4}$
Rovral	$283_{22.1}$	$319_{30.2}$
Ronilan	$304_{23.6}$	$308_{21.2}$
chloroneb	$297_{17.7}$	$307_{16.7}$
control	$299_{30.2}$	$319_{25.0}$

[a] mean no. of acervuli (16 leaf discs)

[b] standard deviation

formulated product. Four hundred seeds from each treatment plus a control were placed on blotters (25/blotter) in Copenhagen germinators and incubated under natural light at 20°C. Ten days later seedlings were examined and percentage infection, seed germination and phytotoxicity recorded and compared with the control.

When applied at full strength all fungicides prevented seedling infection (Table 61). However, with the exception of benomyl and carbendazim there was evidence of significant stunting of seedlings. Dodine was extremely phytotoxic, reducing seedling emergence to 20%.

At half strength (0.5x) all fungicides prevented seedling infection, without evidence of phytotoxicity.

At quarter strength (0.25x) chlorothalonil, captafol and thiram failed to prevent seedling infection.

(C) SEED TREATMENT STUDIES IN SOIL

Seed treatment chemicals may be absorped to colloids and soil particles and their effectiveness reduced accordingly. This reduction is termed - 'the absorption factor' (Mulder, 1943). It is therefore imperative that promising fungicides be tested under field conditions.

Seed samples of *P. deltoides* cv. NE 245 (2.0g approx. 4000 seeds) were contaminated by immersion in 20ml conidial suspensions of *M. brunnea* (200,000 conidia/ml). The seed was vacuum dried and dusted with MBC, carbendazim, benomyl, captafol, dodine, thiophanate, thiram and chlorothalonil at 0.5x and 0.25x. The dusted seed plus a control were sown on sterilized soil and germinated in a mist cabinet. Seedlings were examined for infection and phytotoxic effects ten days later. Germination was assessed by subjective comparison with the control.

The results (Table 62) show that all fungicides tested prevented seedling infections when applied at 0.5x. MBC, benomyl, carbendazim, thiophanate and dodine were also effective when applied to seed at 0.25x. No detrimental effects on seed germination or seedling growth were observed.

TABLE 61: Influence of fungicide treatment of percentage seed germination and percentage seedling infection on blotters

Fungicide Treatment	Fungicide Concentration X[a]		0.5X		0.25X	
	% Germination	% Infection	% Germination	% Infection	% Germination	% Infection
MBC	71.5	0.0	74.5	0.0	78.0	0.0
carbendazim	67.0	0.0	70.0	0.0	78.0	0.0
benomyl	75.0	0.0	76.0	0.0	84.0	0.0
captafol	76.5	0.0	74.0	0.0	80.0	3.5
dodine	20.0	0.0	65.0	0.0	79.5	0.0
thiophanate	66.5	0.0	70.0	0.0	72.0	0.0
chlorothalonil	64.0	0.0	69.0	0.0	73.0	1.5
thiram	69.0	0.0	72.0	0.0	70.5	1.5
talc[c]	79.0	12.0	76.0	14.0	74.0	13.0
control	78.5	13.0	77.0	14.0	74.0	15.0
Mean germination	$66.5_{16.0}$[a]		$72.1_{3.7}$		$76.1_{4.0}$	

[a] X Undiluted formulated product. 0.5X Half formulated product, 0.25X Quarter formulated product

[b] Standard deviation

[c] talc dilutant

TABLE 62: Influence of fungicide treatment on percentage seedling infection in soil

Fungicide Treatment	Fungicide Strength 0.5X[a]	0.25X
MBC	0.5	0.0
carbendazim	0.0	0.0
benomyl	0.0	0.0
captafol	0.0	1.5
dodine	0.0	0.0
thiophanate	0.0	0.0
chlorothalonil	0.0	2.0
Thiram	0.0	1.0
talc	12.0	
control	10.0	

[a] 0.5X = Half formulated product
0.25X = Quarter formulated product

TABLE 63: Influence of fungicide treatment and storage time on percentage seed germination and seedling infection on blotters

Fungicide Treatment	Dusted & Sown % Germination	% Infection	Dusted & Stored (4 months) % Germination	% Infection
MBC	68.0	0.0	71.0	0.0
carbendazim	72.0	0.0	68.0	0.0
benomyl	74.0	0.0	75.0	0.0
captafol	72.0	0.0	73.0	0.0
dodine	63.0	0.0	22.0	0.0
thiophanate	68.0	0.0	65.0	0.0
chlorothalonil	67.0	0.0	66.0	0.0
thiram	70.0	0.0	69.0	0.0
talc	74.0	12.0	70.0	15.0
control	75.0	16.0	73.0	13.0
Mean % Germination	70.3[a] ± 3.8		65.2 ± 15.5	

[a] Standard deviation

(D) INFLUENCE OF SEED TREATMENT ON VIABILITY OF STORED SEED

The effects of seed treatment chemicals on seed viability during storage at sub-zero temperatures were investigated. Seed samples (2.0g approx. 4000 seed) of *P. deltoides* cv. NE 245 were contaminated (200,000 conidia/ml) as in preceeding experiments. The seed was dried and dusted with MBC, carbendazim, benomyl, captafol, chlorothalonil, dodine, thiophanate and thiram diluted to 0.5x. Half of the seed (1.0g) was stored with a desiccant (silica gel) at -18°C for four months. The remaining seed was placed on blotters (400 seed/treatment, 25/blotter) and germinated in Copenhagen germinators under natural light at 20°C. After 10 days percentage germination and percentage seedling infection were recorded. Four months later the fungicide treated seed stored at -18°C was similarly sown on blotters and examined.

With the exception of dodine, storage of treated seed for four months did not affect either germination or seedling vigour (Table 63). Ignoring dodine, the mean percentage germination of seed sown immediately and seed stored for four months was not significantly different (t-test, $P > 0.05$). Storage of treated seed did not diminish the effectiveness of fungicides in controlling *M. brunnea*. Accordingly, with the exception of dodine, poplar seed may be dusted and stored safely with the fungicides listed in Table 63 for at least four months.

DISCUSSION

The solution to all serious poplar diseases is seen in the breeding and selection of resistant cultivars, a difficult task in view of the wide host ranges of *Marssonina brunnea* and *Melampsora* species. The above studies have established that *M. brunnea* was probably imported into New Zealand on the surface of poplar seed as contaminating conidia. Although unproven, it is inviting to speculate that *M. brunnea* was similarly introduced into Europe in the 1960's on poplar seed and/or cuttings imported from the United States. Since breeding programmes are often dependant upon international exchange of seed it is essential that all poplar seed collections be treated with fungicide. The three benzimidazole derived compounds MBC, carbendazim and benomyl were outstandingly effective against *M. brunnea* and should routinely be used to treat seed.

In recent years the development of resistance by fungi to systemic fungicides has become a problem (Bollen & Scholten, 1971; Ruppel & Scott,

1974) a consequence of frequent, uniform applications. However it is speculated that strains of *M. brunnea* resistant to systemic fungicides are unlikely to develop in view of the high cost involved in regularly spraying such large trees. However should resistance develop alternative compounds are available.

The risks of introducing additional species of *Marssonina* and other pathogens into New Zealand on poplar seed would be appreciably reduced if the following procedures were adhered to.

1. If seed collections were made only from mother trees known to be free of disease and preferably resistant to *Marssonina*, *Melampsora* and *Septoria* species.

2. If seed was extracted from the 'cotton' in the country of origin by the apparently more effective air blast method.

3. If prior to dispatch the cleaned seed was dusted with either MBC, carbendazim, benomyl, captafol, dodine, thiophanate, chlorothalonil or thiram applied at half strength of formulated product.

4. If seedlings raised from imported seedlines were grown in isolation from adjacent poplars and inspected regularly for symptoms for the first year.

Should capsules or untreated seed be received, a health test must be conducted using either the Bolley or leaf disc pathogenicity test. It is imperative that such seed be dusted prior to sowing.

CHAPTER 6

PATHOGENESIS OF MARSSONINA SPECIES TO POPLARS

A. Host Penetration

The only available information pertaining to penetration of poplar leaves by *Marssonina* species is provided by *invitro* and *invivo* studies of *M. brunnea* by Heather *et al* (1975) who found that:

"some tubes entered stomata although there was no pronounced trophic growth of tubes towards these. Some tubes formed appressoria and penetrated the host surface directly at the middle lamella of adjoining epidermal cells".

In the following studies penetration of poplar leaves by *M. brunnea* was investigated by light, scanning and electron microscopy.

Materials and Methods

Leaf discs (2.5 cm diam.) from mature leaves of *P. nigra* cv. Italica 'Aurea' were inoculated on the adaxial surface (50,000 conidia/disc) with inoculum of *M. brunnea* from PDA culture. Following incubation at 20°C in continuous light for 12 hours, leaf discs were examined as follows:

(a) Light Microscopy Leaf discs were cleared and stored in a mixture of 95% ethyl alcohol and glacial acetic acid (1:1). The clearing process was facilitated by the naturally low chlorophyll content of *P. nigra* cv. Italica 'Aurea' (golden poplar). Prior to microscopic examination (500x) small squares of leaf tissue were stained with 0.5% acid fuchsin in lactophenol.

(b) Scanning Electron Microscopy Leaf discs were air dried and glued to metal stubs, coated with gold-palladium and examined with a Cwikscan 100 field emission scanning electron microscope. In addition, the technique developed by Staub *et al* (1974) was used to observe penetration holes. This involved covering leaf discs with liquid gelatine (25% aq. w/v) and air drying overnight at 20°C. Twelve hours later the thin, hard gelatine skin was peeled off removing conidia, germtubes and penetration pegs. The peeled leaf discs were further air dried prior to observation in the scanning electron microscope.

(c) Transmission Electron Microscopy Small pieces of leaf tissue ($3mm^2$) were fixed in 3% glutaraldehyde + 2% formaldehyde in phosphate buffer and prepared for electron microscopy (Appendix 8b). Serial sections through germinating conidia were examined with a Philips EM200 Electron Microscope.

RESULTS

(a) Light Microscopy Germinating conidia of *M. brunnea* commonly formed a single germ tube from the larger cell of the conidium. Germ tube extension was limited (<30u) and direct penetration often followed only 10u extension. Contrary to the report by Heather *et al* (1975), appressoria were not formed and penetration was not confined to the middle lamella of adjoining cells (Fig. 119). Germ tubes occasionally penetrated leaves through stomata (Fig. 120). In some instances germ tubes extended directly over stomata.

(b) Scanning Electron Microscopy Emergence of germ tubes involved cracking of the outer conidium wall (Fig. 121). Germ tubes penetrated the leaf surface directly without formation of appressoria (Fig. 122). Removal of conidia with gelatine revealed perforations through which penetration pegs had entered the leaf. The penetration holes were circular and measured 0.6-0.8u in diameter. The edges of the perforations were smooth and inturned with little deformation of the surrounding cuticle. One hole was distinctly bifurcated suggesting that the penetration peg was forked (Fig. 123).

(c) Transmission Electron Microscopy During conidial germination the septal pore became plugged with electron-dense material and sealed by wall overgrowth which effectively isolated the two cells of the conidium (Fig. 124). Cells of germinating conidia were uninucleate and contained extensive endoplasmic reticulum, mitochondria and ribosomes.

Germ tubes arose enteroblastically and as they extended, vacuoles formed in vacated portions of the conidium (Fig. 125). Voided regions of the conidium were lined by a thin layer of cytoplasm bounded by the plasmalemma and tonoplast. Germ tubes conformed closely to surface irregularities, reflecting their plastic structure and were partially or totally surrounded by mucilage.

The first indication that penetration was to be initiated was a darkly stained lower germ tube wall, its structure possibly altered by enzymatic activity. In one instance a lomasome (possibly the organelle responsible for enzyme secretion) was observed between the plasmalemma and the germ tube wall (Fig. 126). The germ tube wall became thinner and a pore (0.5-0.8u diam.) formed in the centre of the germ tube wall in contact with the cuticle (Fig. 127). Prior to dissolution of the germ tube wall the plasmalemma moved upwards away from the germ tube wall at the site of pore formation (Fig. 128).

Penetration involved the formation of a penetration peg which was naked without a cell wall or plasmalemma (Figs. 129, 130). Penetration of the cuticle and epidermis was assumed to be the result of enzymatic action since the infection peg made a clean passage throught the cuticle. The later stages of pentration were not observed. However following passage through the cuticle and epidermal cell wall, hyphae were covered with a plasmalemma and a cell wall.

DISCUSSION

Contrary to the observations of Heather *et al* (1975), penetration by *M. brunnea* frequently occurred while the germ tube was short (<30u), and without the formation of an appressorium. Prior to germination the two cells of the conidium were effectively isolated by septal pore plugging and wall overgrowth. Since both cells of the conidium were capable of forming germ tubes it follows that a single conidium could establish two infection foci.

Transmission electron microscopy showed that germ tubes conformed closely to surface irregularities and did not alter the structure of the cuticle except at the point of penetration. Scanning electron microscope studies confirmed these observations since no germ tube imprint images remained following gelatine treatment, as reported by Staub *et al* (1974) for *Erysiphe graminis*.

Mucilage around the germ tube securely fastened the germ tube to the cuticle because it was not dislodged during electron microscopy preparatory procedures. Essentially similar observations were made by McKeen (1974) for *Botrytis cinerea*.

In general, evidence from electron microscopy indicates that enzymatic activity plays an important role in cell wall penetration (Wheeler, 1975). In this study the infection peg of *M. brunnea* breached the host wall solely by enzymatic action. The zone of host wall alteration was narrow and restricted to the host wall surrounding the infection peg, suggesting that diffusable extracellular enzymes were not produced in large quantities. Further, infection pegs of *M. brunnea* were naked in contrast to those of *Botrytis cinerea* which were covered by the plasmalemma (McKeen, 1974), and those of *Colletotrichum graminicola* (Politis & Wheeler, 1973), *Erysiphe graminis* (Edwards & Allen, 1970), and *Pleiochaeta setosa* (Harvey, 1977) which were covered by a thin cell wall. The naked infection peg further rules out mechanical penetration since the naked peg would not withstand the back thrust required to force the peg through the host wall.

Although stomatal penetration was not observed by electron microscopy hyphae entering stomata would be expected to directly enter cells adjoining the stomatal cavity without formation of an infection peg, as reported by Harvey (1977) for *Pleiochaeta setosa*.

B. PATHOLOGICAL CHANGES IN ULTRASTRUCTURE

There are no published reports specifically describing the establishment and spread of infection of *Marssonina* species within poplar leaves. In the following studies the spread of infection and associated ultrastructural changes of host tissue were investigated by light and transmission electron microscopy.

Materials and Methods

Single isolates of *M. brunnea* (Br 5), *M. populi* (Po 44), and *M. castagnei* (Cs 35), were used to inoculate the following species of poplar:

(i) *M. brunnea* - *P. nigra* cv. Italica 'Aurea', *P. nigra* cv. Vert de Garonne, *P.* x *euramericana* cv. I154 and *P.* x *euramericana* cv. Robusta, *P. alba* x *P. nigra* cv. Sempervirens cv. Mareg 2.

(ii) *M. populi* - as for *M. brunnea* above.

(iii) *M. castagnei* - *P. alba* New Zealand Old Clone and *P. alba* x *P. nigra* cv. Sempervirens cv. Mareg 2.

Leaf discs (2.5 cm diam.) from mature leaves of the above clones inserted into plates of 2% WA were inoculated on the adaxial surface with conidial suspensions (5000 conidia/disc) of the respective *Marssonina* species and incubated at 20°C with a 10 hour white light photoperiod. Following 3, 6 and 12 days incubation small pieces (3mm^2) of infected leaf tissue were fixed in 3% glutaraldehyde + 2% formaldehyde in phosphate buffer and prepared for electron microscopy (Appendix 8b). Thin sections were cut and stained with 0.5% toluidine blue for light microscope examination. For comparative purposes, healthy leaf discs incubated under identical coniditions for 6 days were also prepared for electron microscopy.

RESULTS

(A) Healthy Leaf Tissue

Cells of the epidermis, palisade and mesophyll tissue differed in size, shape, extent of vacuolation and number of chloroplasts. Individual cells contained one or more vacuoles bound by a tonoplast membrane, a nucleus, dictyosomes, peroxysomes, mitochondria and ribosomes both free in the cytoplasm and bound to the endoplasmic reticulum. Chloroplasts numbered from one to twenty five and were circular to elliptical in outline. The chloroplasts were bound by a double membrane enclosing the stroma which contained grana, intergranal lamellae and osmiophilic plastoglobuli (Fig. 131).

An unusual feature occasionally observed in the stroma of chloroplasts were numerous ribosomes arranged in cylinders. As far as the author is aware this phenomenon has not previously been reported for chloroplasts of poplars or other species.

(B) Infected Leaf Tissue

1. *Marssonina brunnea*

Hyphae of *M. brunnea* invaded all four hosts similarly and three days after inoculation were well established in epidermal cells, totally disrupting cellular contents (Fig. 132). Guard and subsidiary cells of stomata were also penetrated and stomata remained closed (Fig. 133). Some penetration of palisade tissue also occurred.

Six days after inoculation hyphae had ramified extensively within epidermal cells penetrating lateral and horizontal walls. Epidermal cells were often completely filled with hyphae (Fig. 134). Palisade and mesophyll cells and intercellular air spaces were also extensively penetrated (Fig. 135).

Within twelve days of inoculation both the epidermal and palisade cells were completely filled with hyphae (Fig. 136). Within the epidermis, lateral walls were eroded by enzymatic action. Pressure by conidia formed from underlying conidiophores caused the cuticle and remains of the upper epidermal cell wall to bulge upwards rupturing the cuticle and epidermal wall, exposing the conidia. Acervuli of *M. brunnea* were thus formed intraepidermally (Figs. 137, 138).

In some instances during early stages of cell penetration, hyphae were confined to vacuoles or were in the cytoplasm causing little disruption to cellular contents (Fig. 139). Eventually hyphae ruptured the tonoplast causing the vacuolar and cytoplasmic contents to be intermixed completely disrupting cell structure. The cytoplasm became granulated and the chloroplasts lost structure (Fig. 140). Initially the thylakoid system disintegrated and later the chloroplast membrane ruptured releasing the contents into the granulated cytoplasm, with only the intergranal lamellae and osmiophilic granules remaining discernable. Other cellular organelles (nucleus, mitochondria, peroxysomes), were not discernable within the granulated cytoplasm and presumably were similarly disrupted. Cells became moribund only following hyphal penetration since severely disrupted and healthy cells were observed adjacent to each other (Fig. 141).

Hyphae penetrated directly through walls of host cells becoming thinner as they passed through wall material. A septum was commonly formed in the penetrating hyphae soon after penetration. Hyphal penetration was principally enzymatic although occasional distortion suggested some mechanical force was involved. The digested ends of host wall microfibrils were commonly observed. Although the zone of cell wall modification was narrow, some leakage of enzymes from hyphae occurred as evidenced by the altered staining properties of cell walls (Fig. 142). The mode of cell wall penetration by intracellular hyphae and hyphae within intercellular air spaces was identical. It is suggested the conidial germ tubes entering intercellular air spaces through stomata would

subsequently penetrate cells directly (as described above), rather than forming an infection peg (Fig. 143).

Lomasomes were frequently observed in the apices of hyphae penetrating cell walls and were simple or complex structures consisting of few or many tightly wound coils of plasmalemma (Fig. 144). In view of their common occurrence in the apices of penetration hyphae these structures may be responsible for the production of enzymes which degrade cell walls. Mitochondria and numerous ribosomes were also commonly observed near the apices of penetration hyphae.

Cells reacted to penetration by hyphae depending on whether the infection was primary or secondary and accordingly the level of cellular degradation. In the case of primary infections, healthy cells reacted to the presence of hyphae adpressed to the outer cell wall by increasing the amounts of endoplasmic reticulum and numbers of ribosomes in the cytoplasm adjacent to the point of contact. Later the plasmalemma moved away from the cell wall enclosing an amorphous electron opaque deposit (Fig. 145). The size of the deposit increased greatly and invading hyphae became embedded in these large complex structures, which often contained membranous inclusions (Fig. 146). In most instances, once hyphae had penetrated these structures they increased diameter, which suggested these bodies offered some resistance to lateral hyphal expansion. These structures were most commonly observed in the apices of palisade cells at the junction of epidermal and palisade tissue. They were also formed inside lateral walls of palisade cells and in mesophyll tissue.

Hyphae of *M. brunnea* were typically electron dense with numerous ribosomes. They were uninucleate, vacuolate and lipid bodies were common. Perforate septa were simple and were commonly flanked by up to five Woronin bodies (Fig. 147).

2. *Marssonina populi*

Hyphae of *M. populi* invaded host tissue in a similar manner to *M. brunnea* and six days after inoculation had spread extensively throughout cells of the palisade and mesophyll tissues (Fig. 148). As with *M. brunnea*, acervuli on *P.* x *euramericana* cv. Robusta, *P.* x *euramericana* cv. I154, and *P. nigra* cv. Vert de Garonne were formed intraepidermally (Fig. 149). However on *P. nigra* cv. Italica 'Aurea', hyphae invaded

epidermal cells both subcuticularly and intraepidermally (Fig. 150). With subcuticular invasion the cuticle became separated from the upper epidermal wall as hyphae ramified extensively under the cuticle, culminating in the formation of conidiophores and conidia (Figs. 151, 152). Hyphae of *M. populi* were morphologically similar to those of *M. brunnea*.

3. *Marssonina castagnei*

Hyphae ramified extensively throughout host tissue of *P. alba* and *P. alba* x *P. nigra* Sempervirens cv. Mareg 2 penetrating palisade and mesophyll tissues within six days following inoculation (Fig. 153). As with *M. brunnea* and *M. populi*, hyphae penetrated cell walls enzymatically totally disrupting cellular contents and large electron opaque structures were commonly formed when hyphae from epidermal cells penetrated underlying healthy palisade cells (Fig. 154). Acervuli of *M. castagnei* were always formed intraepidermally (Fig. 155).

In contrast to hyphae of *M. brunnea* and *M. populi*, hyphae of *M. castagnei* were noticeably more vacuolate and lipid bodies were uncommon (Fig. 156).

DISCUSSION

The ability of hyphae of *Marssonina* species to penetrate cell walls without apparent mechanical force strongly suggests that specific cell wall degrading enzymes are produced. This is not unusual for it is now widely acknowledged that most plant pathogenic fungi produce cell wall degrading enzymes (Wheeler, 1975). Although biochemical tests were not conducted convincing evidence of enzymatic activity was provided by ultrastructural studies which clearly showed digested ends of cell wall microfibrils. In the later stages of pathogenesis there was widespread cell wall degradation which suggested high levels of enzyme production. Cleavage of lateral epidermal cell walls enabled the cuticle and upper epidermal wall to bulge upwards enclosing underlying conidiophores and conidia.

Structures similar to "papillae" reported by Politis & Wheeler (1973) for *Collectotrichium graminicola* infection of maize and "reaction material" reported by Mercer *et al* (1975) for *C. lindemuthianum* infection of french bean were commonly observed in all hosts infected by the three *Marssonina* species. These structures appear to be a common although not

widely reported phenonomen in host-parasitic relationships (Ehrlich & Ehrlich, 1963; Chou, 1970). In the present study these structures were commonly formed by most cells in response to primary infection, contrary to the report by Mercer *et al* (1975) that reaction material developed in the first cell invaded (epidermal) and only rarely in subsequently penetrated cells. They appeared to form in response to stimulation of the cell wall and therefore were always localised in the region directly beneath penetrating hyphae. They have variously been reported to contain cellulose , lignin, suberin and always polysaccharides (Wheeler, 1975). Although conclusive evidence is lacking they are thought to impede or block penetration (Wheeler, 1975). This is supported by the observation that infection hyphae do not enlarge until clear of these structures. In the present study however these structures were always penetrated by invading hyphae, suggesting that their contribution to disease resistance was minimal.

Hanchey & Wheeler (1969) suggested that disruption of the tonoplast and subsequent mixing of vacuolar and cytoplasmic contents would result in cell death as evidenced by granulation of the cytoplasm. This was confirmed since cytoplasmic contents of infected cells became severely disrupted only following rupture of the tonoplast. Prior to rupture cytoplasmic organelles of infected cells appeared normal. Cell death was attributed by Hanchey & Wheeler (1969) to a "rapid loss of cellular osmotic properties". During the initial stages of cell infection hyphae of *Marssonina* species in suceptible tissue occasionally acted more like biotrophic parasites infecting cells without inducing death.

In their studies of the pathogenesis of corn leaves *(Helminthosporium maydis)* and lupin leaves *(Pleiochaeta setosa)*, White *et al* (1973) and Harvey (1977) respectively observed that cellular contents became moribund several cells in advance of invading hyphae. Harvey (1977) logically attributed this response to a diffusion of metabolites in advance of the fungus. Apparently *Marssonina* species observed in this study do not secrete such metabolites since infected and healthy cells were commonly observed in juxtaposition.

It is apparent that enzymes play vital roles in the pathogenesis of *Marssonina* species to poplars. Enzymes are required for the ingress of

infection pegs and subsequently enable hyphae to ramify indiscriminately throughout host tissue.

In view of the important role enzymes play in plant pathogenesis it is surprising that the exact sites of enzyme secretion within hyphae have not been determined. McKeen (1974) suggested that enzymes of *Botrytis cinerea* required for host penetration were produced in tiny vesicles located in an electron-lucent area in the germ tube apex. In the present study lomasomes and vesicles were commonly observed in and near apices of hyphae penetrating cell walls, strongly suggesting that these structures may be the sites of enzyme secretion. Furthermore, mitochondria and a large number of ribosomes were also present thus providing the essential cellular organelles for enzyme synthesis. Bracker (1967) defined lomasomes as 'boundary structures containing a membrane component'. Although the specific function/functions of lomasomes are undetermined they have been implicated in secretion, wall formation, haustorial absorption, glycogen synthesis, membrane proliferation and other roles (Bracker, 1967). In view of the many varying roles assigned to lomasomes, structures appearing to be lomasomes may not all be homologous and therefore may have different specific functions, possibly including enzyme secretion.

With regard to the location of acervuli, Nannfeldt (1932) defined *Marssonina* species as being either subcuticular, intraepidermal or subepidermal whereas according to Sutton (1977, 1980) they were subcuticular. However in the present study acervuli of *M. brunnea* and *M. castagnei* were always intraepidermal whereas those of *M. populi* were both intraepidermal and subcuticular. These observations are essentially in agreement with those of Pirozynski (1974) who reported acervuli of *M. brunnea*, *M. castagnei* and *M. populi* to be intraepidermal.

C. INFLUENCE OF LEAF AGE AND LEAF SURFACE ON HOST SUSCEPTIBILITY

During experiments concerned with the influence of host factors on conidium morphology (Chapt. 1, D) higher levels of infection were obtained on leaf discs from young soft expanding leaves than on leaf discs from mature leaves. Critical examination of poplars infected with *M. brunnea* in the Aokautere Science Centre Research Nursery confirmed these findings. Although for most clones both leaf surfaces were equally infected with

P. candicans, *P. maximowiczii* and *P. trichocarpa* infection levels were generally much higher on the abaxial surface (Fig. 157).

Two experiments were conducted to investigate the influence of both leaf age and leaf surface on susceptibility of poplar clones towards infection by *M. brunnea*.

(A) Influence of Leaf Age and Leaf Surface on Infection Level

Materials and Methods

The influence of leaf age and leaf surface on infection levels of *M. brunnea* was assessed on 55 poplar clones (Table 64). All leaves were picked during mid-January from one year growth from stools. For each clone all leaves were picked from the same shoot and were of three age classes:

(i) young soft, expanding, light-green leaves immediately below the stem apex,

(ii) slightly older, soft, fully expanded, green leaves some distance below leaves of class (i),

(iii) mature, hard, dark green leaves some distance below leaves of class (ii).

Immediately following collection 8 leaf discs (2.5 cm diam.) were punched from single leaves of each age class and inserted into plates of 2% WA (4 discs/plate). Four of the leaf discs were inserted adaxial surface uppermost and four abaxial uppermost. For each clone leaves from two shoots were examined. Leaf discs were inoculated with one ml of inoculum (approx. 6000 conidia) of *M. brunnea* prepared from PDA culture. Inoculum was spread evenly over leaf discs with the pipette tip and plates were incubated in natural light at 20°C for 8 days. Infection levels were assessed by counting the number of lesions over five randomly selected fields of view at 80X (binocular microscope). Within clones, for each treatment data from the two shoots was combined and the overall mean and standard deviation calculated.

TABLE 64: Poplar species and clones used in experiments investigating the influence of leaf age and leaf surface on the level of infection.

Name of Species, Clone of Cultivar	Name of Species, Clone or Cultivar
SECTION AIGEIROS	SECTION TACAMAHACA
A Eurasian Black Poplars	*P. maximowiczii* Henry cv. OJP M1011
P. nigra L. cv. Aurea	" cv. OJP M1016
" cv. Poznan 7	
" cv. Poznan 9	*P. trichocarpa* 74/150/11
" cv. TR 42/80	" 74/150/38
" cv. Y83/66	
" cv. PG 14	*P. candicans*
" cv. Italica F	
" cv. Vert de Garonne	SECTION AIGEIROS X TAMACAHACA
" cv. TR 56/32	*Interamericana (deltoides* x *P. trichocarpa)*
" cv. TR 62/49	" cv. NL 1783
	" cv. S910-4
B American Black Poplars	
P. deltoides cv. NE245	Others
" cv. ANU60/125	*P. deltoides* x *P. maximowiczii* cv. I83/58
" cv. ANU60/129	" x *P. yunnanensis* cv. NZ5004
" cv. NL1660	
" cv. I69/55LUX	*P. koreana* x *P. nigra* cv. K63-125
" cv. I70/51	
" cv. I74/51SP	SECTION LEUCE X AIGEIROS
" cv. Inta 66/71	*P. alba* 'Morocco' x *P. nigra* cv. Sempervirens cv. Mareg 2
" cv. APC67/10-3	
" cv. AGR21-6	
" cv. Mississippi RB	*P. deltoides* x *P. alba* '
" cv. Inta 91/71	" cv. Delmak 22
" cv. Inta 341/69	" cv. Delmak 26
" cv. AGR68-1	
P. x *euramericana* (Dode.) Guinier	
" cv. Cima	
" cv. NL1603	
" cv. San Martino	
" cv. NL925 Dorskamp	
" cv. NL2194	
" cv. Robusta	
" cv. Flevo	
" cv. I154, (140)	
" cv. I154 (923)	
" cv. Bellini	
" cv. Veronese	
" cv. I488	
" cv. NL 2207	
" cv. NL 1610	
" cv. NL 1605	
" cv. Giorgione	
" cv. Robusta Zeeland	
" cv. Eugenei PU	

RESULTS

In most instances infection levels were noticeably higher on soft expanding and soft expanded leaves than on mature leaves. Furthermore within each age class comparable levels of infection were generally recorded on the two leaf surfaces.

1. *P. nigra* (Table 65)

All clones of *P. nigra* were highly susceptible to infection by *M. brunnea*. Infection levels on soft expanding and expanded leaves were significantly ($P>0.05$, t-test) greater than those on mature leaves. Infection levels on soft expanding and soft expanded leaves did not differ significantly ($P>0.05$, t test). Within each leaf age class both leaf surfaces were similarly infected.

2. *P. deltoides* (Table 66)

With the exception of *P. deltoides* cv. NE 245, all clones of *P. deltoides* were resistant to infection by *M. brunnea*. Leaves of the single susceptible clone showed similar patterns of resistance to *M. brunnea* as expressed by clones of *P. nigra*. With many clones significantly higher ($P>0.05$, t test) levels of infection occurred on soft expanding than on soft expanded leaves and mature leaves (Fig. 158). Lesions on soft expanding leaves were minute, consisting of red flecks of pigmented tissue. Conidia were not formed. On clones of *P. deltoides* cv. Inta 341/69: cv. Inta 91/71: cv. Mississippi RB; cv. AGR 68-1, trace levels of infection occurred on leaves of each age class. For all clones of *P. deltoides* within each age class both leaf surfaces were equally susceptible to infection.

3. *P.* x *euramericana* (Table 67)

With the exception of *P.* x *euramericana* cv. Cima, significantly higher ($P>0.05$, t test) levels of infection were recorded on soft expanding and expanded leaves than on mature leaves (Fig. 159). With resistant clones very low levels of infection were recorded on mature leaves. Soft expanding leaves of *P.* x *euramericana* cv. Cima responded in a manner similar to highly resistant clones of *P. deltoides;* that is, lesions were formed without acervuli (Fig. 160). Within each age class both leaf surfaces were similarly infected.

TABLE 65: Influence of leaf age and leaf surface on infection levels of *M. brunnea* on *P. nigra*, following inoculation with approx. 6000 conidia and incubation under natural light at 20°C for 8 days.

Poplar Species and Cultivar	Age of Leaves					
	Soft Expanding		Soft Expanded		Mature	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
P. nigra cv. Aurea	$35.6^{a,b}_{2.9}$	$36.4_{2.3}$	$36.0_{2.2}$	$37.0_{2.8}$	$15.1_{2.1}$	$14.0_{2.5}$
" cv. Poznan 7	$38.4_{5.1}$	$39.8_{4.8}$	$37.6_{3.9}$	$38.1_{5.1}$	$22.5_{3.1}$	$20.2_{2.6}$
" cv. Poznan 9	$36.0_{3.2}$	$36.4_{3.3}$	$35.2_{2.4}$	$35.6_{3.6}$	$21.4_{2.6}$	$22.8_{1.9}$
" cv. TR42/80	$33.6_{3.6}$	$33.6_{4.6}$	$34.2_{4.8}$	$33.2_{3.4}$	$17.0_{2.6}$	$17.5_{2.4}$
" cv. Y83/66	$31.2_{4.6}$	$31.0_{4.0}$	$29.4_{4.1}$	$28.3_{3.7}$	$14.2_{2.8}$	$15.2_{3.0}$
" cv. PG 14	$35.4_{4.7}$	$36.0_{5.0}$	$33.5_{4.1}$	$32.7_{3.8}$	$19.0_{3.8}$	$18.2_{3.6}$
" cv. Italica F	$37.4_{5.6}$	$35.4_{3.8}$	$36.4_{5.1}$	$37.2_{3.7}$	$23.0_{3.6}$	$21.8_{2.6}$
" cv. Vert de Garonne	$40.1_{4.1}$	$42.2_{5.1}$	$39.8_{5.2}$	$36.0_{4.1}$	$19.4_{4.2}$	$20.8_{3.6}$
" cv. TR56/32	$39.6_{3.4}$	$38.6_{6.3}$	$39.6_{4.5}$	$36.0_{4.5}$	$22.0_{3.7}$	$20.8_{3.5}$
" cv. TR62/49	$35.4_{5.6}$	$37.3_{4.8}$	$36.4_{3.1}$	$35.6_{3.7}$	$15.8_{2.8}$	$16.4_{3.0}$
Overall mean	$36.3_{2.7}$	$36.7_{3.1}$	$35.8_{3.0}$	$35.0_{2.9}$	$18.9_{3.2}$	$18.8_{2.9}$

[a] Mean acervuli per field of view 80X (8 discs)

[b] Standard deviation

TABLE 66: Influence of leaf age and leaf surface on infection levels of *M. brunnea* on *P. deltoides* following inoculation with approx. 6000 conidia and incubation under natural light at 20°C for 8 days.

Poplar Species and Cultivar	Age of Leaves					
	Soft Expanding		Soft Expanded		Mature	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
P. deltoides cv. NE245	$31.6^{a}_{4.6}{}^{b}$	$31.8_{5.8}$	$30.2_{5.1}$	$29.6_{5.4}$	$13.0_{3.6}$	$12.4_{4.5}$
" cv. ANU A60/125	$19.6_{2.6}$	$20.0_{2.4}$	$19.0_{2.0}$	$18.6_{2.8}$	$0.4_{0.5}$	$0.8_{0.5}$
" cv. ANU A60/129	$20.0_{2.7}$	$20.8_{2.8}$	$0.6_{0.8}$	$0.6_{0.8}$	$0.4_{0.5}$	$0.4_{0.5}$
" cv. NL 1660	$44.6*_{4.1}$	$45.4*_{4.3}$	$0.8_{0.4}$	$0.8_{0.4}$	$0.4_{0.5}$	$0.4_{0.5}$
" cv. I69/55 LUX	$40.2*_{4.5}$	$38.2*_{3.8}$	$0.6_{0.8}$	$0.4_{0.5}$	$0.4_{0.5}$	$0.4_{0.5}$
" cv. I70/51	$36.4*_{3.1}$	$37.2*_{3.6}$	$0.4_{0.5}$	$0.4_{0.5}$	$0.4_{0.5}$	$0.4_{0.5}$
" cv. I74/51SP	$42.6*_{3.8}$	$39.6_{4.1}$	$0.4_{0.5}$	$0.4_{0.5}$	$0.4_{0.5}$	$0.4_{0.5}$
" cv. Inta 66/71	$37.0*_{4.2}$	$37.8*_{3.6}$	$18.0*_{3.1}$	$18.2*_{2.7}$	$0.4_{0.5}$	$0.4_{0.5}$
" cv. APC67/10-3	$30.2*_{6.2}$	$29.5*_{4.8}$	$0.4_{0.5}$	$0.4_{0.5}$	0.0	0.0
" cv. AGR21-6	$49.8*_{7.3}$	$51.0*_{4.7}$	$1.0_{0.0}$	$0.8_{0.4}$	$0.4_{0.5}$	$0.4_{0.5}$
" cv. Mississippi RB	$1.0_{0.0}$	$1.0_{0.0}$	$0.8_{0.4}$	$0.8_{0.4}$	$0.8_{0.4}$	$0.4_{0.5}$
" cv. Inta 91/71	$0.8_{0.4}$	$0.8_{0.4}$	$0.4_{0.5}$	$0.4_{0.5}$	$0.4_{0.5}$	$0.4_{0.5}$
" cv. Inta 341/69	$0.8_{0.4}$	$1.0_{0.0}$	$0.8_{0.4}$	$0.4_{0.5}$	$0.8_{0.4}$	$0.8_{0.4}$
" cv. AGR68-1	$0.8_{0.4}$	$1.0_{0.0}$	$0.4_{0.5}$	$0.4_{0.5}$	$0.4_{0.5}$	$0.4_{0.5}$
Overall mean	$25.4_{18.0}$	$25.3_{18.0}$	$5.3_{10.0}$	$5.1_{9.5}$	$1.3_{3.3}$	$1.3_{3.2}$

* Minute non-conidium forming lesions

a Mean acervuli per field of view 80X (8 discs)

b Standard deviation

TABLE 67: Influence of leaf age and leaf surface on infection levels of *M. brunnea* on *P.* x *euramericana* following inoculation with approx. 6000 conidia and incubation under natural light at 20°C for 8 days

Poplar Species and Cultivar	Age of Leaves					
	Soft Expanding		Soft Expanded		Mature	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
P. x *euramericana* cv. Cima	$23.0^{*\,ab}_{2\cdot6}$	$22.0^{*}{}_{3\cdot2}$	$0.4_{0\cdot5}$	$0.4_{0\cdot5}$	$0.4_{0\cdot5}$	$0.4_{0\cdot5}$
" cv. NL 1603	$20.2_{3\cdot5}$	$19.8_{3\cdot6}$	$15.2_{3\cdot6}$	$14.8_{2\cdot8}$	$1.0_{0\cdot0}$	$0.8_{0\cdot4}$
" cv. San Martino	$19.4_{2\cdot9}$	$20.0_{3\cdot4}$	$13.2_{2\cdot6}$	$14.4_{3\cdot5}$	$0.4_{0\cdot5}$	$0.4_{0\cdot5}$
" cv. NL925 Dorskamp	$31.0_{4\cdot1}$	$32.1_{3\cdot7}$	$28.7_{2\cdot8}$	$29.0_{3\cdot6}$	$12.0_{2\cdot6}$	$11.2_{2\cdot4}$
" cv. NL2194	$30.2_{3\cdot6}$	$29.0_{3\cdot1}$	$29.2_{4\cdot1}$	$29.0_{3\cdot6}$	$10.2_{1\cdot7}$	$11.2_{2\cdot1}$
" cv. Robusta	$35.0_{4\cdot0}$	$36.2_{3\cdot6}$	$35.3_{3\cdot6}$	$35.4_{2\cdot4}$	$12.4_{2\cdot1}$	$13.0_{2\cdot4}$
" cv. Flevo	$25.2_{2\cdot3}$	$25.5_{2\cdot1}$	$20.6_{2\cdot8}$	$21.2_{2\cdot1}$	$10.4_{1\cdot9}$	$9.3_{2\cdot0}$
" cv. I154 (140)	$30.6_{3\cdot9}$	$29.8_{3\cdot6}$	$30.6_{2\cdot1}$	$29.4_{2\cdot5}$	$11.0_{2\cdot6}$	$11.6_{2\cdot3}$
" cv. I154 (923)	$35.6_{3\cdot6}$	$34.6_{4\cdot1}$	$33.7_{3\cdot5}$	$34.2_{4\cdot1}$	$18.2_{2\cdot7}$	$17.8_{2\cdot4}$
" cv. Bellini	$39.6_{2\cdot5}$	$39.0_{2\cdot7}$	$38.6_{2\cdot0}$	$41.0_{3\cdot8}$	$21.6_{3\cdot3}$	$26.0_{3\cdot0}$
" cv. Veronese	$33.0_{4\cdot0}$	$36.2_{4\cdot2}$	$31.4_{3\cdot9}$	$34.2_{3\cdot8}$	$22.4_{4\cdot4}$	$20.0_{4\cdot4}$
" cv. I488	$31.8_{5\cdot5}$	$32.4_{3\cdot9}$	$30.4_{3\cdot6}$	$28.6_{4\cdot2}$	$14.8_{3\cdot3}$	$15.0_{2\cdot8}$
" cv. NL2207	$32.2_{4\cdot3}$	$34.5_{3\cdot6}$	$31.0_{3\cdot5}$	$31.2_{3\cdot8}$	$14.0_{2\cdot6}$	$14.6_{2\cdot6}$
" cv. NL 1610	$37.1_{5\cdot4}$	$36.5_{4\cdot8}$	$35.4_{3\cdot8}$	$36.2_{3\cdot5}$	$25.2_{3\cdot2}$	$24.7_{3\cdot2}$
" cv. NL1605	$34.4_{3\cdot6}$	$35.2_{3\cdot8}$	$33.7_{3\cdot7}$	$34.2_{4\cdot1}$	$18.7_{2\cdot8}$	$20.2_{2\cdot6}$
" cv. Giorgione	$32.4_{3\cdot8}$	$31.4_{4\cdot3}$	$33.4_{4\cdot2}$	$29.4_{4\cdot1}$	$22.0_{3\cdot7}$	$20.2_{3\cdot4}$
" cv. Robusta Zeeland	$39.4_{5\cdot2}$	$39.2_{4\cdot6}$	$35.6_{3\cdot6}$	$33.2_{3\cdot8}$	$20.0_{4\cdot1}$	$18.8_{3\cdot5}$
" cv. Eugenei PU	$43.0_{4\cdot7}$	$42.6_{4\cdot8}$	$38.7_{4\cdot2}$	$39.3_{4\cdot1}$	$23.0_{3\cdot5}$	$21.6_{3\cdot4}$
Overall mean	$31.8_{6\cdot5}$	$32.0_{6\cdot6}$	$28.6_{10\cdot0}$	$28.6_{10\cdot1}$	$14.7_{7\cdot9}$	$14.2_{7\cdot8}$

* Minute non-conidium forming lesions

[a] Mean acervuli per field of view 80X (8 discs)

[b] Standard deviation

TABLE 68: Influence of leaf age and leaf surface on infection levels of *M. brunnea* on miscellaneous clones following inoculation with approx. 6000 conidia and incubation under natural light at 20°C for 8 days

Poplar Species and Cultivar	Age of Leaves					
	Soft Expanding		Soft Expanded		Mature	
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial
P. trichocarpa 74/150/11	$36.0^{ab}_{3.6}$	$38.4_{3.8}$	$35.2_{3.6}$	$37.0_{4.1}$	$31.8_{2.8}$	$33.7_{4.2}$
" 74/150/38	$28.0_{3.3}$	$26.8_{3.6}$	$24.6_{3.4}$	$24.0_{3.6}$	$12.6_{1.9}$	$13.0_{2.1}$
P. delt. x *P. tricho.* cv. NL1783	$20.1_{2.6}$	$22.4_{3.1}$	$22.6_{2.7}$	$21.5_{3.8}$	$16.4_{2.8}$	$15.8_{2.5}$
" " cv. S910-4	$14.6_{2.7}$	$13.9_{3.2}$	$15.4_{2.8}$	$14.3_{3.2}$	$11.0_{2.7}$	$11.4_{3.0}$
" x *P. yunn.* NZ5004	$34.5_{3.8}$	$36.1_{4.1}$	$34.0_{3.6}$	$33.7_{2.8}$	$25.0_{3.1}$	$26.2_{3.6}$
P. alba x *P. nigra* Sempervirens cv. Mareg 2	$36.6_{2.8}$	$37.2_{3.1}$	$38.8_{2.6}$	$38.0_{3.0}$	$30.2_{2.7}$	$33.2_{3.5}$
P. delt. x *P. maxi.* cv. I83/58	$20.4_{2.9}$	$21.0_{2.7}$	$19.6_{2.9}$	$20.0_{3.1}$	$5.0_{1.8}$	$34.0_{2.0}$
P. candicans	$22.5_{2.0}$	$23.0_{2.1}$	$21.5_{1.5}$	$22.8_{2.1}$	$2.0_{1.0}$	$22.0_{0.3}$
P. maximowiczii cv. OJP. M1011	$4.0_{1.6}$	$18.4_{4.1}$	$3.5_{1.0}$	$20.4_{3.8}$	1.0	$19.0_{3.1}$
" cv. OJP. M1016	$10.0_{2.5}$	$40.5_{4.6}$	$12.0_{2.6}$	$44.3_{5.3}$	$0.8_{0.4}$	$36.8_{3.8}$
P. koreana x *nigra* cv. K63-125	$36.0_{3.2}$	$35.4_{3.5}$	$35.0_{2.8}$	$34.7_{3.5}$	$2.8_{2.0}$	$36.4_{4.1}$
P. alba x *P. delt.* cv. Delmak 22	$10.4_{2.1}$	$19.6_{3.6}$	$2.6_{1.3}$	$20.2_{3.5}$	$0.8_{0.4}$	$24.6_{2.4}$
" " cv. Delmak 26	$11.8_{3.3}$	$29.0_{4.0}$	$5.3_{2.9}$	$29.2_{4.2}$	$1.7_{0.8}$	$26.2_{3.6}$

[a] Mean acervuli per field of view 80X (8 discs)

[b] Standard deviation

4. Miscellaneous Clones (Table 68)

On *P. trichocarpa* 74/150/38, 74/150/11; *P. deltoides* x *P. trichocarpa* cv. NL 1783, cv. S910-4; *P. deltoides* x *P. yunnanensis* cv. NZ 5004; *P. alba* x *P. nigra* Sempervirens cv. Mareg 2, irrespective of leaf age both leaf surfaces were similarly infected. As with clones of *P. nigra*, infection levels recorded on soft expanding and expanded leaves were significantly higher ($P>0.05$, t test) than infection levels recorded on mature leaves.

For the remaining clones listed in Table 64 significantly higher ($P>0.05$, t test) levels of infection were recorded on the abaxial surface of mature leaves. On leaf discs of *P. koreana* x *P. nigra* cv. K63-125, *P. deltoides* x *P. maximowiczii* cv. I83/58 and *P. candicans* infection levels on both surfaces of soft expanding and soft expanded leaves were not significantly different ($P>0.05$, t test). The susceptibility of the abaxial surface of these clones did not diminish with leaf age (Fig. 161). For *P. maximowiczii* cv. M1011, cv. M1016 and *P. deltoides* x *P. alba* cv. Delmak 22 and Delmak 26 the resistance of the adaxial leaf surface increased with leaf maturity whereas infection levels on the abaxial surface remained uniformly high.

(B) Ultrastructural Studies of Epidermal Cells in Relation to Leaf Age

In the following experiment the ultrastructure of epidermal cell walls of both leaf surfaces of young and mature leaves of *P.* x *euramericana* cv. I 154 and *P. candicans* were examined by electron microscopy to determine whether cell wall maturity was responsible for (a) increasing resistance with age, (b) the differential resistance between the two leaf surfaces of mature leaves of *P. candicans*. Additionally the relative spread of hyphae within young and mature host tissue was compared.

Materials and Methods

Leaf discs (2.5 cm diam.) from young expanding and mature leaves of *P.* x *euramericana* cv. I154 and *P. candicans* were inoculated and incubated as outlined for the preceeding experiment. Following 8 days incubation the levels of infection recorded (no. of lesions per 5 randomly selected fields of view at 80X) and small triangular pieces of infected and uninfected leaf tissue were prepared for electron microscopy (Appendix 8b). Additionally, thin sections were cut and stained with 0.5% toluidine blue for light microscopic examination.

RESULTS

1. Infection levels

Infection levels on both leaf surfaces of young soft expanding leaves of *P.* x *euramericana* cv. I154 were significantly higher ($P>0.05$, t test) than those on both leaf surfaces of mature leaves (Table 69). Within each age class infection levels on the two surfaces did not differ significantly (Fig. 162).

On both leaf surfaces of young soft leaves of *P. candicans* the infection levels were not significantly different ($P>0.05$, t test) whereas on mature leaves infection levels were significantly higher ($P>0.05$) on the abaxial surface (Fig. 163). Infection levels on the abaxial surface of mature leaves were however essentially similar to those recorded on both leaf surfaces of young soft leaves.

2. Ultrastructure of Epidermal Cell Walls

(a) *P.* x *euramericana* cv. I154

Transverse sections through epidermal cell walls of both leaf surfaces of young expanding and mature leaves (Fig. 164) showed that within the respective age classes epidermal cell walls of the two leaf surfaces were morphologically similar. Comparison of epidermal cell walls of the two age classes showed that in contrast to soft expanding leaves, cell walls of mature leaves were slightly thicker, more darkly stained with conspicuous microfibrils, and had a thick well developed cuticle. In general, cell walls of soft expanding leaves were amorphous and microfibrils were inconspicuous.

(b) *P. candicans*

Epidermal cell walls on both leaf surfaces of young soft leaves were morphologically similar (Fig. 165). As with young soft leaves of *P.* x *euramericana* cv. I154, cell walls were of amorphous appearance and microfibrils were inconspicuous. Cuticles were poorly developed. Epidermal cell walls of both leaf surfaces of mature leaves were morphologically similar, being of comparable thickness and darkly stained, with conspicuous microfibrils. Cuticles were strongly developed. Epidermal cell walls on the adaxial leaf surface were consistently more darkly stained beneath the cuticle than those of the abaxial surface (Fig. 166). The higher resistance of the adaxial surface of mature leaves to infection was attributed to their greater density.

TABLE 69: Influence of leaf age and leaf surface on infection levels of *M. brunnea* following inoculation with approximately 6000 conidia and incubation under natural light at 20°C for 8 days.

Poplar Species	Age of Leaves			
	Soft Expanding		Mature	
	Adaxial	Abaxial	Adaxial	Abaxial
P. x *euramericana* cv. I154	30.0 [a]	32.0	12.0	14.0
	5.5 [b]	4.2	2.7	3.2
P. candicans	20.0	22.0	<2.0	25.0
	3.5	2.8		4.3

[a] Mean acervuli per field of view 80X (8 discs)

[b] Standard deviation

3. Comparative Spread of Hyphae Within Soft and Mature Leaf Tissue

Hyphae penetrated cell walls of soft expanding leaves of *P.* x *euramericana* cv. I 154 and *P. candicans* more readily than cell walls of mature leaves. The development of symptoms 2-3 days sooner on young soft leaves reflected this difference. However 8 days following inoculation, hyphae had spread equally far throughout both soft and mature leaf tissue, penetrating cells of palisade and mesophyll tissue (Fig. 167).

In soft expanding leaves epidermal cell walls were elastic and were deformed as hyphae ramified extensively in these cells. Cells of the palisade tissue were also breached readily totally disrupting cellular structure. In contrast, the epidermal cells of mature leaves retained their shape and cell walls offered far greater resistance to hyphal penetration. Evidence of enzymatic activity was also more apparent (Figs. 168, 169). Although readily breached, palisade cells of mature leaves offered more resistance to hyphal penetration than palisade cells of soft leaves. As a consequence of the relatively plastic nature of cell walls in young leaves acervuli were generally larger than those formed on mature leaves.

DISCUSSION

The above results clearly demonstrated that resistance of poplar clones to infection by *M. brunnea* increased as leaves matured. Both leaf surfaces of most clones within the same leaf age class were equally susceptible to infection. However the abaxial leaf surface of mature leaves of several clones were more prone to infection. For all clones of *P. nigra* and most clones of *P.* x *euramericana* young soft expanding and soft expanded leaves were equally susceptible. Infection levels were generally 50% higher than those recorded on fully expanded mature leaves. Cellerino & Anselmi (1976) reported essentially similar results with infection levels of *M. brunnea* on *P.* x *euramericana* cv. I-214. Further, they found that the susceptibility of leaves increased as leaves senesced.

Three levels of resistance were exhibited by clones of *P. deltoides*. Firstly, very susceptible clones reacted similarly to clones of *P. nigra* and *P.* x *euramericana*, that is soft expanding and soft expanded leaves were more susceptible to infection than mature leaves. Secondly, trace levels of infection were recorded on leaves of each age class irrespective of their maturity. Finally in the third group of highly resistant clones, abundant

red lesions which did not produce conidia were formed only on soft expanding leaves. This reaction was attributed to the development of resistance factors in young expanded leaves which were not present in young expanding leaves, perhaps enabling limited development of intracellular hyphae and hence symptom expression.

In the first half of this century explanations for resistance were sought in terms of such morphological features as thickness of the cuticle and epidermis, waxy coverings, structure and location of stomata and leaf hairs (see reviews by Akai, 1959; Wood, 1967). In recent years however there has been growing awareness that resistance operates most commonly following pathogen entry and therefore cannot be explained by morphological features. Emphasis has therefore been concentrated on determining the chemical basis of resistance, with little reported success.

It has long been recognised that pathogenic fungi penetrate young leaf tissues more readily than mature tissue. For example, Weinhold & English (1964) and Mence & Hildebrandt (1966) found that resistance of peaches and roses to infection by *Sphaerotheca pannosa* increased markedly as leaves matured. On both hosts increasing resistance was paralleled by an increase in the combined thickness of the cuticle and epidermal cell wall. However, following the observance of haustoria within epidermal cells of mature leaves, Mence & Hildebrand (1966) concluded that penetration was not precluded by the thicker cuticle and epidermal cell wall acting as a morphological barrier. Infection levels on lettuce (*Erysiphe cichoracearum*, Schnathorst, 1959) were also markedly reduced with leaf age, although in this instance infection levels bore no relation to cell wall thickness.

In the present study the higher resistance of mature leaves to infection by *M. brunnea* was attributed to the ultrastructure of the mature cell wall rather than to differences in the cuticle. This conclusion is in keeping with the review by Martin (1964) who concluded that the cuticle provided no serious barrier to penetration. In view of the fact that infection pegs and ramifying hyphae penetrated cell walls principally by enzymatic action and since primary cell walls are more readily hydrolysed by enzymes than secondary walls (Bateman *et al*, 1969) it follows that young expanding primary cell walls should be more readily degraded. Comparative studies on the spread of hyphae within juvenile and mature leaves showed

the relative ease with which cell walls of juvenile tissue were penetrated, as a consequence of which symptoms were expressed 2-3 days sooner.

A corollary arising from the above studies is that in screening cultivars for resistance using the agar leaf disc technique (Spiers, 1978) it is imperative that only leaf discs from mature leaves be used since they more accurately reveal relative susceptibility.

D. HOST RANGE AND PATHOGENICITY STUDIES OF M. BRUNNEA

In New Zealand poplars are primarily used for soil conservation purposes and therefore chemical control of *Marssonina* is impracticable due to the prohibitive costs of fungicide application. Accordingly, as in Europe (Cellerino, 1979) and the United States (Jokela *et al*, 1976) the solution to this problem is seen in the use of resistant clones derived from local breeding programmes and/or from imported cuttings and seed.

Previous studies (Chapt. 1G) established that *M. brunnea* was the most pathogenic species with the widest host range. This species has caused large economic losses to Euramerican poplars in Europe (Castellani & Cellerino, 1964; Zycha, 1965; Cellerino, 1979) and severe damage to *P. deltoides* in the United States (Jokela *et al*, 1976). Although *M. brunnea* was first recorded in New Zealand in February 1976 its importance on poplar clones in this country has not been fully assessed.

Since little is known concerning the relative pathogenicity of New Zealand and overseas isolates of *M. brunnea* three laboratory inoculation experiments were conducted. In the first two the host range and relative pathogenicity of New Zealand isolates of *M. brunnea* were investigated. In the third experiment the relative pathogenicity of New Zealand and overseas isolates was compared. Information arising from these experiments is vital if an effective *Marssonina* resistance breeding programme is to be implemented. As far as the author is aware there are no reports of comparative pathogenicity tests conducted between international isolates of *M. brunnea*.

Materials and Methods

Host range and pathogenicity tests were conducted using the agar leaf-disc technique (Spiers, 1978). Leaf discs (2.5 cm diam.) were punched from mature leaves picked from one year growth on stools at the Aokautere Science Centre Research Nursery. Following inoculation the leaf discs were incubated at 20°C under natural light for 20 days when infection levels were assessed by counting the numbers of lesions/cm^2 leaf area (binocular microscope). According to the number of lesions/cm^2 leaf area the leaf discs were assigned a numerical rating (0-3) (Table 70).

Experiment 1

The host range and pathogenicity of 13 New Zealand *M. brunnea* f.sp. *brunnea* isolates (Table 71) were compared by inoculation of 34 poplar clones (Table 72) on the adaxial surface with 10,000 conidia/leaf disc. Treatments were replicated four times.

Experiment 2

Three hundred and nineteen poplar clones (Table 73) were inoculated on both leaf surfaces with a single New Zealand isolate of *M. brunnea* f. sp. *brunnea* (Br 1 ex. *P. yunnanensis*). Leaf discs were inoculated with 1,000; 5,000; 10,000 and 100,000 conidia/disc and all treatments were replicated four times.

Experiment 3

Thirty-eight poplar clones (Table 74) were inoculated (adaxially, 25,000 conidia/disc) with 17 isolates, 3 from New Zealand (*M. brunnea* f. sp. *brunnea*) and 14 from overseas (11 of *M. brunnea* f. sp. *brunnea* and 3 of *M. brunnea* f. sp. *trepidae*) (Table 75). Individual treatments were replicated 8 times. Where possible single replicates inoculated with the 17 isolates were all from the one leaf.

RESULTS

Experiment 1

Although the 34 host clones differed in susceptibility, on each host the 13 New Zealand isolates of *M. brunnea* f. sp. *brunnea* were similarly pathogenic (Table 76). All clones of *P. nigra* and *P. fremontii* were highly susceptible whereas clones of *P. deltoides* and *P.x euramericana* varied markedly in their resistance. No isolates were pathogenic to *P. tremula, P. tremuloides, P. pseudograndidentata* and *P. alba* x *P. tremula*.

Experiment 2

The results (Tables 77 and 78) show that *M. brunnea* f. sp. *brunnea* has an extremely wide host range attacking representatives from all sections of the genus. These results further show that poplars of the Section *Aigeiros* were more susceptible than poplars of the other Sections *(Leuce, Tacamahaca, Leucoides)*. Within the Section *Aigeiros*, cultivars of the European black poplar *(P. nigra)* were all highly susceptible. By contrast, cultivars of the American black poplar *(P. deltoides)* were uniformly

resistant to highly resistant. As expected, hybrids of *P. nigra* and *P. deltoides (P.* x *euramericana)* were uniformly more susceptible than cultivars of *P. deltoides*. However there were several highly resistant cultivars of *P.* x *euramericana*.

With the Section *Tacamahaca*, cultivars of *P. trichocarpa* were uniformly resistant to highly resistant and hence comparable to *P. deltoides*. Cultivars of *P. maximowiczii* and other species, namely *P. yunnanensis*, *P. ciliata*, *P. szechuanica* and hybrids of species within this section namely, *P. androscoggin* were slightly more resistant to infection than cultivars of *P. trichocarpa*.

Species of the Sections *Leucoides* and *Leuce* were highly resistant to infection. Within the section *Leuce*, species of the sub-section *Trepidae (P. tremula, P. tremuloides)* remained free of infection whereas species of the subsections *Albidae (P. alba)* and *Albidae* x *Trepidae (P. alba* x *P. tremula/P. tremuloides)* were lightly infected.

Cultivars derived from intersectional crosses varied in their resistance to *M. brunnea* f. sp. *brunnea* depending on the resistance of the parent species. That is, cultivars arising from crosses between the Sections *Leuce* x *Tacamahaca* were more resistant than cultivars derived from crosses between *Leuce* x *Aigeiros* and *Tacamahaca* x *Aigeiros*.

Although both leaf surfaces of most cultivars of the Sections *Leuce*, *Leucoides* and *Aigeiros* were equally susceptible to infection, certain species of the Section *Tacamahaca* namely, *P. maximowiczii*, *P. candicans* and *P. simonii* var. *fastigiata* were noticeably more susceptible on the abaxial leaf surface. Furthermore, certain cultivars from the intersectional crosses *Aigeiros* x *Tacamahaca (Koreana* x *nigra, maximowiczii* x *nigra)*, and *Leuce* x *Aigeiros, (deltoides* x *alba)* were also more heavily infected on the abaxial surface.

From the mean overall infection levels (Table 78) it is obvious that infection levels were higher as the inoculum load increased. Up to an inoculum level of 10,000 conidia/disc there was a substantial increase in infection. However an increase in the inoculum load from 10,000 to 100,000 conidia/disc produced only a negligible increase of infection intensity.

TABLE 70: Disease rating scale and susceptibility classification for assessing poplars for resistance to *Marssonina* species in the laboratory

Disease Rating	Lesions per cm^2 Leaf Area	Infection Level	Susceptibility Classification
0	0	Nil	Highly Resistant
1	1-10	Light	Resistant
2	>10<25	Medium	Susceptible
3	>25	Heavy	Very susceptible

TABLE 71: New Zealand isolates of *M. brunnea* used to inoculate poplars in host range and pathogenicity studies (experiment one).

Isolate	Host Species	Origin	Date
NZ1 = Br1	*P. yunnanensis*	Palmerston North	10.1.78
NZ2 = Br2	*P.* x *euramericana*	Pahiatua	10.1.78
NZ3 = Br3	*P. fremontii* cv. ANU61/48	Palmerston North	10.1.78
NZ4 = Br4	*P. fremontii* x *P. nigra* Sempervirens cv. ANU66/9	" "	10.1.78
NZ5 = Br5	*P.* x *euramericana* cv. I214	" "	10.1.78
NZ6 = Br6	*P. alba*	" "	10.1.78
NZ7 = Br7	*P.* x *euramericana* cv. Flevo	" "	10.1.78
NZ8 = Br8	*P.* x *euramericana* cv. NL2194	" "	10.1.78
NZ9 = Br9	*P. delt.* x *P. maxi.* cv. I83/58	" "	10.1.78
NZ10 NA	*P. deltoides*	" "	10.1.78
NZ11 NA	*P. alba* x *P. nigra* Sempervirens cv. Mareg	" "	10.1.78
NZ12 NA	*P. maximowiczii*	" "	10.1.78
NZ13 NA	*P. nigra* cv. Italica	" "	10.1.78

NA = Not Applicable

TABLE 72: Poplar species, clones tested for susceptibility to 13 New Zealand isolates of *M. brunnea* (Experiment one).

Name of Species, Clone or Cultivar	Remarks - Parentage, Origin or Source
SECTION LEUCE	
Populus alba L. cv. NZ old clone	Italian selections
cv. B02	" "
P. alba var. hickeliana Dode, cv. PE90	
P. pseudograndidentata	
P. tremuloides	Seedlot ex. Wiscon. USA
P. alba L. x *P. tremula*	Hungary
P. x *canescens* Smith	*P. alba* L. x *P. tremula* L.
P. alba x *P. glandulosa*	
SECTION AIGEIROS	
P. deltoides Marsh spp. Angulata Ait. cv. I69/55	Italian Selection
I72/51 SP	" "
I74/51 SP	" "
NL2180	Dutch Selection
P. fremontii Wats. cv. ANU61/48	Australian
P. nigra L. cv. Italica	
" cv. TR56/52	Turkey
SECTION AIGEIROS X AIGEIROS	
P. x *euramericana* (Dode.) Guinier cv. Flevo	Holland
I154	Italy
NL925 (Dorskamp)	Holland
NL1603	"
NL2196	"
ANU 65-29	Australia *deltoides* x *nigra* sempervirens
ANU 66/8	" *fremontii* x *nigra* "
ANU 66/9	" " " "
SECTION TACAMAHACA	
P. maximowiczii Henry cv. Kew	Japanese selection
cv. OJP M1011	
P. trichocarpa Torr. & Gray. cv. S617-41	Belgium
P. yunnanensis	China
SECTION AIGEIROS X TACAMAHACA	
S909-19	Belgium (*delt.* x *tricho.*)
NL1785 (Borgh)	Dutch (*delt.* x *tricho.*)
SECTION LEUCE X AIGEIROS	
Delbo U	Australian (*deltoides* x *alba*)
Delmak 20	" " "
Mareg 2	" (*alba* x *nigra* Sempervirens)
SECTION LEUCE X TACAMAHACA	
Mayu 1	Australian (*alba* x *yunnanensis*)

TABLE 73: Poplar clones and species screened for susceptibility to *M. brunnea* isolate Br 1 (Experiment 2)

Name of Species, Clone or Cultivar
SECTION LEUCE
A. *ALBIDAE*
P. alba cv. NZ old clone
cv. I40/57 Italian selections
I42/57 " "
I49/51 " "
I59/1 " "
BO 2 " "
P. alba L. var. hickeliana Dode. cv. PE90
" L. var. pyramidalis Bunge
Clone 73/76/8 New Zealand selections
" 71/3/2 " " "
" 72/8/3 " " "
" 73/77/5 " " "
B. *TREPIDAE*
P. pseudograndidentata
P. tremula L. cv. FRI
P. tremula L. cv. Nelson
P. tremula L. cv. Wok.
P. tremuloides. Michx. seed ex. Wisconsin
C. *ALBIDAE* x *TREPIDAE*
P. alba L. x *P. tremula* L. Hungary
P. canescens B. Smith (*P. alba* x *P. tremula*)
P. alba x *P. glandulosa* cv. K65-22-4
cv. K66-20-1
SECTION AIGEIROS
A. American Black Poplars
P. deltoides AGr 21-6.
AGr 28-8
AGr 61-58
AGr 68-1
ANU 28-8
ANU 60/110
ANU 60/129
ANU 60/135
ANU 60/166
ANU 60/103
ANU 60/125
ANU 60/146
APC 67/1-3
APC 67/5-1
APC 67/5-2
APC 67/5-6

(CONTINUED)

TABLE 73 Continued: Poplar clones and species screened for susceptibility to *M. brunnea* isolate Br 1 (Experiment 2)

Name of Species, Clone or Cultivar
P. deltoides APC 67/5-7
APC 67/5-13
APC 67/7-1
APC 67/7-2
APC 67/7-12
APC 67/10-3
APC 67/12-4
APC 67/16-8
APC 67/24-2-3
APC 67/28-4
APC 67/28-6
APC 67/28-8
APC 67/28-11
APC 67/31-1
APC 67/40-1
APC 67/40-2
APC 67/40-5
APC 67/47-2
APC 67/51-1
APC 67/51-5
APC 67/51-6
APC 67/65-14
APC 67/119A-8
APC 67/119A-9
APC 67/123A-8
P. deltoides Marsh spp. angulata Ait. cv. Carolinensis
Chautagne
G3
G48
Mississippi RB
P. deltoides Harvard
I63/51
I69/55 (Lux)
I70/51
I72/51 SP
I74/51 SP
P. deltoides Marsh spp. monilifera cv. Frimley
P. deltoides Inta 14/71
Inta 16/69
Inta 39/71
Inta 66/67
Inta 71/67
Inta 79/71
Inta 91/71
Inta I58/69
Inta 341/69
Inta 372/69
NE 245
NL 1454

(CONTINUED)

TABLE 73 Continued: Poplar clones and species screened for susceptibility to *M. brunnea* isolate Br 1 (Experiment 2)

Name of Species, Clone or Cultivar
P. deltoides Continued
NL 1660
NL 2180
NL 2243
NL 2515
Stoneville 62
Stoneville 66
Stoneville 70
Stoneville 71
Stoneville 81
Stoneville 92
P. fremontii Wats. cv. ANU 61/48
P. fremontii Wats. Seedling ex Utah.
B Eurasian Black Poplars
P. nigra L. var. Caudina Tenore.
L. var. Blanc de Garonne
P. nigra cv. CE9
TR 42/80
Italica
Italica Aurea
Italica F
LP1
MC18
MC20
PG14
PG22
Poznan 9
R103
Sempervirens
Thevestina
TR56/32
TR56/52
TR56/72
TR56/75
TR62/27
TR62/49
TR62/52
TR62/57
TR62/127
TR62/140
TR62/149
TR62/154
TR62/191
Vert de Garonne
Y83/66
Y95/67
Granaracci
P. x *euramericana* (Dode.) Guinier cv. (d. x n.)
ANU 65-1 (d. x ns.)
ANU 65-5 (d. x ns.) (CONTINUED)

TABLE 73 Continued: Poplar clones and species screened for susceptibility to *M. brunnea* isolate Br 1 (Experiment 2)

Name of Species, Clone or Cultivar
P. x *euramericana* Continued
ANU 65-14 (d. x ns.)
ANU 65-19 (d. x ns.)
ANU 65-24 (d. x ns.)
ANU 65-26 (d. x ns.)
ANU 65-29 (d. x ns.)
ANU 65-70 (d. x ns.)
ANU 66/8 (fremontii x ns.)
ANU 66/9 "
Altichiero
Bellini
BL Costanzo
Boccalari
Carpaccio
Cima
EC028
Eugenei PU (Reg. x NI.)
Eugenei UL "
Fierolo
Flevo
Fogolino
Gelrica HA
Glorgione
Guardi
Guariento
Harff (Schroek 290)
I30
I65 (Gwydyr)
I 74D
I78
I154
I214
I455
I488
I45/51
I92/40
Laevigiata
Leipzig
Longhi
Marilandica F
Marilandica ID26
NL 925 (Dorskamp)
NL 1070
NL 1601
NL 1602
NL 1603
NL 1605
NL 1610
NL 1775 (Spijk)
NL 2170
NL 2171
NL 2193
NL 2194
NL 2195

(CONTINUED)

TABLE 73 Continued: Poplar clones and species screened for susceptibility to *M. brunnea* isolate Br 1 (Experiment 2)

Name of Species, Clone or Cultivar
P. x *euramericana* Continued
NL 2196
NL 2197
NL 2200
NL 2202
NL 2205
NL 2206
NL 2207
NL 2217
NL 2220
NL 2223
NL 2226
OP 66
OP 223AH
Pacher
Regenerata 360 (Neupotz) (N. x Serotina)
Regenerata Nepia "
Regenerata WV "
Reinbeck LK 82 "
Robusta Bacherlierii H (d. x NP.)
Robusta cook "
Robusta PH "
Robusta H "
Robusta Zeeland "
Rubra
San Martino
Schiavone
Serotina VB
Serotina de Champagne (Reg. x Serotina)
Serotina du Poitu "
Tiepolo
Triplo
Veneziano
Veronese
SECTION TACAMAHACA
P. maximowiczii Henry cv. Kew
OJP M106
OJP M108
OJP M1011
OJP M1012
OJP M1020
OJP MA-3
OJP MC-22
P. trichocarpa Torr. & Gray var. hastata (Dode.) Henry
" Torr. & Gray cv. CF
LA99
WV
V235
S617-16
S617-41
S617-88

(CONTINUED)

TABLE 73 Continued: Poplar clones and species screened for susceptibility to *M. brunnea* isolate Br 1 (Experiment 2)

Name of Species, Clone or Cultivar
P. yunnanensis Dode.
P. candicans Ait.
P. ciliata
P. simonii Carr.
" var. fastigiata
P. szechuanica Gibbs.
" tibetica
P. androscoggin (maxi. x tricho.)
P. koreana x *P. trichocarpa*
P. tacamahaca x *P. trichocarpa* 32
" " 37AF
SECTION AIGEIROS X TACAMAHACA
A. Interamericana - *P. deltoides* x *P. trichocarpa*
cv. NE205
NE207
NL 1616
NL 1623 (Barn)
NL 1626
NL 1647
NL 1650
NL 1656
NL 1783
NL 1785 (Borgh)
NL 2228
NL 2233
S909-1
S909-10
S909-12
S909-16
S909-19
S910-1 (Unal 7)
S910-2 (Unal 8)
S910-4
S910-5
S910-8
S910-10
69042-1
69042-2
69042-3
69042-4
69042-5
69042-6
69043-1
69043-2
69043-3
69043-4
69044-2
B. Others Andover (n. x t.)
ANU 70-2 (d. x y.)
Berry 65/16 (gen. x y.)
Berry 65/26 (gen. x y.)

(CONTINUED)

TABLE 73 Continued: Poplar clones and species screened for susceptibility to *M. brunnea* isolate Br 1 (Experiment 2)

Name of Species, Clone or Cultivar
Others Continued
Frye (n. x laurifolia)
Geneva (maxi. x berol.)
I83/58 (maxi. x d.)
K62-9 (n. x maxi.)
K63-100 (Koreana x n.)
K63-125 "
K63-130 "
K63-129 (Koreana x NI.)
Laurel 52 (d. x taca.) x laurifolia
Maine (t. x berol.)
Oxford (maxi. x berol.)
Rochester (maxi. x nP.)
Roxbury (n. x t.)
NZ5001 (d. x y.)
Waipuki 1 (d. x y.)
Waipuki 2 (d. x y.)
SECTION LEUCOIDES
P. lasiocarpa Oliv.
P. wilsonii Schneid.
SECTION LEUCE X AIGEIROS
P. alba L. x *P. nigra* L.
P. deltoides x *P. alba* cv. Maktar
cv. Delmak 13
Delmak 16
Delmak 18
Delmak 19
Delmak 20
Delmak 22
Delmak 26
P. deltoides x *P. alba* var. pyramidalis
cv. Delbo U
Delbo 9
P. alba 'Morocco' x *P. nigra* Sempervirens
cv. Mareg 2
SECTION LEUCE X TACAMAHACA
P. alba 'Morocco' x *P. yunnanensis* ANU
cv. Mayu 1
Mayu 2

TABLE 73 Continued: Poplar clones and species screened for susceptibility to *M. brunnea* isolate Br 1 (Experiment 2).

KEY TO ABBREVIATIONS

berol.	berolinensis
d.	deltoides
gen.	generosa
maxi.	maximowiczii
n.	nigra
nI.	nigra Italica
nP.	nigra Plateriensis
nS.	nigra Sempervirens
tricho. (t)	trichocarpa
taca.	tacamahaca
y.	yunnanensis
Reg.	Regenerata

TABLE 74: Poplar species, clones inoculated with New Zealand and and overseas isolates of *M. brunnea* (Experiment 3)

Section	Name of Species, Clone or Cultivar
LEUCE	*P. alba* L. NZ old clone
	" 72/8/3 (NZ selection)
	P. tremuloides
	P. tremula
AIGEIROS	*P. deltoides* cv. ANU 60/110
	" ANU 60/125
	" ANU 60/129
	" ANU 60/146
	" ANU 60/166
	" APC 67/28-4
	" APC 67/28-6
	" APC 67/31-1
	" APC 67/65-14
	P. deltoides spp. *angulata* cv. Chautagne
	" " cv. G3
	" " cv. G48
	" " cv. Mississippi Rough Bark
	P. deltoides I69/55 (Lux)
	" I70/51
	" I72/51SP
	" I74/51SP
	" Inta 66/71
	" Inta 91/71
	" Inta 341/69
	" Inta 372/69
	" NL 1660
	" Stoneville 81
	P. fremontii cv. ANU 61/48
	P. nigra cv. Italica
	" cv. Blanc de Garonne
	P. x *euramericana* cv. ECO 28
	" cv. Fierolo
	" cv. Flevo
	" cv. NL 925
	" cv. NL 1070
	" cv. NL 1603
	" cv. NL 2194
TACAMAHACA	*P. maximowiczii*

TABLE 75: Isolates of *M. brunnea* used to inoculate poplars in host range and pathogenicity studies (Experiment 3)

Isolate	Host Species	Origin
Br 5	*P.* x *euramericana* cv. I214	Palmerston North, NZ
Br 5L1	Large conidium variant	15%V8
Br 6	*P. alba*	Palmerston North, NZ
Br 10	*P. deltoides*	Illinois, USA
Br 11	"	Illinois, USA
Br 12	"	Illinois, USA
Br 13	"	Iowa, USA
Br 14	*P. trichocarpa*	Minnesota, USA
Br 15	*P. deltoides*	Colorado, USA
Br 16	*P. nigra*	Surrey, England
Br 17	*P.* x *euramericana* cv. Robusta	Dublin, Ireland
Br 18	"	Nancy, France
Br 19	*P. deltoides*	Hees, Holland
Br 20	*P.* x *euramericana*	Ankara, Turkey
Br 21	*P. tremuloides*	Colorado, USA
Br 23	"	Alaska, USA
Br 24	"	Minnesota, USA

TABLE 76: Susceptibility of poplar clones to New Zealand isolates of *M. brunnea* following inoculation with 10,000 conidia/disc and incubation for 20 days at 20°C in natural light (Experiment one)

Host Species, Clone or Cultivar	New Zealand Isolate of *M. brunnea*												
	NZ1	NZ2	NZ3	NZ4	NZ5	NZ6	NZ7	NZ8	NZ9	NZ10	NZ11	NZ12	NZ13
P. alba NZ old clone	1 [a]	2	1	1	1	1	1	2	1	1	1	1	1
" var. hickeliana	0	0	1	0	0	1	0	0	1	1	0	0	0
" cv. BO 2	0	0	0	0	0	0	0	1	1	0	0	0	0
P. pseudograndidentata	0	0	0	0	0	0	0	0	0	0	0	0	0
P. tremuloides	0	0	0	0	0	0	0	0	0	0	0	0	0
P. tremula	0	0	0	0	0	0	0	0	0	0	0	0	0
P. alba x *P. tremula*	0	0	0	0	0	0	0	0	0	0	0	0	0
P. canescens	0	0	1	1	0	1	0	1	1	0	0	0	0
P. alba x *P. glandulosa*	0	0	0	0	0	0	1	1	1	0	0	0	0
P. deltoides cv. I69/55	2	2	3	2	2	2	2	2	2	3	2	2	2
" I72/51SP	0	0	0	1	0	0	0	0	0	0	0	0	0
" I74/51SP	1	1	1	1	1	1	1	1	1	1	0	1	1
" NL2180	2	3	3	3	3	3	3	3	3	3	3	3	2
P. fremontii cv. 61/48	3	3	3	3	3	3	3	3	3	3	3	3	3
P. nigra cv. Italica	3	3	3	3	3	3	3	3	3	3	3	3	3
" cv. TR56/52	3	3	3	3	3	3	3	3	3	3	3	3	3
P. xeuramericana cv. Flevo (d x n)	1	1	1	1	1	1	1	1	1	1	1	1	1
" I154	3	3	3	3	3	3	3	3	3	3	3	3	2
" NL925	0	1	0	1	0	0	1	1	1	1	1	1	0
" NL1603	1	1	1	1	1	1	1	1	1	1	1	1	1
" NL2196	3	3	3	3	3	3	3	3	3	3	3	2	3
" ANU65-29	3	3	3	3	3	2	2	2	2	3	3	3	3
" ANU66/8	3	3	3	3	3	3	3	3	3	3	3	3	3
" ANU66/9	3	3	3	3	3	3	3	3	3	3	3	3	3
P. maximowiczii cv. Kew	1	1	1	1	1	1	1	1	1	1	1	1	1
" cv. OJPM1011	1	1	1	2	1	2	1	2	1	1	1	1	1
P. trichocarpa cv. 617-41	2	2	3	3	2	3	2	3	3	2	2	2	2
P. yunnanensis	1	1	1	1	1	1	1	1	1	1	1	1	1
S909-19 (d x t)	2	2	3	3	2	3	2	3	3	2	2	2	2
NL1785 "	3	3	2	2	3	3	3	3	3	2	2	2	3
Delbo U (d. x a.)	1	1	1	1	1	1	1	1	1	1	1	1	1
Delmak 20 "	2	3	3	3	2	2	3	3	3	2	2	2	2
Mareg 2 (a x n)	3	3	3	3	3	3	3	3	3	3	3	3	3
Mayu 1 (a x y)	1	1	1	1	1	1	1	1	1	1	1	1	1
TOTAL	49	53	55	56	50	55	53	59	57	52	49	49	48
MEAN	1.4	1.5	1.6	1.6	1.4	1.6	1.5	1.7	1.6	1.5	1.4	1.4	1.4

[a] 0 = no infection 1 = 1-10 acervuli/cm² 2 = >10<25 acervuli/cm² 3 = >25 acervuli/cm²

a = *P. alba* *d* = *P. deltoides* *n* = *P. nigra* *y* = *P. yunnanensis*

TABLE 77: Relative susceptibility of poplar clones to *M. brassicae* Br 1 following inoculation with 1000, 5000, 10000, 100000 conidia and 20 days incubation at 20°C under natural light (Experiment 2)

Host Species, Clone or Cultivar	Inoculum Level & Leaf Surface							
	10^3		5×10^3		10^4		10^5	
	AD	AB	AD	AB	AD	AB	AD	AB
LEUCE - *ALBIDAE*								
P. alba cv. NZ old clone	1	1	1	1	1	1	1	1
" cv. BO 2	0	0	0	0	0	0	0	0
" cv. I40/57	0	0	0	0	1	V	1	V
" cv. I42/57	0	0	0	0	1	0	1	0
" cv. I49/51	0	0	0	0	0	0	1	0
" cv. I59/1	0	0	0	0	0	0	0	0
P. alba var. hickeliana	0	0	1	0	1	0	1	0
P. alba var. pyramidalis	0	0	0	0	1	0	1	0
73/76/8	0	0	0	0	1	0	1	0
71/3/2	1	0	1	0	1	1	1	2
72/8/3	1	0	1	0	1	1	1	0
73/77/5	0	1	1	0	1	1	1	1
TREPIDAE								
P. pseudograndidentata	0	0	0	0	0	0	0	0
P. tremula FRI	0	0	0	0	0	0	0	0
" Nelson	0	0	0	0	0	0	0	0
" WOK	0	0	0	0	0	0	0	0
P. tremuloides	0	0	0	0	0	0	0	0
ALBIDAE x *TREPIDAE*								
Alba x *tremula*	0	0	0	0	0	0	0	0
P. canescens	0	0	0	0	0	0	0	0
P. alba x *glandulosa*	0	0	0	0	0	0	0	0
K65-22-4	0	0	0	0	0	0	0	0
K66-20-1	0	0	0	0	0	0	0	0
MEAN LEUCE	0.1	0.1	0.2	0.04	0.4	0.2	0.4	0.2
AIGEIROS								
P. deltoides cv. AGr21-6	1	1	2	2	3	3	3	3
" AGr 28 8	1	1	1	1	1	1	2	2
" AGr 61-58	1	1	1	1	1	1	1	1
" AGr 68-1	1	1	1	1	1	1	2	2
" AGr 28-8	1	1	2	2	2	2	3	3
" ANU 60/110	1	1	1	1	2	1	2	2
" ANU 60/129	1	1	1	1	1	1	1	1
" ANU 60/135	1	1	1	1	2	2	3	3
" ANU 60/166	1	1	2	2	2	2	2	2
" ANU 60/103	1	1	1	1	1	1	2	2
" ANU 60/125	1	1	1	1	1	1	1	1
" ANU 60/146	0	0	0	0	1	1	1	1
" APC 67/1-3 La.	1	1	1	1	1	1	2	2
" APC 67/5-1 La.	1	1	1	1	2	2	2	2
" " 67/5-2 La.	1	1	2	2	3	3	3	3
" " 67/5-6 La.	1	1	2	2	3	3	3	3
" " 67/5-7 La.	1	1	2	2	3	3	3	3
" " 67/5-13 La.	1	1	2	2	3	3	3	3
" " 67/7-1 La.	1	1	1	1	2	2	2	2

TABLE 77 Continued: Relative susceptibility of poplar clones to *M. brunnea* Br 1 following inoculation with 1000, 5000, 10000, 100000 conidia and 20 days incubation at 20°C under natural light (Experiment 2)

Host Species, Clone or Cultivar	Inoculum Level & Leaf Surface							
	10^3		5×10^3		10^4		10^5	
	AD	AB	AD	AB	AD	AB	AD	AB
P. deltoides cv. APC67/7-2 La.	1	1	2	2	2	2	2	2
" cv. APC 67/7-12 La.	1	1	2	2	2	2	3	3
" " 67/10-3 La.	1	1	2	2	2	2	3	3
" " 67/12-4 La.	1	1	2	2	2	2	2	2
" " 67/16-8 La.	1	1	2	2	2	2	3	3
" " 67/24-2 Miss.	1	1	2	2	2	2	3	3
" " 67/28-4 Miss.	0	0	0	0	0	0	0	0
" " 67/28-6 Miss.	0	0	1	0	1	1	1	1
" " 67/28-8 Miss.	1	1	1	1	2	2	2	2
" " 67/28-11 Miss.	0	0	0	0	0	0	1	1
" " 67/31-1 Miss.	1	1	1	1	1	1	1	1
" " 67/40-1 Miss.	1	1	2	2	2	2	2	2
" " 67/40-2 Miss.	1	1	2	2	3	3	3	3
" " 67/40-5 Miss.	1	1	2	2	2	2	2	2
" " 67/47-2 Miss.	1	1	1	1	1	1	1	1
" " 67/51-1 Miss.	1	1	2	2	2	2	3	3
" " 67/51-5 Miss.	2	2	2	2	2	2	2	2
" " 67/51-6 Miss.	2	2	3	3	3	3	3	3
" " 67/65-14 Miss.	0	0	0	0	0	0	1	0
" " 67/119A-8 Ill.	0	0	1	0	1	1	1	1
" " 67/119A-9 Ill.	0	0	0	0	1	1	1	1
" " 67/123A-8 Ill.	2	2	2	3	3	3	3	3
P. deltoides Angulata cv.								
" Carolinensis	1	1	1	2	2	2	2	2
" Chautagne	1	1	1	2	2	2	3	3
" G3	1	1	1	1	1	1	1	1
" G48	0	0	0	0	1	1	1	1
" Mississippi RB	1	1	1	1	1	1	1	1
" Harvard	1	1	1	1	2	2	3	3
" I63/51	1	1	1	1	2	2	2	2
" I69/55(Lux)	1	1	2	2	2	2	3	3
" I70/51	0	0	0	0	0	0	1	0
" I72/51SP	0	0	0	0	0	1	1	1
" I74/51SP	0	0	0	0	1	1	1	1
P. delt. spp. *monilifera*								
cv. Frimley	1	1	2	2	2	2	3	3
" Gwavas	2	2	3	3	3	3	3	3
P. deltoides Inta 14/71	1	1	2	2	3	3	3	3
" " 16/69	1	1	2	2	2	3	2	3
" " 39/71	1	1	1	1	1	1	1	1
" " 66/71	0	0	0	0	0	0	0	0
" " 71/67	0	0	0	0	0	0	0	0
" " 79/71	1	1	1	1	1	1	2	2
" " 91/71	0	0	0	0	0	0	0	0
" " 158/69	1	1	1	1	1	1	2	2
" " 341/69	0	0	0	0	0	0	1	1
" " 372/69	0	0	0	0	0	0	0	0
" NE 245	1	1	2	2	2	2	3	3
" NL 1454	1	1	1	1	1	1	1	1
" NL 1660	1	1	1	1	1	1	1	1
" cv. NL 2180	2	2	3	3	3	3	3	3
" NL 2243	2	2	3	3	3	3	3	3
" NL 2433	2	2	3	3	3	3	3	3
" NL 2515	1	2	2	2	2	2	2	2
" cv. Stoneville 62	1	1	2	2	3	3	3	3
" " 66	1	1	2	2	2	2	2	2

TABLE 77 Continued: Relative susceptibility of poplar clones to *M. brunnea* Br 1 following inoculation with 1000, 5000, 10000, 100000 conidia and 20 days incubation at 20°C under natural light (Experiment 2)

Host Species, Clone or Cultivar	Inoculum Level & Leaf Surface							
	10^3		5×10^3		10^4		10^5	
	AD	AB	AD	AB	AD	AB	AD	AB
P. deltoides cv. Stoneville 70	1	1	1	2	2	2	2	2
" " 71	1	1	1	1	2	2	2	2
" " 81	1	1	1	1	1	1	1	1
" " 92	2	2	2	2	3	3	3	3
P. fremontii cv. ANU 61/48	3	3	3	3	3	3	3	3
" seedling Utah	3	3	3	3	3	3	3	3
MEAN P. DELTOIDES	0.9	0.9	1.3	1.3	1.6	1.6	1.8	1.8
P. nigra cv. Caudina	1	1	2	2	3	3	3	3
" Blanc de Garonne	1	1	2	2	3	3	3	3
" CE9	2	2	3	3	3	3	3	3
" Italica	3	3	3	3	3	3	3	3
" Italica Aurea	2	3	3	3	3	3	3	3
" Italica F	2	2	3	3	3	3	3	3
" LP1	1	1	2	3	2	3	2	3
" MC18	2	2	2	2	3	3	3	3
" MC20	2	2	2	2	3	3	3	3
" PG 14	1	1	2	2	3	3	3	3
" PG 22	2	2	3	2	3	3	3	3
" Poznan 9	2	2	2	3	3	3	3	3
" R103	2	2	3	3	3	3	3	3
" Sempervirens	2	3	3	3	3	3	3	3
" Thevestina	2	2	3	3	3	3	3	3
" TR56/32	1	1	2	2	2	2	3	3
" TR56/52	2	2	2	2	3	3	3	3
" TR56/72	2	2	2	2	2	2	3	3
" TR56/75	2	2	3	3	3	3	3	3
" TR62/27	3	3	3	3	3	3	3	3
" TR62/49	2	2	3	3	3	3	3	3
" TR62/52	3	3	3	3	3	3	3	3
" TR62/57	2	2	3	3	3	3	3	3
" TR62/127	2	2	3	3	3	3	3	3
" TR62/140	1	1	2	2	3	3	3	3
" TR62/149	2	2	3	3	3	3	3	3
" TR62/154	2	2	3	3	3	3	3	3
" TR62/191	1	1	2	2	3	3	3	3
" Vert de Garonne	3	3	3	3	3	3	3	3
" Y83/66	2	2	3	3	3	3	3	3
" Y95/67	2	2	3	3	3	3	3	3
" Granaracci	2	2	2	2	3	3	3	3
MEAN P. NIGRA	2.0	2.0	2.5	2.6	2.9	2.9	2.9	3.0
P. x euramericana cv. ANU 65-1	1	2	2	2	3	3	3	3
" 65-5	1	1	1	2	2	2	2	3
" 65-14	1	2	2	2	2	2	2	2
" 65-19	1	2	2	2	3	3	3	3
" 65-24	1	2	2	2	3	3	3	3
" 65-26	1	2	3	3	3	3	3	3
" 65-29	2	2	2	3	3	3	3	3
" 65-70	1	2	2	3	3	3	3	3
" 66/8	3	3	3	3	3	3	3	3
" 66/9	3	3	3	3	3	3	3	3
" Altichiero	1	1	1	1	2	2	3	3
" Bellini	2	2	2	2	3	3	3	3
" B L Costanzo	2	2	2	2	2	2	2	3

TABLE 77 Continued: Relative susceptibility of poplar clones to *M. brunnea* Br 1 following inoculation with 1000, 5000, 10000, 100000 conidia and 20 days incubation at 20°C under natural light (Experiment 2)

Host Species, Clone or Cultivar	Inoculum Level & Leaf Surface							
	10^3		5×10^3		10^4		10^5	
	AD	AB	AD	AB	AD	AB	AD	AB
P. x *euramericana* Boccalari	2	2	2	2	2	3	3	3
" Carpaccio	1	1	2	2	3	3	3	3
" Cima	1	1	1	1	2	2	3	3
" Eco 28	0	0	0	1	1	1	1	1
" Eugenei PU	1	2	2	3	3	3	3	3
" cv. Eugenei UL	1	1	2	2	3	3	3	3
" Fierolo	1	1	1	1	1	1	1	1
" Flevo	0	0	1	0	1	1	1	1
" Fogolino	1	1	2	2	2	2	3	3
" Gelrica HA	1	1	2	2	3	3	3	3
" Giorgione	1	1	1	1	2	3	3	3
" Guardi	1	1	1	1	2	2	3	3
" Guariento	1	1	2	2	3	3	3	3
" Harff (Schroek 290)	1	1	2	2	2	2	3	3
" I30	2	2	3	3	3	3	3	3
" I65	1	2	2	3	3	3	3	3
" I74D	1	1	2	2	2	3	2	3
" I78	3	3	3	3	3	3	3	3
" I-154	1	1	2	2	3	3	3	3
" I214	1	1	2	2	3	3	3	3
" I455	2	2	3	3	3	3	3	3
" I488	1	1	3	3	3	3	3	3
" I45/51	1	1	2	2	3	3	3	3
" I92/40	1	1	2	2	2	3	3	3
" Laevigiata	1	1	2	2	3	3	3	3
" Leipzig	1	1	2	2	3	3	3	3
" Longhi	1	1	2	2	3	3	3	3
" Marilandica F	2	2	2	2	3	3	3	3
" Marilandica ID26	2	2	3	3	3	3	3	3
" NL925 (Dorskamp)	0	0	0	0	1	0	1	1
" NL1070	0	0	1	0	1	1	1	1
" NL1601	1	2	2	2	2	2	2	3
" NL1602	2	2	2	3	3	3	3	3
" NL1603	1	1	1	1	1	1	1	1
" NL1605	2	2	3	3	3	3	3	3
" NL1610	2	2	3	3	3	3	3	3
" NL1775 (Spijk)	1	2	2	2	3	3	3	3
" NL2170	2	2	3	3	3	3	3	3
" NL2171	1	1	2	2	2	2	3	3
" NL2193	2	2	3	3	3	3	3	3
" NL2194	1	2	2	2	3	3	3	3
" NL2195	1	1	2	2	2	2	3	3
" NL2196	2	2	3	3	3	3	3	3
" NL2197	2	2	3	3	3	3	3	3
" NL2200	1	1	2	3	3	3	3	3
" NL2202	2	2	3	3	3	3	3	3
" NL2205	1	1	2	2	2	2	3	3
" NL2206	1	1	2	2	2	2	3	3
" NL2207	1	1	2	2	3	3	3	3
" NL2217	1	2	2	2	3	3	3	3
" NL2220	1	2	2	2	2	2	3	3
" NL2223	1	1	2	2	3	3	3	3
" NL2226	2	2	2	2	3	3	3	3
" OP66	1	1	2	2	2	3	3	3
" OP223AH	1	1	2	2	3	3	3	3
" Pacher	1	1	1	1	2	2	2	2
" Regenerata 360	2	2	3	3	3	3	3	3
" Regenerata Nepia	1	1	2	2	3	3	3	3

TABLE 77 Continued: Relative susceptibility of poplar clones to *M. brunnea* Br 1 following inoculation with 1000, 5000, 10000, 100000 conidia and 20 days incubation at 20°C under natural light (Experiment 2)

Host Species, Clone or Cultivar	Inoculum Level & Leaf Surface							
	10^3		5×10^3		10^4		10^5	
	AD	AB	AD	AB	AD	AB	AD	AB
AIGEIROS x TACAMAHACA								
P. x interamericana cv.								
NE205	1	1	2	3	2	3	2	3
" NE207	1	1	1	1	2	3	2	3
" NL1616	1	1	2	2	3	3	3	3
" NL1623	1	1	1	2	2	2	2	2
" NL1626	1	1	1	1	1	1	1	1
" NL1647	1	1	2	1	3	2	3	2
" NL1650	1	1	1	1	1	1	1	1
" NL1656	1	1	1	1	2	2	2	2
" NL1783	1	1	2	2	2	2	2	2
" NL1785	2	2	3	3	3	3	3	3
" NL2228	1	1	1	1	1	1	1	1
" NL2233	1	1	1	2	1	2	1	3
" S909-1	1	1	1	1	1	1	1	1
" S909-10	1	1	2	1	2	1	2	1
" S909-12	1	0	2	1	3	1	3	1
" S909-16	1	1	2	1	3	1	3	1
" S909-19	1	0	1	1	1	1	1	1
" S910-1 (Una17)	1	1	2	1	3	1	3	1
" S910-2 (Una18)	1	0	2	1	2	1	3	1
" S910-4	1	0	2	0	2	1	2	1
" S910-5	1	1	2	2	3	3	3	3
" S910-8	1	0	2	1	2	1	3	1
" S910-10	1	0	2	1	2	1	2	1
" 69042-1	1	0	1	1	1	1	1	1
" 69042-2	1	1	1	1	1	1	2	1
" 69042-3	1	0	2	1	3	1	3	1
" 69042-4	1	0	1	1	1	1	2	1
" 69042-5	1	1	1	1	1	1	2	1
" 69042-6	1	1	1	1	2	1	3	2
" 69043-1	1	1	2	1	2	1	2	1
" 69043-2	1	1	3	2	3	2	3	3
" 69043-3	1	1	1	1	1	1	2	2
" 69043-4	1	1	1	1	1	1	2	2
" 69044-2	1	1	2	1	3	1	3	1
MEAN INTERAMERICANA	1.1	0.8	1.5	1.3	2.0	1.5	2.2	1.6
OTHERS Andover	1	1	2	3	2	3	2	3
ANU70-2	0	1	0	1	1	1	1	1
Berry 65/16	1	1	1	2	1	2	1	2
Berry 65/26	1	1	2	2	3	3	3	3
Frye	1	1	2	2	3	3	3	3
Geneva	1	2	2	2	2	3	3	3
I83/58	1	1	1	1	2	2	2	2
K62-9	1	1	1	1	2	2	2	2
K63-100	0	0	0	0	0	0	0	0
K63-125	1	1	2	2	2	3	2	3
K63-130	1	2	2	3	2	3	2	3
K63-129	1	1	1	2	1	2	1	3
K63-131	0	0	1	1	1	1	1	1
Laurel S2	2	2	3	3	3	3	3	3
Maine	1	1	1	2	1	3	1	3
Oxford	1	1	1	1	1	1	1	1
Rochester	1	1	1	2	1	3	1	3
Roxbury	2	2	2	3	3	3	3	3

TABLE 77 Continued: Relative susceptibility of poplar clones to *M. brunnea* Br 1 following inoculation with 1000, 5000, 10000, 100000 conidia and 20 days incubation at 20°C under natural light (Experiment 2)

Host Species, Clone or Cultivar	Inoculum Level & Leaf Surface							
	10^3		5×10^3		10^4		10^5	
	AD	AB	AD	AB	AD	AB	AD	AB
P. x *euramericana* cv.								
" Regenerata WV	1	1	2	2	2	3	2	3
" Reinbeck LK82	2	2	3	3	3	3	3	3
" Robusta Bacherlierii	1	1	2	2	3	3	3	3
" Robusta Cook	2	2	2	2	3	3	3	3
" Robusta PH	1	2	2	3	3	3	3	3
" Robusta H	2	2	2	2	3	3	3	3
" Robusta Zeeland	1	2	2	2	3	3	3	3
" Rubra	1	1	2	2	3	3	3	3
" San Martino	1	1	2	2	3	3	3	3
" Schiavone	1	1	1	2	2	2	2	3
" Serotina VB	2	2	2	3	3	3	3	3
" Serotina de Champagne	1	1	2	2	3	3	3	3
" Serotina du Poitu	1	1	2	2	2	3	3	3
" Tiepolo	1	1	1	1	2	2	2	2
" Triplo	1	1	2	2	2	2	3	3
" Veneziano	1	1	2	2	2	2	2	3
MEAN P. x EURAMERICANA	1.3	1.5	2.1	2.3	2.5	2.7	2.7	2.8
MEAN AIGEIROS	1.4	1.5	2.0	2.1	2.3	2.4	2.5	2.5
TACAMAHACA								
P. mari. Kew	0	0	1	1	1	1	1	1
" OJP M106	1	1	1	1	1	1	1	1
" " M108	1	2	1	2	1	2	2	2
" " M109	1	1	1	1	1	1	1	2
" " M1011	1	2	1	2	2	3	2	3
" " M1012	1	1	1	1	1	1	1	2
" " M1020	1	1	1	1	1	1	1	1
" " MA-3	1	1	1	1	1	1	1	1
" " MC-22	1	1	1	2	1	2	1	2
MEAN P. MAXIMOWICZII	0.9	1.2	1.0	1.4	1.1	1.5	1.2	1.6
P. trichocarpa var. Hastata	1	1	1	1	1	1	2	2
" cv. CF	1	1	1	2	2	3	2	3
" LA99	1	1	1	1	2	2	2	2
" WV	1	1	1	1	1	2	2	2
" V235	1	1	1	1	2	1	2	1
" S617-16	1	1	1	1	2	1	2	1
" S617-41	1	1	2	1	2	1	2	1
" S617-88	1	1	1	1	2	1	2	2
MEAN P. TRICHOCARPA	1.0	1.0	1.1	1.1	1.7	1.5	2.0	1.8
P. yunnanensis	0	0	0	0	0	0	1	1
P. candicans	1	1	1	1	1	3	2	3
P. ciliata	0	0	0	0	0	0	0	0
P. simonii	1	1	1	2	3	3	3	3
P. simonii var. fastigatata	1	1	1	1	1	2	1	2
P. szechuanica	1	1	1	1	1	1	1	2
P. szechuanica tibetica	1	1	1	1	1	1	1	1
P. androscoggin	1	1	1	1	1	1	1	1
P. koreana x *P. trichocarpa*	1	1	1	1	1	1	1	1
P. tacamahaca x *P. trichocarpa* 32	1	1	1	1	1	1	1	1
P. taca. x *P. tricho.* 37AF	1	1	1	2	2	2	2	2
MEAN	0.8	0.8	0.8	1.0	1.1	1.3	1.3	1.5
MEAN TACAMAHACA	0.9	1.0	1.0	1.2	1.3	1.4	1.5	1.7

TABLE 77 Continued: Relative susceptibility of poplar clones to *M. brunnea* Br 1 following inoculation with 1000, 5000, 10000, 100000 conidia and 20 days incubation at 20°C under natural light (Experiment 2)

Host Species, Clone or Cultivar	Inoculum Level & Leaf Surface							
	10^3		5×10^3		10^4		10^5	
	AD	AB	AD	AB	AD	AB	AD	AB
OTHERS NZ 5001	2	2	3	3	3	3	3	3
Waipuk 1	1	1	1	1	1	1	1	1
Waipuk 2	1	1	1	1	1	1	1	1
MEAN OTHERS	1.0	1.1	1.4	1.5	1.7	2.2	1.8	2.2
MEAN AIGEIROS X TACAMAHACA	1.1	1.0	1.4	1.4	1.8	1.8	2.0	1.9
LEUCOIDES								
P. lasiocarpa	0	0	0	0	1	0	1	0
P. wilsonii	0	0	0	0	0	0	0	0
MEAN	0	0	0	0	0.5	0	0.5	0
LEUCE X AIGEIROS								
P. alba x *P. nigra*	0	0	0	0	1	0	1	1
P. delt. x *P. alba* 'Maktar'								
cv. Delmak 13	1	1	1	2	1	2	1	2
" 16	1	1	1	1	2	3	2	3
" 18	1	1	2	2	2	3	2	3
" 19	1	1	1	2	2	3	2	3
" 20	1	1	2	2	3	3	3	3
" 22	1	1	1	1	1	1	1	2
" 26	1	1	1	2	1	2	2	3
P. delt. x *P. alba* pyramidalis								
cv. Delbo U	1	1	1	1	1	1	1	1
Delbo 9	1	1	1	2	1	2	2	2
P. alba x *P. nigra* Sempervirens								
cv. Mareg 2	2	2	3	3	3	3	3	3
MEAN LEUCE X AIGEIROS	1.0	1.0	1.3	1.6	1.6	2.0	1.8	2.4
LEUCE X TACAMAHACA								
P. alba x *P. simonii*								
cv. Mayu 1	1	1	1	1	1	1	1	1
Mayu 2	1	1	1	1	1	1	1	1
MEAN	1	1	1	1	1	1	1	1

TABLE 78: Relative susceptibility of poplars to *M. brunnea* isolate Br 1 following inoculation of leaf discs on the ad/abaxial leaf surface with 1000; 5000; 10,000; 100,000; conidia and incubation at 20°C under natural light for 20 days.

Name of Section or Poplar Species	1000 Conidia		5,000 Conidia		10,000 Conidia		100,000 Conidia		Total	Mean
	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial	Abaxial		
LEUCE										
Albidae	0.2*	0.2	0.4	0.1	0.7	0.4	0.8	0.4	3.2	0.4
Trepidae	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Albidae x *Trepidae*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
MEAN	0.07	0.07	0.1	0.03	0.2	0.1	0.3	0.1	0.9	0.1
AIGEIROS										
P. deltoides	0.9	0.9	1.3	1.3	1.6	1.6	1.8	1.8	11.2	1.4
P. nigra	2.0	2.0	2.5	2.6	2.9	2.9	2.9	3.0	20.8	2.6
P. x *euramericana*	1.3	1.5	2.1	2.3	2.5	2.7	2.7	2.8	18.0	2.2
MEAN	1.4	1.5	2.0	2.1	2.3	2.4	2.5	2.5	17.0	2.1
TACAMAHACA										
P. maximowiczii	0.9	1.2	1.0	1.4	1.1	1.5	1.2	1.6	10.0	1.2
P. trichocarpa	1.0	1.0	1.1	1.1	1.7	1.5	2.0	1.8	11.2	1.4
Other cultivars	0.8	0.8	0.8	1.0	1.1	1.3	1.3	1.5	8.6	1.1
MEAN	0.9	1.0	1.1	1.2	1.3	1.4	1.5	1.6	9.9	1.2
LEUCOIDES										
MEAN	0.0	0.0	0.0	0.0	0.5	0.0	0.5	0.0	1.0	0.1
AIGEIROS X TACAMAHACA										
P. x *Interamericana*	1.1	0.8	1.5	1.3	2.0	1.5	2.2	1.6	12.0	1.5
Others	1.0	1.1	1.4	1.5	1.7	2.2	1.8	2.2	12.9	1.6
MEAN	1.1	1.0	1.4	1.4	1.8	1.8	2.0	1.9	12.4	1.5
LEUCE X AIGEIROS										
MEAN	1.0	1.0	1.3	1.6	1.6	2.0	1.8	2.4	12.7	1.6
LEUCE X TACAMAHACA										
MEAN	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	8.0	1.0
TOTAL	11.2	11.5	14.5	15.3	18.4	18.6	20.0	20.0		
Overall Mean	0.8	0.8	1.0	1.1	1.3	1.3	1.4	1.4		

* 0 = No infection 1 = 1-10 acervuli/cm² 2 = >10<25 acervuli/cm² 3 = >25 acervuli/cm²

TABLE 79: Relative pathogenicity of New Zealand and overseas isolates of *M. brunnea* to selected poplar clones following inoculation with 25,000 conidia/disc and incubation at 20°C in natural light for 20 days (Experiment 3)

Species, Clone	Isolate of Marssonina brunnea																
	Br5	Br5L1	Br6	Br10	Br11	Br12	Br13	Br14	Br15	Br16	Br17	Br18	Br19	Br20	Br21	Br23	Br24
P. alba NZ old clone	1*	1	2	1	1	1	1	1	1	2	1	2	1	1	1	0	1
" 72/8/3	1	1	1	1	1	1	1	1	1	1	1	2	1	1	1	1	1
P. tremuloides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
P. tremula	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
P. deltoides ANU60/110	1	1	1	1	2	2	1	1	1	1	1	1	1	1	0	0	0
" ANU60/125	1	1	3	3	3	3	3	2	1	2	2	2	2	1	0	0	0
" ANU60/129	1	1	1	1	1	1	1	0	0	1	1	0	1	1	0	0	0
" ANU60/146	0	0	0	0	1	V	1	0	0	0	1	0	0	0	0	0	0
" ANU60/166	1	1	3	1	1	3	1	1	1	3	3	1	1	1	0	0	0
" APC67/28-4	0	0	0	V	3	2	1	0	0	1	V	0	0	0	0	0	0
" APC67/28-6	V	V	V	1	3	3	2	1	0	V	3	1	V	V	0	0	0
" APC67/31-1	0	0	3	3	3	3	3	1	1	3	3	1	2	1	0	0	0
" APC67/65-14	1	2	2	1	2	3	2	1	1	3	3	1	2	2	0	0	0
P. delt. angulata cv. Chautagne	2	2	3	3	3	3	3	2	1	3	3	1	2	3	0	0	0
" cv. G3	1	1	2	1	1	2	1	1	1	1	1	1	1	0	0	0	0
" cv. G48	1	1	2	1	1	2	1	0	0	2	2	0	1	1	0	0	0
" cv. Mississippi RB	1	1	2	1	1	3	1	0	1	1	1	0	1	1	0	0	0
P. deltoides I69/55 (Lux)	1	1	2	2	3	3	2	2	1	3	3	2	2	2	0	0	0
" I70/51	1	1	1	1	1	1	1	1	0	1	1	0	1	0	0	0	0
" I72/51SP	1	1	1	1	3	3	V	1	V	V	V	V	1	1	0	0	0
" I74/51SP	1	1	2	1	1	3	1	1	1	1	2	1	1	1	0	0	0
" Inta 66/71	1	1	1	1	3	3	2	1	1	1	1	1	1	1	0	0	0
" Inta 91/71	0	0	1	1	3	3	1	0	0	V	V	0	1	V	0	0	0
" Inta 341/69	0	0	V	1	2	2	1	1	V	V	V	1	1	V	0	0	0
" Inta 372/69	1	1	1	1	3	3	1	1	1	1	2	1	1	1	0	0	0
" NL1660	V	V	V	V	3	3	3	3	V	V	V	V	V	V	0	0	0
" Stoneville 81	1	1	3	2	3	3	3	0	1	3	3	1	3	3	0	0	0
P. fremontii ANU61/48	3	3	3	3	3	3	3	3	3	3	3	3	3	3	2	2	2
P. nigra cv. Italica	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	1	1
" Blanc de Garonne	3	3	3	3	3	3	3	3	3	3	3	3	3	3	1	1	1
P. x euramericana cv. EC028	1	0	3	3	3	3	1	1	1	3	3	3	2	3	0	0	0
" cv. Fierolo	1	1	2	1	1	1	0	1	0	2	2	0	1	0	0	0	0
" cv. Flevo	1	1	3	3	3	3	1	3	3	3	3	3	3	3	0	0	0
" cv. NL925	V	V	2	3	1	3	0	2	3	3	3	2	3	2	0	0	0
" cv. NL1070	1	1	3	3	1	3	0	1	2	3	3	1	3	1	0	0	0
" cv. NL1603	0	0	V	V	3	3	3	V	0	V	3	V	V	V	0	0	0
" cv. NL2194	3	3	3	3	3	3	3	3	3	3	3	3	3	3	0	0	0
P. maximowiczii	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
TOTAL	38	39	67	59	77	89	57	45	40	69	74	46	57	51	7	6	8
Mean	1.0	1.0	1.8	1.5	2.0	2.3	1.5	1.2	1.0	1.8	1.9	1.2	1.5	1.3	0.2	0.2	0.2

* 0 = No infection 1 = 1-10 acervuli/cm² 2 = >10<25 acervuli/cm² 3 = >25 acervuli/cm² V = acervuli on veins only

Experiment 3

The results (Table 79) show that isolates of *M. brunnea* from throughout the world were not uniformly pathogenic and provided further justification for recognition of the special form, *M. brunnea* f. sp. *trepidae*. That is, isolates from *P. tremuloides* (Br 21, Br 23, Br 24) were pathogenic to *P. tremula* but in no instance infected cultivars of *P. deltoides*.

The four highly susceptible clones, (*P. fremontii*, *P. nigra* cv. Italica, *P. nigra* cv. Blanc de Garonne and *P.* x *euramericana* cv. NL2194) were heavily infected by all isolates of *M. brunnea* f. sp. *brunnea*. The remaining highly resistant cultivars were variously attacked. Isolate Br 12 (ex. *P. deltoides*, Illinois) was outstandingly pathogenic. Isolate Br 8 was clearly the most pathogenic New Zealand isolate despite having been repeatedly subcultured (PDA) for at least a year prior to inoculation. Isolate Br 5 and its large conidium variant (Br5L1) were of comparable pathogenicity.

On some highly resistant clones, namely *P. deltoides* cv. NL 1660, cv. I72/51 and *P.* x *euramericana* cv. NL 1603, acervuli were formed only on veins, not on leaf tissue, suggesting a difference in resistance between these tissues.

DISCUSSION

Host range and pathogenicity studies with first generation New Zealand isolates of *M. brunnea* f. sp. *brunnea* demonstrated their comparable host range and pathogenicity, which was possibly indicative of their common origin (Chapt. 5).

Detailed host range and pathogenicity studies with a single New Zealand isolate of *M. brunnea* f. sp. *brunnea* revealed the wide host range and strong pathogenicity of this species. Promising levels of resistance however were shown by certain species from the Sections *Aigeiros*, *(P. deltoides, P.* x *euramericana); Tacamahaca, (P. trichocarpa, P. maximowiczii, P. ciliata, P. szechuanica, P. androscoggin),* and intersectional hybrids; *Aigeiros* x *Tacamahaca (P.* x *interamericana, P. deltoides* x *P. maximowiczii); Leuce* x *Aigeiros (P. alba* x *P. deltoides)* and *Leuce* x *Tacamahaca (P. alba* x *P. yunnanensis).* Many of these

observations are essentially in agreement with those of Castellani & Cellerino (1969) of infected field plantings in Italy, based on 5 years observation. Although Cellerino (1979) did not observe infections of the Section *Leuce* with *M. brunnea* f. sp. *brunnea*, in the present study some species of this section were infected.

Pinon (1974) reported that the susceptibility of poplars to infection by *M. brunnea* f. sp. *brunnea* both in the field and in the laboratory increased with increasing levels of inoculum. He further found that at inoculum strengths greater than 10,000 conidia/ml even resistant clones became severely infected and concluded that low inoculum levels (1000 conidia/ml) more accurately reflected field differences in susceptibility. These latter results contrasted with those of the present study which showed that differences of susceptibility were still apparent when inoculum levels of 10,000 conidia/ml and greater were applied.

Comparative pathogenicity tests of New Zealand and overseas isolates of *M. brunnea* f. sp. *brunnea* revealed in many instances gross differences in relative pathogenicity. This was clearly demonstrated by severe infection of *P.* x *euramericana* cv. NL 925 and NL 1603 by some isolates. In New Zealand these clones have remained free from infection despite constant exposure to extremely high levels of inoculum for three years. Further there is evidence to suggest that isolates of *M. brunnea* f. sp. *brunnea* can become more pathogenic following repeated subculturing. For example immediately following isolation, isolates Br 5 and Br 6 were similarly pathogenic (experiment one) however more than a year later (following repeated subculturing) isolate Br 6 was clearly more pathogenic. It is therefore important that all pathogenicity tests be conducted using first generation isolates. As most isolates used in experiment three were not first generation isolates it is unknown whether the observed differences in pathogenicity were innate or culturally induced. To obtain further information concerning the occurrence of pathotypes, isolates from throughout the world should be compared in one locality on a series of highly resistant clones. Such information would assist poplar breeders by indicating the possibility of their selections being resistant in other countries.

In the opinion of the writer the selection of *M. brunnea* resistant poplars would be facilitated if there was international standardisation of

methods used to screen new selections for resistance. This should be possible under the co-ordination of the International Poplar Commission which under the auspices of the Food and Agriculture Organisation of the United Nations (FAO) is the body responsible for co-ordinating research on this important lumber genus. Previous screening techniques have involved floating of leaf discs on water and inoculum has been applied with an atomiser (Gremmen, 1964; Anselmi *et al* 1975). However with this method the exact inoculum load is unknown and nutrients leach rapidly from leaf discs into the water altering their resistance to *Marssonina* (Gremmen, 1964). The agar leaf disc technique (Spiers, 1978) overcomes these problems. Cultivars with a disease rating of 2(>10<25 lesions/cm^2 leaf area) 20-days following inoculation with 10,000 conidia/disc (2.5 cm diam.) are considered sufficiently resistant to warant further field testing. Inoculation experiments should always include reference clones of known field resistance. In addition to identifying resistant cultivars the results would enable the pathogenicity of isolates of *M. brunnea* to be compared between countries.

KEY OF APPENDIX 1

ATCC	American Type Culture Collection, Maryland, USA.
B	Botanischer Garten and Museum, Berlin - Dahlem, Germany.
BR	Jardin Botanique National de Belgique, Meise, Belgium.
DAOM	National Mycological Herbarium, Canada Agriculture, Ontario, Canada.
FH	Farlow Herbarium, Harvard University, Massachusetts, USA.
K	The Herbarium, Royal Botanic Gardens, Kew, Surrey, England.
L	Rijksherbarium, Leiden, Netherlands.
O	Botanisk Museum, Oslo, Norway.
PC	Laboratoire de Cryptogamie, Musée National d'Histoire Naturelle, Paris, France.
PS	Herbarium Prof. P. Schutt, Forstbotanisches Institut, Munchen, Germany.
UPS	Institute of Systematic Botany, Uppsala, Sweden.
ZT	Institut für Spezielle Botanik, ETH, Zurich, Switzerland.
Z	Botanischer Garten und Institut für Systematische Botanik der Universität, Zurich, Switzerland.

APPENDIX 1a: Herbarium specimens of *M. brunnea* examined.

Specimen No.	Origin	Collector	Host	Collection Date	Identified By	Comments
1	Italy	G. Cellerino	*P.* x *eura. cv.* I214	/1965	Collector	-
2 PS	Geesthacht, Germany	Zycha	*P. berolinensis*	19/8/1965	Collector	-
3 PS	Winseldorf, Germany	Zycha	*P. marilandica*	13/8/1965	Collector	-
4 PS	Volksmanen, Germany	Zycha	*P. nigra*	25/2/1965	Collector	-
5 PS	Weede Schieren, Germany	Zycha	*P. Robusta*	14/8/1965	Collector	-
6 PS	Tessin, Germany	H. Butin	*P.* x *eura.*	20/9/1964	Collector	-
7 PS	Reinbek, Germany	Zycha	*P.* x *eura. cv.* I488	16/8/1965	Collector	-
8 PS	Reinbek, Germany	Zycha	*P. regenerata*	16/8/1965	Collector	-
9	Urbana, Illinois, USA	J.J. Jokela	*P. deltoides*	28/7/1978	Collector	Collection no. 78075
10	Urbana, Illinois, USA	J.J. Jokela	*P. deltoides*	28/7/1978	Collector	Collection no. 7803
11	Urbana, Illinois, USA	J.J. Jokela	*P. deltoides*	28/7/1978	Collector	Collection no. 78071
12	Urbana, Illinois, USA	J.J. Jokela	*P. deltoides*	28/7/1978	Collector	Collection no. 78076
13	Urbana, Illinois, USA	J.J. Jokela	*P. deltoides*	28/7/1978	Collector	Collection no. 78074
14	Urbana, Illinois, USA	J. Paxton	*P. deltoides*	2/8/1978	Collector	-
15 ATCC	New Field, New Jersey, USA	Ellis & Everhart	*P. candicans*	/8/1889	Collector	Type specimen
16	Rosemount, Minnesota, USA	A.L. Schipper	*P. trichocarpa*	/1978	Collector	-
17	Budapest, Hungary	T. Bertalan	*P.* x *euramericana*	8/1/1979	Collector	-
18	Columbus, Ohio, USA	L.H. Rhodes	*P. deltoides*	/1978	Collector	-
19 UPS	Guelph, Ontario, Canada	J. Dearness	*P. deltoides*	30/7/1913	D.B.O. Savile	DAOM 130529
20 UPS	Chilliwack, British Columbia, Canada	D.H. Ruppell	*P. regenerata*	3/8/1962	D.B.O. Savile	DAOM 92821
21 FH	Trempealeau, Wisconsin, USA	J.J. Davis	*P. deltoides*	23/8/1920	Author	Herb. Univ. of Wiscon.
22 FH	London, Ontario, Canada	J. Dearness	*P. balsamifera*	3/8/1893	Author	Herb. W.G. Farlow
23 FH	Mendata, Wisconsin, USA	J.J. Davis	*P. canidicans*	6/9/1922	Collector	-
24 FH	Sacramento, USA	H.E. Parks & W.S. Fields	*P. fremontii*	8/6/1924	Author	Herb. Uni. Calif. no. 2254
25 FH	Brighton, Utah, USA	G.D. Darker	*P. aurea*	4/8/1936	Author	-

APPENDIX 1b: Herbarium specimens of *M. brunnea* examined.

Specimen No.	Origin	Collector	Host	Collection Date	Identified By	Comments
26 FH	Wisconsin, USA	J.J. Davis	*P. candensis*	24/8/1926	Author	-
27 FH	Ft. Collins, Colorado, USA	C.F. Baker	*P. deltoides*	30/9/1892	Author	Ex Herb. Ellis
28 FH	London, Ontario, Canada	J. Dearness	*P. balsamifera*	/8/1893	Author	Herb. W.G. Farlow
29 FH	Madison, Wisconsin, USA	H.C. Greene	*P. deltoides*	16/8/1955	Author	Herb. Univ. Wiscon.
30 FH	Lafayette, Indiana, USA	L.D. Kerns	*P. deltoides*	16/7/1910	Author	Herb. Elam Bartholomew
31 FH	Geneva, New York, USA	A.B. Seymour	*P. deltoides*	8/8/1900	Author	Reliquiae Seymourianae
32 FH	Knoxville, Tennessee, USA	J.K. Underwood	*P. balsamifera*	2/7/1937	Author	Flor. of Tennessee no. 10887
33 FH	Madison, Wisconsin, USA	H.G. Greene	*P. deltoides*	7/9/1945	Author	Herb. Univ. Wiscon.
34 FH	Black River, Wisconsin, USA	J.J. Davis	*P. deltoides*	20/6/1916	Author	Herb. Univ. Wiscon.
35 FH	Madison, Wisconsin, USA	E. Bartholomew	*P. candicans*	27/8/1919	Author	-
36	Farnham, Surrey, England	D.A. Burdekin	*P. Laevigiata*	27/7/1979	Collector	-
37	Farnham, Surrey, England	D.A. Burdekin	*P. Robusta*	27/7/1979	Collector	-
38 K	New Field, New Jersey, USA	Ellis & Everhart	*P. candicans*	/10/1897	Collector	Ell. & Ev. Fungi Columbianic no. 1267
39 K	New Field, New Jersey, USA	Ellis & Everhart	*P. candicans*	/8/1889	Collector	Type specimen
40	Ankara, Turkey	M. Vural	*P.* x *eura. cv.* I214	/12/1979	Collector	-
41	Colorado, USA	T.E. Hinds	*P. deltoides*	30/10/1979	Collector	-
42	Palmerston North, NZ	Author	*P. eugenei*	20/4/1980	Author	-
43	Palmerston North, NZ	Author	*P. trichocarpa*	20/4/1980	Author	-
44	Palmerston North, NZ	Author	*P. regenerata*	20/4/1980	Author	-

APPENDIX 1c: Herbarium specimens of *M. brunnea* examined.

Specimen No.	Origin	Collector	Host	Collection Date	Identified By	Comments
45	Palmerston North, NZ	Author	*P. szechuanica*	20/4/1980	Author	-
46	Palmerston North, NZ	Author	*P.* x *eura. cv.* I154	20/4/1980	Author	-
47	Palmerston North, NZ	Author	*P.* x *eura. cv.* I30	20/4/1980	Author	-
48	Palmerston North, NZ	Author	*P. nigra*	20/4/1980	Author	-
49	Palmerston North, NZ	Author	*P. nigra*	20/4/1980	Author	-
50	Palmerston North, NZ	Author	*P.* x *eura. cv.* I455	20/4/1980	Author	-
51	Palmerston North, NZ	Author	*P. Mareg* 2	20/4/1980	Author	-
52	Palmerston North, NZ	Author	*P.* x *eura. cv.* NL2195	20/4/1980	Author	-
53	Palmerston North, NZ	Author	*P. simonii*	20/4/1980	Author	-
54	Palmerston North, NZ	Author	*P. fremontii*	20/4/1980	Author	-
55	Palmerston North, NZ	Author	*P. alba*	20/4/1980	Author	-
56	Palmerston North, NZ	Author	*P. Robusta*	20/5/1980	Author	-
57	Palmerston North, NZ	Author	*P. candicans*	20/4/1980	Author	-
58 ZT	Wisconsin, USA	J.J. Davis	*P. tremuloides*	16/7/1921	Author	Ex Herb. Univ. of Wisconsin
59 DAOM	New Brunswick, Canada	W.R. Newell	*P. tremuloides*	-	Author	DAOM 4305
60	Victoria, British Columbia, Canada	E.G. Harvey	*P, alba*	18/8/1961	Author	DAOM 88055
61	Fairbanks, Alaska, USA	T. Hinds - T. Laurent	*P. tremuloides*	8/8/1977	Author	Pathology Herb. Ft. Collins, no. 4606-F
62 UPS	Klamath, Oregon, USA	Lee Bonar	*P. tremuloides*	15/8/1949	Author	Uni. of Calif. Fungi of Oregon no. 786
63 UPS	Beaverley, British Columbia, Canada	E.V. Morris	*P. tremuloides*	23/8/1960	Author	DAOM. 75117
64 UPS	Yukon, Canada	J. Holms	*P. tremuloides*	21/8/1962	Author	DAOM. 92823
65 UPS	Kirton, British Columbia, Canada	J. Grant	*P. tremuloides*	11/9/1963	Author	DAOM. 107716
66 FH	Sierra Madre Mtns., Wyoming, USA	W.G. Solheim - E. Andrews	*P. tremuloides*	12/8/1966	Author	Mycol. Herb. Wilhelm G. Solheim no. 6460
67 FH	Holy Cross, Colorado, USA	G.E. Hedgecock	*P. tremuloides*	/9/1917	Author	Herb. E. Bartholomew No. 3942

APPENDIX 1d: Herbarium specimens of *M. brunnea* examined.

Specimen No..	Origin	Collector	Host	Collection Date	Identified By	Comments
68 FH	Albany Co., Wyoming, USA	W.G. Solheim	*P. tremuloides*	16/9/1940	Author	The Rocky Mtn. Herb. No. 1939 Uni. Wyoming
69 FH	Sierra Madre Mtns., Wyoming, USA	W.G. Solheim	*P. tremuloides*	12/8/1966	Author	Mycol. Herb. W.G. Solheim No. 6454
70 FH	Teton Co., Wyoming, USA	W.G. Solheim	*P. tremuloides*	23/8/1949	Author	Mycol. Herb. W.G. Solheim No. 2686
71 FH	Raciue, Wisconsin, USA	J.J. Davis	*P. tremuloides*	25/9/1894	Author	Herb. J.J. Davis
72 FH	Mt. Timpanagos, Utah USA	A.O. Garrett	*P. tremuloides*	9/8/1928	Author	Ex. Herb. A.O. Garrett No. 3466
73	Sacramento, California, USA	W.S. Fields H.E. Parks	*P. tremuloides*	8/6/1924	Author	Herb. Uni. Calif.
74 FH	Fallen Leaf Lake, California, USA	G.D. Darker	*P. tremuloides*	7/9/1929	Author	Farlow Herb., Harvard Uni.
75 FH	Albany, Wyoming, USA	Unknown	*P. tremuloides*	-	Author	Herb. Uni. Calif.
76 FH	Barron Co., Wisconsin, USA	J.J. Davis	*P. tremuloides*	1/9/1916	Author	Herb. J.J. Davis
77 FH	Blair, Wisconsin, USA	J.J. Davis	*P. tremuloides*	13/8/1916	Author	Herb. Uni. Wiscon.
78 FH	Blair, Wisconsin, USA	J.J. Davis	*P. tremuloides*	13/8/1916	Author	Herb. Uni. Wiscon.
79 FH	Cameron, Wisconsin, USA	J.J. Davis	*P. tremuloides*	1/9/1916	Author	Herb. Uni. Wiscon.
80 FH	Pullman, Washington, USA	C. Piper	*P. tremuloides*	21/7/1894	Author	-
81 FH	Webster, Wisconsin, USA	J.J. Davis	*P. tremuloides*	31/8/1916	Author	Herb. Uni. Wiscon.
82 FH	Pullman, Washington, USA	G.L. Zundel	*P. tremuloides*	26/8/1921	Author	Herb. J. Franklin Collins
83 FH	Madison, Wisconsin, USA	E. Bartholomew	*P. tremuloides*	30/8/1919	Author	Nth. Am. Fungi Elam Bartholomew no. 6706
84 FH	Bozeman, Montana, USA	D.B. Swingle	*P. tremuloides*	21/9/1911	Author	Nth. Am. Fungi Elam Bartholomew
85 FH	Pullman, Washington, USA	C.V. Piper	*P. tremuloides*	31/8/1894	Author	Washington Flor. no. 239
86	Rosemount, Minnesota, USA	M.E. Ostry	*P. tremuloides*	19/7/1979	Author	-

APPENDIX 1e: Herbarium specimens of *M. brunnea* examined.

Specimen No.	Origin	Collector	Host	Collection Date	Identified By	Comments
87 DAOM	Guelph, Ontario, Canada	J. Dearness	*P. grandidentata*	8/8/1913	Author	DAOM 130530
88 DAOM	London, Ontario, Canada	J. Dearness	*P. grandidentata*	28/8/1913	Author	DAOM 130531
89 UPS	Guelph, Ontario, Canada	J. Dearness	*P. grandidentata*	8/8/1913	Author	DAOM 130530
90 FH	Columbia Co., Wisconsin, USA	H.C. Greene	*P. grandidentata*	15/10/1945	Author	Uni. of Wiscon.
91 FH	Madison, Wisconsin, USA	J.J. Davis	*P. grandidentata*	17/9/1912	Author	Nth. Am. Fungi. Elam Bartholomew
92 FH	Shrevesport, Louisiana, USA	J.D. Lee	*P. alba* var. *canescens*	10/7/1912	Author	Herb. J. Franklin Collins
93 ZT	Gloria Str., Zurich, Switzerland	R.H. Rimpau	*P. tremula*	20/10/1960	Collector	-
94 ZT	Hansiried, Zurich, Switzerland	R.H. Rimpau	*P. tremula*	22/10/1960	Collector	-
95 ZT	Waldegg, Zurich, Switzerland	R.H. Rimpau	*P. tremula*	23/10/1960	Collector	-
96 ZT	Brandenburg, Germany	H. Sydow	*P. tremula*	17/8/1919	Author	Mycotheca Germanica no. 1729
97 PS	Berchtesgarten, Germany	H. Butin	*P. tremula*	19/9/1965	Author	-
98 UPS	London, Ontario, Canada	J. Dearness	*P. tremula*	/9/1923	Author	F. Petrak, Mycotheca Generalis no. 1244
99 Z	London, Ontario, Canada	J. Dearness	*P. tremula*	/9/1923	Author	F. Petrak, Mycotheca Generalis no. 1244
100 Z	Brandenburg, Germany	H. Sydow	*P. tremula*	26/9/1908	Author	Sydow, Mycotheca Germanica no. 834
101 Z	Hamburg, Germany	P. Magnus	*P. tremula*	23/9/1895	Author	-
102 Z	Brandenburg, Germany	H. Sydow	*P. tremula*	17/8/1919	Author	Mycotheca Germanica no. 1729
103 L	Verona, Italy	C. Massalongo	*P. tremula*	Autumn/ 1905	Author	D. Saccardo, Mycotheca Italica
104 L	Brandenburg, Germany	H. Sydow	*P. tremula*	26/9/1908	Author	Sydow, Mycotheca Germanica no. 834

APPENDIX 1f: Herbarium specimens of *M. brunnea* examined.

Specimen No.	Origin	Collector	Host	Collection Date	Identified By	Comments
105 FH	Brandenburg, Germany	H. Sydow	*P. tremula*	26/9/1908	Author	Sydow, Mycotheca Germanica no. 834
106 FH	Brandenburg, Germany	H. Sydow	*P. tremula*	17/8/1919	Author	Sydow, Mycotheca Germanica no. 1279
107 K	London, Ontario, Canada	J. Dearness	*P. tremula*	/9/1923	Author	-
108 K	London, Ontario, Canada	J. Dearness	*P. tremula*	/9/1923	Author	F. Petrak, Mycotheca Generalis no. 1244
109 K	Pavia, Italy	B.E. Cavara	*P. tremula*	1890	Author	Briosie Cavara no. 149
110 K	Brandenburg, Germany	H. Sydow	*P. tremula*	26/9/1908	Author	Mycotheca Germanica no. 834
111 BR	Westfalen, Germany	A. Ludwig	*P. tremula*	20/9/1923	Author	Sydow, Mycotheca Germanica No. 2219
112 BR	Westfalen, Germany	A. Ludwig	*P. tremula*	20/9/1923	Author	Sydow, Mycotheca Germanica No. 2219
113 BR	France	C. Roumequère	*P. tremula*	-	Author	Fungi Selecti Galliaei
114 BR	France	C. Roumequère	*P. tremula*	-	Author	Fungi Selecti Galliaei No. 1757
115 ZT	Mythenquai, Zurich, Switzerland	R.H. Rimpau	*P. alba*	3/8/1960	Author	Mixed infection with *M. castagnei*
116 O	Norway	I. Jorstad	*P. alba*	27/7/1927	Author	-

APPENDIX 1g: Herbarium specimens of *M. populi* examined.

Specimen No.	Origin	Collector	Host	Collection Date	Identified By	Comments
1 DAOM	British Columbia, Canada	C.S. Wood	*P. trichocarpa*	30/7/1969	K.A. Pirozynski	DAOM. 138632
2 ZT	Hannovers Münden, Germany	H. Butin	*P. berolinensis*	12/5/1956	Collector	-
3 ZT	Kassel, Germany	H. Butin	*P. regenerata*	15/10/1956	Collector	-
4 ZT	Hannovers Münden, Germany	H. Butin	*P. marilandica*	20/8/1955	Collector	-
5 ZT	Zurich, Switzerland	R.H. Rimpau	*P. nigra*	3/8/1960	Collector	-
6 ZT	Zurich, Switzerland - Affoltern, Wehntaler	R.H. Rimpau	*P. nigra*	17/8/1960	Collector	-
7 ZT	Zurich, Switzerland	R.H. Rimpau	*P. nigra*	7/9/1960	Collector	-
8 ZT	Niederdonau, Germany	F. Petrak	*P. nigra*	/9/1939	Collector	F. Petrak, Mycotheca Generalis
9 ZT	Hamburg, Germany	A. Ludwig	*P. nigra*	/8/1927	Collector	F. Petrak, Mycotheca Generalis
10 PS	Bayern, Germany	H. Butin	*P. gelrica*	/8/1959	-	-
11 PS	Rothenburg, Hannover, Germany	Zycha	*P. simonii*	17/8/1966	Collector	-
12 PS	Herrmannsberg, Germany	Zycha	*P. nigra*	16/8/1965	Collector	-
13 PS	Geesthacht, Germany	Zycha	*P. berolinensis*	19/8/1965	Collector	-
14 DAOM	British Columbia, Canada	R.J. Andrews	*P. tremuloides*	18/7/1961	D.B.O. Savile	DAOM 88060
15 DAOM	Bear Is., Ontario, Canada	G.E. Thompson	*P. balsamifera*	25/8/1931	Collector	Ex. Herb. Roy F. Cain, DAOM 81786
16	Harpenden, England	J.S.W. Dickens	*P. balsamifera*	13/11/1978	Collector	-
17	Munchen, Germany	Prof. P. Schutt	*P. x berolinensis*	14/9/1978	Collector	-
18 UPS	Cuxhaven, Germany	A. Ludwig	*P. nigra*	/8/1927	F. Petrak	F. Petrak, Mycotheca Generalis
19 UPS	USSR	T.C. Hebotobek	*P. nigra*	8/9/1950	-	-

APPENDIX 1h: Herbarium specimens of *M. populi* examined.

Specimen No.	Origin	Collector	Host	Collection Date	Identified By	Comments
20 UPS	Niederdonau, Germany	F. Petrak	*P. nigra*	/9/1939	F. Petrak	F. Petrak, Mycotheca Generalis
21 UPS	Denmark	F.G. Rostrup	*P. nigra*	18/8/1902	Collector	-
22 UPS	Stockholm, Sweden	J. Eriksson	*P. nigra*	5/8/1884	Author	Fungi Parasitici Scandinavici
23 PC	France	J.B. Desmazieres	*P. nigra*	/1849	Author	Coll.Desm. No. 1729
24 O	Norway	I. Jorstad	*P. nigra*	25/9/1927	Collector	-
25 O	Norway	I. Jorstad	*P. nigra*	10/9/1962	Collector	-
26 O	Norway	I. Jorstad	*P. simonii*	10/8/1944	Collector	-
27 O	Madlalia, Norway	I. Jorstad	*P. nigra*	12/9/1976	Collector	-
28 O	Bolsay, Norway	I. Jorstad	*P. berolinensis*	1/9/1953	Collector	-
29 O	Linsin, Norway	I. Jorstad	*P. berolinensis*	5/9/1925	Collector	-
30 O	Norway	I. Jorstad	*P. berolinensis*	10/9/1931	Collector	-
31 O	Norway	I. Jorstad	*P. berolinensis*	10/8/1944	Collector	-
32 O	Norway	I. Jorstad	*P. nigra*	29/8/1965	Collector	-
33 O	Norway	I. Jorstad	*P. berolinensis*	27/9/1957	Collector	-
34 O	Norway	I. Jorstad	*P. nigra*	/8/1926	Collector	-
35 O	Norway	I. Jorstad	*P. nigra*	4/7/1934	Collector	-
36 O	Norway	I. Jorstad	*P. nigra*	19/8/1920	Collector	-
37 O	Norway	I. Jorstad	*P. nigra*	7/9/1923	Collector	-
38 O	Norway	Unknown	*P. nigra*	21/8/1908	Collector	-
39 O	Norway	I. Jorstad	*P. nigra*	25/9/1927	Collector	-
40 O	Norway	I. Jorstad	*P. nigra*	21/9/1927	Collector	-
41 O	Norway	I. Jorstad	*P. nigra*	29/8/1927	Collector	-
42 O	Norway	I. Jorstad	*P. nigra*	27/7/1927	Collector	-
43 O	Norway	I. Jorstad	*P. nigra*	21/7/1925	Collector	-
44 O	Bergen, Norway	I. Jorstad	*P. nigra*	/1935	Collector	-
45 O	Norway	I. Jorstad	*P. nigra*	21/6/1932	Collector	-
46 O	Norway	I. Jorstad	*P. nigra*	16/8/1929	Collector	-
47 O	Norway	I. Jorstad	*P. nigra*	12/9/1931	Collector	-
48 O	Norway	I. Jorstad	*P. nigra*	10/8/1944	Collector	-

APPENDIX 1i: Herbarium specimens of *M. populi* examined.

Specimen No.	Origin	Collector	Host	Collection Date	Identified By	Comments
49 O	Bergen, Norway	I. Jorstad	*P. nigra*	7/10/1944	Collector	-
50 O	Norway	I. Jorstad	*P. nigra*	26/8/1939	Collector	-
51 O	Madlalia, Norway	I. Jorstad	*P. nigra*	23/7/1973	Collector	-
52 Z	Westfalen, Germany	A. Ludwig	*P. nigra*	18/9/1937	Collector	Sydow, Mycotheca Germanica
53 Z	France	M.A. Libert	*P. nigra*	1834	Collector	Type Specimen
54 Z	Niederdonau, Germany	F. Petrak	*P. nigra*	/9/1939	Collector	Mycotheca Generalis no. 1334
55 Z	Vindobonensi, Germany	F. Petrak	*P. nigra*	/8/	Collector	F. Petrak Cryptogamae exsiccatue 3374
56 Z	Hamburg, Germany	A. Ludwig	*P. nigra*	/8/1927	Collector	F. Petrak M. Generalis no. 120
57 L	Holland	Unknown	*P. nigra*	Unknown	-	Ex The Rijksherbarium, Leiden
58 L	Verona, Italy	D. Saccardo	*P. nigra*	/9/1905	Collector	D. Saccardo, Mycotheca Italica
59 L	Maria-Einsiedel, Germany	A. Allescher	*P. nigra*	/9/1896	Collector	Allescher & Schnabl., Fungi Bavarici
60 L	Groningen, Netherlands	D. Saccardo	*P. nigra*	/10/1877	Collector	Saccardo, Mycotheca Veneta no. 1237
61 L	Groningen, Netherlands	D. Saccardo	*P. nigra*	/9/1894	Collector	-
62 L	Groningen, Netherlands	Unknown	*P. nigra*	/1871	-	-
63 L	Netherlands	Unknown	*P. nigra*	12/9/1871	-	-
64 L	Netherlands	Unknown	*P. nigra*	/9/1894	-	-
65 L	Unknown, Netherlands	Unknown	*P. nigra*	1/6/1872	-	-
66 FH	Munchen, Germany	A. Allescher	*P. nigra*	/9/1896	Collector	-
67 FH	Wyoming, USA	W.G. Solheim	*P. angustifolia*	4/10/1952	Collector	Herb. W.G. Solheim, no. 3433
68 FH	Wisconsin, USA	J.J. Davis	*P. balsamifera*	1/8/1925	Collector	-
69 FH	Normandy, France	L. Boberge	*P. nigra*	/1896	-	Herbier, Barbey Bossier No. 2412
70 FH	Washington, USA	C.V. Piper	*P. trichocarpa*	26/10/1894	-	Washington Flora, E. Bartholomew

APPENDIX 1j: Herbarium specimens of *M. populi* examined.

Specimen No.	Origin	Collector	Host	Collection Date	Identified By	Comments
71 FH	Washington, USA	C.V. Piper	*P. trichocarpa*	26/10/1894	-	Washington Flora, E. Bartholomew
72	Surrey, England	D.A. Burdekin	*P. nigra*	27/7/1979	Collector	-
73 K	Rushton, England	M.J. Berkeley	*P. nigra*	1879	-	Herb. Berk. - *Asteroma Labes*
74 K	Bloemendaal, Netherlands	J. Gremmen	*P. nigra*	/9/1963	Collector	J. Gremmen No. 2232
75 K	Hyddersfield, England	L.H. Broadhead	*P. nigra*	/9/1907	-	Herb. C. Crossland
76 K	Rushton, England	M.J. Berkeley	*P. nigra*	-	Sub.*Asteroma Labes*.	Herb. C.E. Broome
77 K	Cheshire, England	J.W. Ellis	*P. deltoides?*	30/8/1913	-	Herb. W.B. Grove
78 K	Vindobonensi, Germany	F. Petrak	*P. nigra*	/8/	-	Cryptogamie exisiccatae, no. 3379
79 K	France	M.A. Libert	*P. nigra*	/1834	-	Type specimen
80 BR	France	J.B. Desmazières	*P. nigra*	/1849	-	Desmazières, Plantes Cryptogames de France, no. 2129
81 BR	Rushton, England	M.J. Berkeley	*P. nigra*	8/4/1843	Sub.*Asteroma Labes*.	-
82 BR	France	M.A. Libert	*P. nigra*	/1834	-	Type specimen
83 BR	Belgium	E. Osquinet	*P. nigra*	28/9/1855	-	-
84 BR	Vindobonensi, Germany	F. Petrak	*P. nigra*	/8/	-	F. Petrak no. 3379
85 BR	Westfalen, Germany	A. Ludwig	*P. nigra*	18/9/1937	Sydow	Sydow, Mycotheca Germanica no. 3385
86 BR	Italy	V. Mouton	*P. nigra*	/10/1877	-	Saccardo, Mycotheca Veneta no. 1237
87	Ankara, Turkey	M. Vural	*P. nigra*	/12/1979	Collector	-
88	Milton, England	A.G. Bailey	*P. nigra*	10/11/1979	Collector	-
89	Dublin, Ireland	F. O'Riordain	*P. nigra*	31/10/1979	Collector	-

APPENDIX 1k: Herbarium specimens of *M. castagnei* examined.

Specimen No.	Origin	Collector	Host	Collection Date	Identified By	Comments
1 DAOM	Mt. Horeb, Wisconsin, USA	R.F. Cain	*P. alba*	6/9/1953	Collector	DAOM 40831
2 ZT	Ankara, Turkey	H. Bremer	*P. alba*	25/9/1941	Collector	-
3 ZT	Lubmin, Germany	F.W. Neger	*P. alba*	Unknown	Collector	-
4 ZT	Zurich, Switzerland	A. Volkart	*P. alba*	7/9/1907	Collector	-
5 ZT	Germany	H. Sydow	*P. alba*	8/1919	Collector	Ex. Herb. Dr. F. Petrak
6 ZT	Zurichberg, Zurich, Switzerland	R.H. Rimpau	*P. alba*	27/7/1960	Collector	-
7 ZT	Mythenquai, Zurich, Switzerland	R.H. Rimpau	*P. alba*	3/8/1960	Collector	-
8 ZT	Bellevue-Platz, Zurich, Switzerland	R.H. Rimpau	*P. alba*	20/8/1960	Collector	-
9 DAOM	Kings Co., Nova Scotia, Canada	D. Creelman	*P. alba*	3/9/1953	Collector	DAOM 44004
10	Columbus, Ohio, USA	C.W. Ellett	*P. alba*	Unknown	Collector	-
11 UPS	Aix, France	J.L. Castagne	*P. alba*	Unknown	Collector	Herb. E. Fries
12 UPS	Bouches du Rhone, Miramas, France	J.L. Castagne	*P. alba*	Unknown	Collector	Herb. E. Fries
13 UPS	Latvia, USSR	K. Starcs	*P. alba*	/9/1934	Collector	Cryptogamae exsicc. editae Museo. Natr. Vindobensi, no. 3615
14 UPS	Denmark	F.G.E. Rostrup	*P. alba*	27/8/1888	Author	-
15 UPS	Gare de Guillou, France	Fautrey	*P. alba*	/9/1894	Author	-
16 UPS	Montubeccaria, Italy	C. Pollini	*P. alba*	/1888	Author	Herb. Agri. Ticinensis
17 UPS	Stockholm, Sweden	J. Eriksson	*P. alba*	5/8/1884	Collector	Eriksson, Fungi parasitici Scandinavici, no. 384
18	Stockholm, Sweden	J. Eriksson	*P. alba*	5/8/1884	Collector	-
19 UPS	Ottawa, Ontario, Canada	D.B.O. Savile	*P. alba*	20/8/1073	Collector	DAOM 144431
20 UPS	Uppsala, Sweden	J. Nannfeldt	*P. alba*	21/8/1930	Collector	Flora Suecica, 3805
21 UPS	Munchen, Germany	K.W. Fuckel	*P. alba*	/1894	Collector	Herbier Barbey-Bossier, 2478

APPENDIX 1L: Herbarium specimens of *M. castagnei* examined.

Specimen No.	Origin	Collector	Host	Collection Date	Identified By	Comments
22 UPS	Nurnberg, Germany	K. Starcs	*P. alba*	6/9/1948	Collector	Herb. Starcs, Flora Bavarica no. 9285
23 PC	France	Unknown	*P. alba*	1849	Collector	Collection Mougeot, no. 1484
24 O	Norway	I. Jorstad	*P. alba*	5/8/1931	Author	-
25 O	Norway	I. Jorstad	*P. alba*	9/9/1927	Author	-
26 O	Norway	Unknown	*P. alba*	28/9/1957	Author	-
27 O	Norway	Unknown	*P. alba*	8/10/1941	Author	-
28 O	Norway	Unknown	*P. alba*	10/8/1944	Author	-
29 O	Bergen, Norway	V.J. Roll-Hansen	*P. alba*	13/9/1944	Author	-
30 Z	Frankfurt, Germany	P. Magnus	*P. alba*	17/9/1873	Collector	-
31 Z	Temesvar, Hungary	G.V. Moesz	*P. alba*	/9/1925	Collector	F. Petrak, Mycotheca Generalis.
32 Z	Mähr-Weisskirchen Czechoslovakia	F. Petrak	*P. alba*	27/8/1924	Collector	F. Petrak Flora Bohemiac & Moraviac, no. 1932
33 Z	Tharandt, Germany	F.W. Neger	*P. alba*	-	Collector	Herb. Fisher-Sigwart
34 Z	Brandenburg, Germany	H. Sydow	*P. alba*	18/8/1918	Collector	Sydow, Mycotheca Germanica, no. 1728
35 Z	Thuringen, Germany	G. Oertel	*P. alba*	6/10/1903	Collector	Sydow, Mycotheca Germanica, no. 187
36 L	Brandenburg, Germany	H. Sydow	*P. alba*	18/8/1918	Collector	Sydow, Mycotheca Germanica, no. 1728
37 L	Nurnberg, Germany	K. Starcs	*P. alba*	6/9/1948	Collector	-
38 L	Tharandt, Germany	F.W. Neger	*P. alba*	-	Collector	-
39 L	Chester Co., Philadelphia, USA	Dr. Martin	*P. alba*	/7/1882	Collector	Ellis, Nth. Am. Fungi, No. 1172
40 L	Nunspeek, Belgium	Unknown	*P. alba*	18/8/1896	Collector	-
41 L	Nunspeek, Belgium	Unknown	*P. alba*	6/9/1902	Collector	-
42 L	Nunspeek, Belgium	Unknown	*P. alba*	8/1894	Collector	-
43 FH	Shrevesport, Louisiana USA	J.D. Lee	*P. alba*	10/7/1912	Author	Herb. J. Franklin Collins

APPENDIX 1m: Herbarium specimens of *M. castagnei* examined.

Specimen No.	Origin	Collector	Host	Collection Date	Identified By	Comments
44 FH	Hays, Kansas, USA	E. Bartholomew	*P. alba*	10/10/1930	Author	E. Bartholomew Herb. no. 11284
45 FH	Columbus, Kansas, USA	E. Bartholomew	*P. alba*	16/9/1920	Author	-
46 FH	Stillwater, Oklahoma, USA	W.W. Ray	*P. alba*	21/7/1943	Author	Herb. Okla. A.& M. Coll. no. 2902
47 FH	Emporia, Kansas, USA	E. Bartholomew	*P. alba*	13/10/1932	Author	E. Bartholomew Herb.
48 FH	Racine, Wisconsin, USA	J.J. Davis	*P. alba*	16/9/1893	Author	Herb., J.J. Davis
49 FH	Ogle Co., Illinois, USA	E.F. Guba	*P. alba*	29/8/1922	Author	Farlow Herb.
50 FH	London, Ontario, Canada	J. Dearness	*P. alba*	30/9/1892	Author	Herb. W.G. Farlow
51 FH	London, Ontario, Canada	J. Dearness	*P. alba*	13/9/1923	Author	Herb. Elam Bartholomew
52 FH	Westboro, Ontario, Canada	D.B.O. Savile	*P. alba*	29/7/1945	Collector	Mycol. Herb. Can. Dept. Agric., No. 15,557
53 FH	Ottawa, Ontario, Canada	D.B.O. Savile	*P. alba*	20/8/1973	Collector	DAOM 144431
54 FH	Chester Co., Philadelphia, USA	Dr. Martin	*P. alba*	/7/1882	Collector	Ellis, Nth, Am. Fungi
55 FH	Block Is., Rhode Island, USA	G.G. Hedgecock	*P. alba*	5/8/1919	Collector	Herb. J. Franklin Collins
56 FH	Aiken, South Carolina, USA	E. Bartholomew	*P. alba*	14/10/1924	Collector	Elam Bartholomew, Herb. no. 9005
57 FH	Hattenheim, Germany	K.W. Fuckel	*P. alba*	1894	Collector	Herbier Barbey Boissier, no. 2478
58 FH	Temesvar, Hungary	G.V. Moesz	*P. alba*	/9/1925	Collector	F. Petrak, Mycotheca generalis, no. 20
59 FH	Temesvar, Hungary	G.V. Moesz	*P. alba*	27/9/1923	Collector	Herb. Musei. Nat. Hungar. Budapest
60 FH	Brandenburg, Germany	H. Sydow	*P. alba*	18/8/1918	Collector	Sydow, Mycotheca Germanica, no. 1728
61 FH	Brandenburg, Germany	G.V. Moesz	*P. alba*	8/9/1927	Collector	Herb. Musei. Nat. Hungar. Budapest
62 K	Chester Co., Philadelphia, USA	Dr. Martin	*P. alba*	/7/1882	Collector	Ellis, Nth. Am. Fungi. no. 1172

APPENDIX 1n: Herbarium specimens of *M. castagnei* examined.

Specimen No.	Origin	Collector	Host	Collection Date	Identified By	Comments
63 K	Stockholm. Sweden	J. Eriksson	*P. alba*	25/8/1884	Collector	Eriksson, Fungi Scandinavici no. 3846
64 K	Thuringen, Germany	G. Gertel	*P. alba*	6/10/1903	Collector	Sydow, Mycotheca Germanica, no. 187
65 K	Tamsel, Germany	P. Vogel	*P. alba*	2/9/1921	Collector	Herb. Rev. P.G.M. Rhodes
66 K	Polperro, Italy	P. Rhodes & E. Rilstone	*P. alba*	21/7/1929	Collector	Herb. W.B. Grove, 4101
67 BR	Aix, France	Desm. & Mont.	*P. alba*	/1849	Collector	Type specimen
68 BR	Thuringen, Germany	G. Oertel	*P. alba*	6/10/1903	Collector	Sydow, Mycotheca Germanica, no. 187
69 BR	Fontainebleau, France	C. Roumeguere	*P. alba*	6/1884	Author	Fungi Gallici exsiccati, no. 3189
70 BR	Palmengarten, Frankfurt, Germany	P. Magnus	*P. alba*	7/9/1873	Collector	-
71 BR	Pavia, Italy	C. Pollini	*P. alba*	/1888	Author	-
72 BR	Parma, Italy	G. Passerini	*P. alba*	/1874	Author	-
73 BR	Bois de la Cambe, France	Unknown	*P. alba*	/7/1882	Author	-
74 BR	Boitsport, France	Unknown	*P. alba*	/8/1879	Author	-
75 BR	Vibroix, France	Putleman	*P. alba*	25/9/1917	Author	Collection Maurice Beeli, no. 639
76 BR	Watermail, France	E. Bommer & M. Rousseau	*P. alba*	18/7/1880	Collector	Herb. E. Bommer & M. Rousseau
77 BR	Mahr-Weisskirchen, Czechoslovakia	F. Petrak	*P. alba*	27/8/1924	Collector	F. Petrak, Flora Bohemiae & Moraviae, no. 1932
78 BR	France	C. Roumeguere	*P. alba*	Unknown	Collector	Fungi Gallici exsiccati, no. 317
79	Ankara, Turkey	M. Vural	*P. alba*	/12/1979	Collector	-
80	Corvallis, USA	C.W.S. van van Kraayenoord	*P. alba*	8/1979	Author	-
81	Dublin, Ireland	F. O'Riordain	*P. alba*	31/10/1979	Collector	-

APPENDIX 2a: Conidial dimensions of *M. brunnea* from Herbarium specimens

Number	Mean Length (u)	Mean Breadth (u)	L:B Ratio	TL:S Ratio	LS:B Ratio	% Sept.	Number	Mean Length (u)	Mean Breadth (u)	L:B Ratio	TL:S Ratio	LS:B Ratio	% Sept.
1	$15.2_{1.2}$[a]	$4.6_{0.3}$	3.30	2.86	1.20	34.9	22	$16.6_{1.5}$	$4.4_{0.3}$	3.80	3.20	1.20	31.3
2	$15.4_{1.2}$	$4.0_{0.2}$	3.90	3.00	1.30	33.4	23	$15.2_{1.3}$	$4.4_{0.3}$	3.50	3.20	1.08	31.3
3	$14.0_{0.8}$	$4.5_{0.3}$	3.06	3.03	1.01	33.0	24	$14.6_{1.1}$	$4.3_{0.3}$	3.40	3.28	1.03	30.5
4	$15.0_{1.5}$	$4.1_{0.3}$	3.70	2.90	1.30	34.0	25	$15.1_{1.4}$	$4.0_{0.3}$	3.70	3.13	1.20	32.0
5	$14.9_{0.8}$	$4.0_{0.2}$	3.60	3.20	1.16	31.2	26	$15.4_{1.8}$	$4.3_{0.3}$	3.60	3.31	1.08	30.2
6	$14.2_{1.0}$	$4.0_{0.2}$	3.50	3.14	1.12	31.7	27	$16.0_{1.3}$	$4.6_{0.5}$	3.51	3.12	1.12	32.0
7	$14.8_{0.9}$	$4.0_{0.2}$	3.70	3.23	1.15	31.0	28	$17.0_{2.0}$	$4.7_{0.6}$	3.70	3.30	1.12	30.3
8	$14.0_{0.9}$	$4.1_{0.3}$	3.40	3.08	1.10	32.4	29	$14.2_{1.5}$	$4.4_{0.4}$	3.20	3.20	1.00	31.2
9	$13.6_{1.4}$	$4.4_{0.3}$	3.00	3.00	1.02	33.0	30	$15.0_{1.3}$	$4.3_{0.3}$	3.51	3.16	1.10	31.7
10	$14.1_{1.6}$	$4.1_{0.3}$	3.42	3.13	1.10	32.0	31	$15.2_{2.5}$	$4.7_{0.4}$	3.20	3.00	1.06	33.2
11	$15.6_{1.6}$	$4.1_{0.2}$	3.80	3.00	1.24	33.0	32	$14.8_{1.4}$	$4.7_{0.4}$	3.10	3.18	1.00	31.4
12	$14.2_{1.5}$	$4.1_{0.3}$	3.40	3.13	1.10	32.0	33	$14.4_{1.0}$	$4.3_{0.3}$	3.40	3.37	1.04	31.0
13	$13.5_{2.0}$	$4.2_{0.2}$	3.20	3.00	1.05	33.0	34	$14.6_{1.5}$	$4.3_{0.3}$	3.40	3.31	1.04	30.2
14	$13.9_{0.8}$	$4.3_{0.3}$	3.20	3.20	1.00	31.4	35	$15.3_{1.3}$	$4.6_{0.3}$	3.34	3.20	1.04	31.2
15	$15.1_{1.0}$	$4.5_{0.4}$	3.32	3.01	1.10	33.1	36	$13.5_{1.0}$	$4.2_{0.3}$	3.23	3.50	0.90	28.6
16	$14.0_{1.0}$	$4.5_{0.2}$	3.12	3.23	1.00	31.0	37	$13.9_{1.2}$	$4.0_{0.2}$	3.50	3.70	0.94	27.1
17	$14.2_{0.7}$	$4.4_{0.2}$	3.21	3.02	1.06	33.0	38	$15.6_{1.4}$	$4.7_{0.5}$	3.30	3.16	1.04	31.6
18	$13.7_{0.9}$	$4.7_{0.4}$	2.91	3.18	0.90	31.4	39	$14.5_{1.2}$	$4.7_{0.7}$	3.00	3.17	1.00	31.5
19	$15.3_{1.5}$	$4.1_{0.6}$	3.70	3.07	1.20	32.6	40	$13.0_{2.0}$	$4.2_{0.3}$	3.06	3.14	0.97	31.8
20	$14.3_{0.8}$	$4.4_{0.3}$	3.24	3.16	1.02	31.6	41	$14.7_{1.0}$	$4.6_{0.3}$	3.18	3.03	1.05	33.0
21	$14.7_{0.5}$	$4.5_{0.5}$	3.24	3.15	1.03	31.7	42	$15.0_{1.1}$	$4.4_{0.2}$	3.44	3.37	1.02	29.6

APPENDIX 2b: Conidial dimensions of *M. brunnea* from Herbarium specimens

Number	Mean Length (u)	Mean Breadth (u)	L:B Ratio	TL:S Ratio	LS:B Ratio	% Sept.
43	$14.3_{0.6}$	$4.2_{0.2}$	3.31	3.31	1.02	30.2
44	$14.6_{1.2}$	$4.4_{0.3}$	3.31	3.33	1.00	29.9
45	$14.9_{1.2}$	$4.4_{0.3}$	3.40	3.23	1.05	30.1
46	$14.5_{0.7}$	$4.1_{0.2}$	3.51	3.28	1.07	30.5
47	$15.3_{0.8}$	$4.3_{0.3}$	3.58	3.31	1.08	30.2
48	$14.7_{1.0}$	$4.4_{0.3}$	3.35	3.30	1.01	30.3
49	$14.3_{0.7}$	$4.0_{0.2}$	3.40	3.30	1.03	30.3
50	$14.7_{0.8}$	$4.4_{0.2}$	3.34	3.30	1.01	30.3
51	$14.9_{0.7}$	$4.0_{0.2}$	3.66	3.35	1.09	29.8
52	$14.7_{0.7}$	$4.1_{0.2}$	3.60	3.25	1.10	30.8
53	$14.7_{1.0}$	$3.9_{0.4}$	3.71	3.29	1.12	30.3
54	$14.9_{0.9}$	$4.0_{0.2}$	3.67	3.24	1.13	30.8
55	$15.0_{1.0}$	$4.3_{0.3}$	3.52	3.13	1.12	31.9
56	$14.7_{0.9}$	$4.2_{0.2}$	3.52	3.27	1.07	30.6
57	$14.9_{1.5}$	$4.2_{0.2}$	3.55	3.30	1.06	30.0
58	$15.2_{1.2}$	$4.4_{0.3}$	3.43	3.00	1.13	33.6
59	$14.0_{0.7}$	$4.0_{0.2}$	3.55	3.00	1.20	33.6
60	$14.7_{1.1}$	$4.2_{0.3}$	3.48	3.21	1.08	31.1
61	$14.6_{1.3}$	$4.0_{0.3}$	3.68	3.20	1.15	31.3
62	$16.0_{1.5}$	$4.4_{0.2}$	3.63	3.02	1.20	33.1
63	$16.0_{1.8}$	$4.1_{0.2}$	3.90	2.85	1.36	35.0
64	$16.3_{1.2}$	$4.2_{0.2}$	3.90	2.95	1.31	34.0
65	$15.8_{1.3}$	$4.3_{0.3}$	3.71	3.00	1.24	33.6
66	$14.5_{1.3}$	$4.4_{0.3}$	3.30	3.00	1.10	33.6
67	$13.8_{1.4}$	$3.7_{0.2}$	3.76	3.20	1.17	31.1
68	$14.3_{1.5}$	$4.1_{0.4}$	3.46	3.11	1.11	32.1
69	$14.4_{1.5}$	$4.2_{0.5}$	3.45	3.20	1.08	31.3
70	$14.9_{1.6}$	$4.2_{0.2}$	3.52	3.08	1.14	32.5
71	$15.0_{1.4}$	$4.1_{0.3}$	3.66	3.23	1.13	31.0
72	$14.9_{1.8}$	$4.3_{0.3}$	3.50	3.12	1.11	32.0
73	$14.0_{0.9}$	$4.3_{0.2}$	3.23	3.32	1.00	30.1
74	$15.6_{1.3}$	$4.0_{0.3}$	3.90	3.36	1.16	29.7
75	$15.3_{1.4}$	$4.2_{0.4}$	3.70	3.15	1.17	32.7
76	$15.2_{1.2}$	$4.5_{0.4}$	3.40	3.06	1.11	32.7
77	$14.6_{1.3}$	$4.0_{0.3}$	3.60	3.05	1.18	32.7
78	$14.7_{1.6}$	$4.0_{0.4}$	3.64	3.20	1.14	31.3
79	$14.7_{1.4}$	$4.4_{0.3}$	3.32	3.01	1.10	33.2
80	$16.0_{1.4}$	$4.6_{0.5}$	3.46	3.03	1.14	33.0
81	$15.3_{2.8}$	$4.3_{0.3}$	3.54	2.93	1.21	34.1
82	$15.6_{1.5}$	$4.2_{0.3}$	3.68	3.31	1.11	30.2
83	$14.7_{1.4}$	$4.2_{0.3}$	3.54	3.07	1.15	32.6
84	$14.4_{1.3}$	$4.1_{0.4}$	3.50	3.06	1.15	32.7

APPENDIX 2c: Conidial dimensions of *M. brunnea* Herbarium specimens

Number	Mean Length (u)	Mean Breadth (u)	L:B Ratio	TL:S Ratio	LS:B Ratio	% Sept.
85	$15.8_{1.6}$	$4.4_{0.5}$	3.56	3.04	1.17	32.9
86	$13.2_{1.0}$	$3.9_{0.4}$	3.40	3.10	1.09	32.2
87	$15.6_{1.4}$	$5.0_{0.4}$	3.20	2.95	1.03	33.8
88	$14.3_{1.0}$	$4.0_{0.2}$	3.60	3.10	1.15	32.3
89	$14.2_{1.2}$	$3.8_{0.2}$	3.70	3.07	1.29	32.6
90	$14.0_{1.1}$	$3.9_{0.3}$	3.60	3.15	1.14	31.7
91	$13.7_{1.3}$	$4.0_{0.4}$	3.40	3.91	1.16	34.4
92	$14.0_{1.2}$	$4.2_{0.5}$	3.30	3.05	1.08	32.7
93	$16.0_{1.3}$	$4.9_{0.4}$	3.27	2.85	1.20	35.0
94	$16.6_{1.8}$	$5.3_{0.6}$	3.21	2.60	1.30	38.5
95	$17.2_{1.8}$	$5.5_{0.6}$	3.21	2.60	1.30	38.6
96	$16.4_{1.3}$	$5.1_{0.8}$	3.27	2.80	1.13	35.0
97	$17.1_{1.6}$	$4.8_{0.4}$	3.50	2.80	1.24	35.5
98	$14.7_{0.8}$	$4.2_{0.3}$	3.50	3.02	1.15	33.0
99	$14.7_{1.3}$	$4.1_{0.2}$	3.60	3.11	1.15	32.0
100	$15.6_{1.3}$	$5.0_{0.5}$	3.14	3.02	1.04	33.0
101	$15.2_{0.9}$	$5.2_{0.5}$	2.93	2.81	1.04	35.5
102	$15.3_{1.2}$	$4.7_{0.3}$	3.25	2.98	1.09	33.5
103	$16.3_{1.2}$	$4.5_{0.3}$	3.61	2.90	1.25	34.6
104	$15.2_{1.5}$	$5.1_{0.5}$	2.94	2.90	1.01	34.4
105	$15.7_{1.6}$	$5.1_{0.5}$	3.10	2.95	1.04	33.8
106	$15.6_{1.5}$	$4.5_{0.3}$	3.43	3.00	1.14	33.2
107	$14.1_{1.3}$	$4.2_{0.3}$	3.37	3.20	1.06	31.5
108	$14.6_{1.8}$	$4.3_{0.4}$	3.40	3.00	1.13	33.3
109	$15.6_{1.4}$	$4.6_{0.5}$	3.35	3.20	1.05	31.2
110	$16.0_{1.5}$	$4.7_{0.4}$	3.34	3.13	1.07	32.0
111	$15.2_{1.6}$	$4.8_{0.6}$	3.16	3.08	1.03	32.4
112	$15.9_{1.7}$	$4.7_{0.6}$	3.37	3.00	1.12	33.2
113	$16.0_{1.5}$	$4.7_{0.5}$	3.36	2.90	1.15	34.4
114	$16.3_{1.2}$	$5.0_{0.4}$	3.30	3.10	1.07	32.4
115	$13.9_{0.9}$	$4.1_{0.3}$	3.40	3.46	0.95	29.0
116	$14.3_{1.4}$	$4.1_{0.2}$	3.45	3.30	1.04	30.2
Mean	$15.0_{0.9}$	$4.4_{0.3}$	$3.42_{0.2}$	$3.10_{0.2}$	$1.10_{0.10}$	$32.4_{1.5}$

[a] Standard deviation

APPENDIX 2d: Conidial dimensions of *M. populi* from herbarium specimens

Number	Mean Length (u)	Mean Breadth (u)	L:B Ratio	TL:S Ratio	LS:B Ratio	% Sept.	Number	Mean Length (u)	Mean Breadth (u)	L:B Ratio	TL:S Ratio	LS:B Ratio	% Sept.
1	$16.5_{1.2}$[a]	$7.0_{0.8}$	2.42	3.60	0.66	27.8	22	$19.5_{1.8}$	$6.6_{0.5}$	2.96	3.85	0.77	26.0
2	$21.0_{2.0}$	$6.7_{0.7}$	3.10	3.23	1.00	31.0	23	$20.0_{1.4}$	$6.7_{0.6}$	2.97	3.60	0.82	27.7
3	$23.2_{2.2}$	$6.7_{0.6}$	3.40	3.04	1.14	32.8	24	$18.7_{1.5}$	$6.4_{0.5}$	2.90	3.52	0.82	28.3
4	$20.8_{1.5}$	$6.8_{0.5}$	3.10	3.24	0.96	31.0	25	$20.0_{1.8}$	$6.8_{0.8}$	2.93	3.63	0.80	27.5
5	$20.8_{1.8}$	$6.6_{0.6}$	3.21	3.18	1.01	31.4	26	$19.8_{2.0}$	$6.6_{0.4}$	2.98	3.40	0.90	29.4
6	$18.4_{1.5}$	$6.0_{0.5}$	3.10	3.33	0.96	30.0	27	$19.7_{2.2}$	$6.7_{0.5}$	2.95	3.56	0.83	28.1
7	$20.5_{2.0}$	$6.0_{0.6}$	3.35	3.25	1.11	30.7	28	$19.7_{1.8}$	$6.3_{0.5}$	3.10	3.44	0.90	29.0
8	$21.5_{1.7}$	$7.2_{0.7}$	2.97	3.17	0.94	31.5	29	$17.9_{1.5}$	$6.0_{1.0}$	2.94	3.84	0.76	26.0
9	$19.6_{1.6}$	$6.7_{0.7}$	2.96	3.21	0.90	31.0	30	$19.4_{1.3}$	$6.6_{0.4}$	2.92	3.65	0.80	27.3
10	$19.3_{1.9}$	$6.7_{0.8}$	2.88	3.87	0.77	25.8	31	$21.0_{2.0}$	$7.2_{0.7}$	2.91	3.51	0.83	28.5
11	$21.0_{2.2}$	$6.2_{0.8}$	3.40	3.25	1.06	30.7	32	$18.7_{1.6}$	$6.9_{0.5}$	2.70	3.40	0.80	29.5
12	$20.3_{1.4}$	$6.8_{0.5}$	3.00	3.35	0.93	30.0	33	$19.7_{1.5}$	$6.5_{0.4}$	3.00	3.80	0.80	26.3
13	$20.0_{1.9}$	$6.0_{0.5}$	3.40	3.40	1.00	29.2	34	$19.0_{1.7}$	$6.3_{0.4}$	3.03	3.27	0.92	30.5
14	$22.2_{1.4}$	$9.0_{0.9}$	2.47	3.27	0.76	30.6	35	$17.8_{1.8}$	$6.5_{0.6}$	2.71	3.61	0.75	27.7
15	$19.8_{1.1}$	$6.3_{0.5}$	3.13	3.70	0.85	27.1	36	$24.3_{1.6}$	$6.9_{0.5}$	2.67	3.61	0.74	27.7
16	$19.1_{1.8}$	$5.3_{0.4}$	3.61	3.55	1.02	28.2	37	$20.0_{1.4}$	$7.3_{0.7}$	2.74	3.62	0.76	27.6
17	$21.4_{1.4}$	$6.8_{0.5}$	3.05	3.37	0.90	29.6	38	$19.5_{1.8}$	$6.7_{0.6}$	2.92	3.64	0.80	27.5
18	$20.7_{1.4}$	$6.7_{0.5}$	3.11	3.57	0.87	28.0	39	$20.4_{1.6}$	$6.6_{0.7}$	3.08	3.64	0.84	27.4
19	$21.5_{2.0}$	$6.8_{0.5}$	3.14	3.50	0.90	28.5	40	$20.2_{2.0}$	$6.4_{0.5}$	3.15	3.37	0.93	29.7
20	$21.3_{1.5}$	$6.8_{0.8}$	3.11	3.43	0.90	29.2	41	$18.6_{1.3}$	$6.6_{0.7}$	2.80	3.31	0.84	30.2
21	$20.0_{1.4}$	$6.5_{0.6}$	3.04	3.40	0.90	29.3	42	$18.7_{1.3}$	$7.0_{0.5}$	2.70	3.44	0.80	29.0

APPENDIX 2e: Conidial dimensions of *M. populi* from herbarium specimens

Number	Mean Length (u)	Mean Breadth (u)	L:B Ratio	TL:S Ratio	LS:B Ratio	% Sept.	Number	Mean Length (u)	Mean Breadth (u)	L:B Ratio	TL:S Ratio	LS:B Ratio	% Sept.
43	$17.7_{1.8}$	$7.1_{0.6}$	2.50	3.55	0.70	28.1	63	$18.6_{1.8}$	$6.6_{1.0}$	2.81	3.32	0.84	30.0
44	$17.7_{1.0}$	$6.4_{0.4}$	2.75	3.39	0.80	29.4	64	$18.4_{1.7}$	$6.5_{0.7}$	2.82	3.50	0.80	28.5
45	$18.4_{1.4}$	$6.3_{0.4}$	2.92	3.52	0.83	28.4	65	$19.7_{2.3}$	$7.0_{1.0}$	2.82	3.35	0.84	29.8
46	$18.2_{1.5}$	$6.6_{1.0}$	2.80	3.44	0.80	29.1	66	$20.7_{1.8}$	$6.7_{0.7}$	3.08	3.54	0.87	28.2
47	$18.6_{1.3}$	$6.9_{0.5}$	2.70	3.64	0.74	27.4	67	$16.3_{1.5}$	$6.4_{0.6}$	2.55	3.33	0.76	30.0
48	$20.0_{1.4}$	$6.4_{1.1}$	3.13	3.45	0.90	28.9	68	$22.3_{2.3}$	$8.9_{0.8}$	2.51	3.30	0.76	30.5
49	$18.6_{1.4}$	$6.5_{0.3}$	2.86	3.60	0.80	27.9	69	$19.0_{1.7}$	$6.8_{0.7}$	2.80	3.66	0.76	27.3
50	$17.9_{1.0}$	$6.5_{0.5}$	2.73	3.30	0.83	30.4	70	$18.1_{1.9}$	$6.7_{0.6}$	2.72	3.60	0.76	27.9
51	$17.3_{1.0}$	$6.8_{0.4}$	2.53	3.41	0.74	29.3	71	$19.3_{2.1}$	$7.4_{0.8}$	2.61	3.78	0.70	26.4
52	$20.4_{1.4}$	$6.3_{1.0}$	3.25	3.74	0.87	26.7	72	$18.1_{2.2}$	$6.2_{0.5}$	2.94	3.75	0.78	26.6
53	$20.5_{2.0}$	$6.5_{0.8}$	3.14	3.55	0.88	28.1	73	$18.4_{1.5}$	$7.0_{0.6}$	2.60	4.03	0.64	24.8
54	$20.4_{1.5}$	$7.3_{0.6}$	2.82	3.37	0.83	29.6	74	$21.0_{2.0}$	$6.1_{0.5}$	3.41	3.37	1.01	29.6
55	$18.8_{1.4}$	$6.7_{0.6}$	2.80	3.91	0.71	25.5	75	$18.3_{1.3}$	$6.4_{0.6}$	2.87	3.55	0.80	28.1
56	$19.3_{1.5}$	$6.4_{0.5}$	3.02	3.78	0.80	26.4	76	$19.0_{2.0}$	$6.6_{0.7}$	2.86	3.90	0.74	25.7
57	$20.8_{2.0}$	$6.8_{0.6}$	3.04	3.30	0.92	30.3	77	$18.7_{1.8}$	$6.6_{1.1}$	2.85	3.76	0.76	26.6
58	$18.4_{1.4}$	$6.6_{0.7}$	2.80	3.60	0.80	27.7	78	$20.0_{2.0}$	$6.9_{0.5}$	2.90	3.63	0.80	27.5
59	$20.5_{1.6}$	$7.1_{1.0}$	2.90	3.50	0.82	28.6	79	$20.2_{2.0}$	$6.6_{0.8}$	3.06	3.43	0.90	29.0
60	$18.6_{1.6}$	$6.3_{0.9}$	2.95	3.50	0.84	28.6	80	$20.7_{2.0}$	$6.7_{0.7}$	3.08	3.70	0.83	27.0
61	$16.8_{1.2}$	$6.7_{0.6}$	2.50	3.50	0.71	28.5	81	$17.8_{2.1}$	$6.7_{0.8}$	2.70	4.08	0.65	24.5
62	$20.4_{1.7}$	$6.5_{1.0}$	3.12	3.40	0.91	29.4	82	$19.8_{2.3}$	$6.6_{0.7}$	3.00	3.73	0.80	26.8

APPENDIX 2f: Conidial dimensions of *M. populi* from herbarium specimens

Number	Mean Length (u)	Mean Breadth (u)	L:B Ratio	TL:S Ratio	LS:B Ratio	% Sept.
83	$20.7_{1.7}$	$6.5_{0.7}$	3.20	3.72	0.85	26.9
84	$19.7_{2.0}$	$7.0_{0.8}$	2.80	3.77	0.75	26.5
85	$20.2_{1.5}$	$6.8_{0.8}$	2.95	3.75	0.78	26.6
86	$17.2_{1.7}$	$6.6_{0.7}$	2.60	3.64	0.71	27.4
87	$18.4_{1.4}$	$6.8_{1.0}$	2.71	3.59	0.75	27.8
88	$18.9_{2.1}$	$6.2_{0.4}$	3.06	3.49	0.88	28.7
89	$21.4_{2.0}$	$6.0_{1.2}$	3.59	3.70	0.96	26.9
Mean	$19.6_{1.4}$	$6.7_{0.5}$	$2.93_{0.2}$	$3.53_{0.2}$	$0.83_{0.09}$	$28.1_{3.2}$

[a] Standard deviation

APPENDIX 2g: Conidial dimensions of *M. castagnei* from herbarium specimens

Number	Mean Length (u)	Mean Breadth (u)	L:B Ratio	TL:S Ratio	LS:B Ratio	% Sept
1	$18.0_{1.6}^{a}$	$6.0_{0.7}$	3.13	2.46	1.17	40.5
2	$19.0_{1.6}$	$6.0_{0.6}$	3.15	2.45	1.32	40.7
3	$17.0_{1.5}$	$5.9_{0.5}$	2.82	2.50	1.21	40.0
4	$18.5_{2.0}$	$6.2_{0.5}$	3.00	2.45	1.23	40.8
5	$17.7_{1.8}$	$6.2_{0.4}$	2.82	2.41	1.15	41.4
6	$17.0_{1.4}$	$5.8_{0.4}$	2.93	2.41	1.17	41.4
7	$16.8_{1.8}$	$5.4_{0.5}$	3.08	2.43	1.27	41.2
8	$16.4_{1.1}$	$5.6_{0.4}$	2.90	2.50	1.16	39.8
9	$19.0_{1.4}$	$5.9_{0.5}$	3.21	2.45	1.31	40.8
10	$17.4_{1.5}$	$6.0_{0.4}$	2.91	2.40	1.21	41.6
11	$19.2_{1.6}$	$6.4_{0.3}$	3.00	2.30	1.32	44.0
12	$16.7_{1.1}$	$6.1_{0.3}$	2.73	2.40	1.13	41.6
13	$16.7_{1.3}$	$6.2_{0.4}$	2.70	2.47	1.10	40.5
14	$18.5_{1.6}$	$5.8_{0.8}$	3.14	2.43	1.30	41.1
15	$19.3_{1.7}$	$5.8_{0.6}$	3.31	2.53	1.30	39.4
16	$18.5_{2.0}$	$6.0_{0.5}$	3.05	2.53	1.20	39.6
17	$18.5_{1.6}$	$6.0_{0.4}$	3.08	2.53	1.22	39.5
18	$23.0_{1.8}$	$7.9_{0.4}$	2.93	2.30	1.28	43.7
19	$16.3_{1.6}$	$5.6_{0.5}$	2.91	2.45	1.18	40.7
20	$18.7_{1.8}$	$6.0_{0.4}$	3.10	2.42	1.28	41.3
21	$18.8_{2.0}$	$6.0_{0.4}$	3.17	2.47	1.28	40.5
22	$17.0_{1.2}$	$5.7_{0.3}$	3.00	2.52	1.18	39.6
23	$14.6_{0.9}$	$4.8_{0.3}$	3.00	2.90	1.05	35.0
24	$18.8_{2.0}$	$6.0_{1.1}$	3.17	2.34	1.35	42.6
25	$16.8_{1.4}$	$5.5_{0.4}$	3.03	2.40	1.26	41.6
26	$18.0_{1.6}$	$5.9_{0.3}$	3.02	2.66	1.13	37.5
27	$16.2_{1.3}$	$5.5_{0.4}$	2.93	2.85	1.03	35.0
28	$18.5_{1.6}$	$5.7_{0.5}$	3.20	2.38	1.35	42.0
29	$16.7_{1.4}$	$5.6_{0.4}$	3.00	2.73	1.10	36.5
30	$19.4_{1.8}$	$6.1_{0.6}$	3.14	2.35	1.33	42.5
31	$18.5_{1.8}$	$5.9_{0.4}$	3.12	2.53	1.23	39.5
32	$18.0_{1.6}$	$5.7_{0.5}$	3.12	2.55	1.22	39.1
33	$15.5_{1.1}$	$5.1_{0.4}$	3.00	2.76	1.10	36.1
34	$18.0_{2.1}$	$6.0_{0.5}$	3.00	2.45	4.21	40.1
35	$17.5_{1.5}$	$5.6_{0.4}$	3.11	2.72	1.14	37.0
36	$17.2_{1.5}$	$6.0_{0.5}$	2.57	2.36	1.08	42.4
37	$16.8_{1.5}$	$5.8_{0.5}$	2.87	2.57	1.11	38.8
38	$15.9_{1.2}$	$5.5_{0.5}$	2.86	2.80	1.03	36.0
39	$19.0_{1.6}$	$6.4_{0.6}$	2,94	2.36	1.24	42.3
40	$18.4_{1.5}$	$5.7_{0.4}$	3.23	2.51	1.30	39.8
41	$16.2_{1.5}$	$5.5_{0.4}$	2.92	2.62	1.11	38.2
42	$18.1_{1.6}$	$6.0_{0.5}$	3.06	2.35	1.30	42.5

APPENDIX 2h: Conidial dimensions of *M. castagnei* from herbarium specimens

Number	Mean Length (u)	Mean Breadth (u)	L:B Ratio	TL:S Ratio	LS:B Ratio	% Sept.
43	$17.4_{1.6}$	$6.0_{0.4}$	2.93	2.68	1.09	37.2
44	$19.4_{1.7}$	$6.0_{1.0}$	3.22	2.40	1.32	42.0
45	$17.6_{1.7}$	$5.7_{1.0}$	3.08	2.55	1.21	39.2
46	$16.8_{1.4}$	$5.7_{0.4}$	2.93	2.70	1.10	37.1
47	$17.3_{2.3}$	$5.9_{0.5}$	2.93	2.66	1.10	37.6
48	$17.2_{2.0}$	$5.6_{0.4}$	3.06	2.55	1.20	39.2
49	$19.2_{1.5}$	$5.7_{0.4}$	3.34	2.46	1.36	40.7
50	$19.0_{1.6}$	$6.1_{0.7}$	3.10	2.46	1.26	40.7
51	$19.1_{2.0}$	$6.3_{0.5}$	3.02	2.33	1.29	43.0
52	$18.4_{2.4}$	$6.0_{0.5}$	3.04	2.50	1.22	40.1
53	$17.4_{1.4}$	$5.8_{0.5}$	3.00	2.56	1.17	39.0
54	$18.5_{1.3}$	$6.4_{0.7}$	2.90	2.54	1.14	39.4
55	$19.0_{2.0}$	$6.1_{0.4}$	3.12	2.60	1.20	38.7
56	$20.1_{2.0}$	$6.3_{0.4}$	3.21	2.42	1.32	41.3
57	$18.8_{2.0}$	$6.1_{0.7}$	3.10	2.53	1.22	39.5
58	$19.2_{2.0}$	$6.1_{0.5}$	3.14	2.46	1.28	40.6
59	$19.1_{2.1}$	$6.2_{0.6}$	3.06	2.50	1.24	40.4
60	$18.5_{2.3}$	$6.1_{0.5}$	3.03	2.33	1.30	42.8
61	$17.4_{1.8}$	$5.7_{0.5}$	3.07	2.56	1.20	39.0
62	$18.2_{1.9}$	$6.2_{0.5}$	2.92	2.48	1.18	40.3
63	$18.5_{1.5}$	$6.3_{0.6}$	2.94	2.57	1.14	39.0
64	$17.2_{1.5}$	$5.4_{0.4}$	3.17	2.63	1.20	38.0
65	$18.3_{1.8}$	$5.7_{0.4}$	3.17	2.56	1.24	39.0
66	$17.6_{1.5}$	$5.7_{0.4}$	3.09	2.74	1.12	36.4
67	$17.2_{1.7}$	$6.1_{0.4}$	2.80	2.44	1.15	41.0
68	$17.1_{1.6}$	$5.9_{0.6}$	2.91	2.67	1.09	37.4
69	$18.9_{2.0}$	$6.4_{0.6}$	2.96	2.65	1.11	37.6
70	$18.7_{1.8}$	$6.2_{0.7}$	3.02	2.61	1.56	38.3
71	$17.2_{1.6}$	$6.1_{0.5}$	2.82	2.70	1.05	37.2
72	$18.0_{2.0}$	$6.2_{0.6}$	2.90	2.53	1.14	40.0
73	$18.0_{1.6}$	$6.0_{0.8}$	3.02	2.54	1.19	39.4
74	$17.0_{1.6}$	$5.7_{0.7}$	3.00	2.59	1.15	38.6
75	$18.0_{2.0}$	$6.0_{0.6}$	3.00	2.60	1.15	38.7
76	$18.2_{1.8}$	$6.0_{0.4}$	3.00	2.58	1.16	38.8
77	$17.3_{1.5}$	$6.1_{0.5}$	2.85	2.60	1.10	38.5
78	$16.5_{1.3}$	$5.0_{0.6}$	3.32	2.70	1.24	37.4
79	$17.3_{1.7}$	$6.2_{0.5}$	2.81	2.61	1.07	38.3
80	$18.1_{1.9}$	$5.7_{0.4}$	3.19	2.60	1.22	38.4
81	$17.6_{1.4}$	$5.4_{0.6}$	3.26	2.84	1.48	35.2
Mean	$17.9_{1.2}$	$5.9_{0.4}$	$3.02_{0.1}$	$2.53_{0.1}$	$1.20_{0.2}$	$39.6_{2.2}$

[a] Standard deviation

APPENDIX 3a: Influence of culture medium on conidial dimensions of *Marssonina* species following 10 days incubation at 20°C under a 12 hour white light photoperiod.

Treatment	Length (u)	Breadth	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea* Br 1 *P. yunnanensis* NZ					
CDA	$15.8_{1.3}$	$4.7_{0.4}$	3.33	2.74	1.23	36.5
PDA	$15.0_{1.5}$	$5.0_{0.4}$	3.03	3.14	0.96	31.8
PCA	$15.3_{1.3}$	$4.0_{0.3}$	3.80	2.90	1.30	34.4
PC-10	$15.7_{1.2}$	$4.1_{0.3}$	3.80	2.96	1.28	33.7
15%V8	$15.5_{1.4}$	$4.0_{0.3}$	3.86	2.95	1.30	33.9
Mean	$15.5_{0.3}$	$4.4_{0.5}$	3.56	2.94	1.21	34.1
Isolate	*M. brunnea* Br 2 *P.* x *euramericana* NZ					
CDA	$15.5_{1.1}$	$5.5_{0.4}$	2.82	3.00	0.94	33.5
PDA	$15.7_{1.4}$	$4.9_{0.3}$	3.20	3.20	0.98	31.4
PCA	$14.9_{1.3}$	$4.0_{0.5}$	3.70	2.90	1.30	34.4
PC-10	$14.7_{1.1}$	$4.2_{0.3}$	3.40	2.93	1.20	34.0
15%V8	$15.4_{1.1}$	$3.9_{0.3}$	3.95	3.00	1.32	33.6
Mean	$15.2_{0.4}$	$4.6_{0.7}$	3.41	3.00	1.15	33.4
Isolate	*M. brunnea* Br 3 *P. fremontii* cv. 61/93 NZ					
CDA	$14.7_{1.0}$	$4.4_{0.2}$	3.33	3.08	1.10	32.4
PDA	$15.1_{1.5}$	$4.5_{0.4}$	3.33	2.90	1.16	34.5
PCA	$14.1_{1.0}$	$4.0_{0.2}$	3.52	2.99	1.18	33.4
PC-10	$14.0_{0.8}$	$4.1_{0.2}$	3.43	3.00	1.14	33.3
15%V8	$14.1_{0.9}$	$3.8_{0.2}$	3.67	3.20	1.16	31.2
Mean	$14.4_{0.5}$	$4.2_{0.3}$	3.45	3.03	1.15	33.0
Isolate	*M. brunnea* Br 4 *P. fremontii* cv. 66/9 NZ					
CDA	$15.0_{1.0}$	$4.7_{0.3}$	3.16	3.03	1.04	32.9
PDA	$15.1_{1.1}$	$4.7_{0.2}$	3.18	3.08	1.03	32.4
PCA	$14.2_{1.0}$	$4.0_{0.2}$	3.56	2.95	1.21	34.0
PC-10	$14.5_{0.9}$	$3.8_{0.2}$	3.76	3.08	1.21	32.4
15%V8	$15.0_{0.9}$	$4.0_{0.2}$	3.75	3.04	1.24	32.8
Mean	$14.7_{0.4}$	$4.2_{0.4}$	3.48	3.04	1.15	32.9

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea* Br 5 *P.* x *euramericana* cv. I-214 NZ					
CDA	$15.4_{0.8}$	$5.0_{0.2}$	3.06	3.02	1.00	33.0
PDA	$15.5_{0.8}$	$4.7_{0.2}$	3.31	2.95	1.13	34.0
PCA	$15.0_{1.2}$	$4.1_{0.2}$	3.70	3.02	1.22	33.0
PC-10	$14.9_{1.0}$	$4.1_{0.2}$	3.65	2.96	1.23	33.7
15%V8	$15.3_{1.2}$	$4.2_{0.3}$	3.64	2.93	1.26	34.0
Mean	$15.2_{0.2}$	$4.4_{0.4}$	3.47	2.97	1.17	33.5
Isolate	*M. brunnea* Br 6 *P. alba* NZ					
CDA	$17.6_{1.5}$	$5.1_{0.3}$	3.45	2.98	1.15	33.5
PDA	$19.0_{1.5}$	$5.1_{0.3}$	3.73	3.00	1.24	33.3
PCA	$16.7_{1.2}$	$4.2_{0.2}$	4.02	3.14	1.27	32.0
PC-10	$16.5_{1.4}$	$4.1_{0.2}$	4.02	2.96	1.35	33.8
15%V8	$17.4_{1.4}$	$4.2_{0.3}$	4.10	3.00	1.37	33.3
Mean	$17.4_{1.0}$	$4.5_{0.5}$	3.86	3.44	1.28	33.2
Isolate	*M. brunnea* Br 7 *P.* x *euramericana* cv. Flevo NZ					
CDA	$15.4_{1.0}$	$4.9_{0.3}$	3.15	3.25	0.96	30.7
PDA	$15.3_{1.2}$	$4.6_{0.3}$	3.32	3.22	1.03	31.0
PCA	$15.0_{1.2}$	$3.9_{0.5}$	3.87	3.20	1.21	31.3
PC-10	$14.8_{1.1}$	$4.0_{0.2}$	3.72	3.20	1.16	31.3
15%V8	$15.0_{1.0}$	$4.1_{0.2}$	3.27	2.70	1.20	35.0
Mean	$15.1_{0.2}$	$4.3_{0.4}$	3.47	3.11	1.11	32.0
Isolate	*M. brunnea* Br 8 *P.* x *euramericana* cv. NL2194 NZ					
CDA	$17.6_{1.8}$	$4.8_{0.3}$	3.68	3.08	1.19	32.4
PDA	$17.3_{1.4}$	$4.5_{0.3}$	3.66	3.08	1.19	32.4
PCA	$16.3_{1.5}$	$4.0_{0.2}$	4.06	3.14	1.29	31.7
PC-10	$16.7_{1.4}$	$4.0_{0.2}$	4.17	3.07	1.36	32.5
15%V8	$16.4_{1.4}$	$4.2_{0.2}$	3.96	2.96	1.33	33.7
Mean	$16.8_{0.5}$	$4.3_{0.3}$	3.91	3.07	1.27	32.5

APPENDIX 3b: Influence of culture medium on conidial dimensions of *Marssonina* species following 10 days incubation at 20°C under a 12 hour white light photoperiod.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 9 P. delt. x P. maxi.* cv. I83/58 NZ					
CDA	$14.7_{1.8}$	5.0	2.96	3.10	0.95	32.3
PDA	$15.4_{1.3}$	5.0	3.10	3.14	0.98	31.8
PCA	$14.9_{1.1}$	4.0	3.76	3.15	1.20	31.7
PC-10	$15.2_{1.2}$	4.0	3.80	3.03	1.25	33.0
15%V8	$15.5_{1.3}$	4.0	3.86	3.05	1.26	32.7
Mean	$15.1_{0.3}$	$4.4_{0.5}$	3.50	3.09	1.13	32.3
Isolate	*M. brunnea Br 10 P. deltoides* USA					
CDA	$15.3_{1.0}$	$5.4_{0.2}$	2.80	2.94	0.95	34.0
PDA	$14.4_{0.6}$	$4.8_{0.3}$	2.98	2.99	1.00	33.4
PCA	$14.1_{0.8}$	$4.0_{0.2}$	3.53	3.33	1.07	30.0
PC-10	$14.1_{0.8}$	$4.0_{0.2}$	3.47	3.15	1.10	31.7
15%V8	$13.9_{0.8}$	$4.0_{0.3}$	3.40	3.08	1.10	32.4
Mean	$14.4_{0.5}$	$4.4_{0.6}$	3.24	3.10	1.04	32.3
Isolate	*M. brunnea Br 11 P. deltoides* USA					
CDA	$14.8_{1.0}$	$5.5_{0.4}$	2.70	2.97	0.90	33.6
PDA	$14.8_{0.9}$	$5.0_{0.4}$	2.92	3.07	0.95	32.5
PCA	$14.2_{0.8}$	$4.0_{0.2}$	3.53	3.03	1.16	33.0
PC-10	$14.0_{1.0}$	$3.9_{0.2}$	3.60	3.08	1.17	32.4
15%V8	$14.7_{0.9}$	$4.2_{0.2}$	3.50	2.94	1.17	34.0
Mean	$14.5_{0.4}$	$4.5_{0.7}$	3.25	3.02	1.07	33.1
Isolate	*M. brunnea Br 12 P. deltoides* USA					
CDA	$15.2_{1.1}$	$5.6_{0.3}$	2.70	3.00	0.92	33.3
PDA	$15.0_{1.0}$	$5.2_{0.5}$	2.90	3.00	1.00	33.3
PCA	$14.3_{0.8}$	$4.1_{0.5}$	3.50	3.12	1.12	32.0
PC-10	$14.5_{1.0}$	$4.2_{0.3}$	3.45	3.00	1.03	33.3
15%V8	$15.0_{1.2}$	$4.2_{0.3}$	3.60	3.07	1.17	32.5
Mean	$14.8_{0.4}$	$4.7_{0.7}$	3.23	3.04	1.05	32.9

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 13 P. deltoides* USA					
CDA	$15.3_{0.7}$	$5.0_{0.2}$	3.05	2.99	1.02	33.4
PDA	$15.5_{1.1}$	$5.1_{0.2}$	3.01	2.99	1.00	33.4
PCA	$14.5_{1.0}$	$4.0_{0.2}$	3.60	3.16	1.13	31.6
PC-10	$15.0_{1.3}$	$4.0_{0.2}$	3.73	3.13	1.19	32.0
15%V8	$15.1_{1.0}$	$4.1_{0.2}$	3.70	3.02	1.21	33.0
Mean	$15.1_{0.4}$	$4.4_{0.6}$	3.42	3.06	1.11	32.7
Isolate	*M. brunnea Br 14 P. trichocarpa* USA					
CDA	$16.0_{0.8}$	$4.7_{0.2}$	3.40	3.11	1.08	32.1
PDA	$16.2_{1.0}$	$4.6_{0.3}$	3.50	3.01	1.16	33.2
PCA	$14.6_{0.6}$	$3.9_{0.2}$	3.74	3.16	1.18	31.6
PC-10	$15.4_{0.8}$	$3.8_{0.2}$	3.99	3.03	1.31	33.0
15%V8	$15.3_{0.7}$	$4.0_{0.1}$	3.82	3.15	1.21	31.7
Mean	$15.5_{0.6}$	$4.2_{0.4}$	3.69	3.09	1.19	32.3
Isolate	*M. brunnea Br 15 P. deltoides* USA					
CDA	$15.1_{0.7}$	$5.1_{0.2}$	2.96	2.94	1.07	34.0
PDA	$15.5_{0.9}$	$4.8_{0.3}$	3.25	3.03	1.07	33.0
PCA	$14.0_{1.1}$	$4.0_{0.3}$	4.40	3.96	1.12	25.4
PC-10	$14.9_{1.0}$	$4.2_{0.2}$	3.52	3.08	1.14	32.5
15%V8	$14.7_{0.9}$	$4.2_{0.2}$	3.45	3.12	1.10	32.0
Mean	$14.8_{0.5}$	$4.4_{0.4}$	3.52	3.23	1.10	31.4
Isolate	*M. brunnea Br 16 P. Robusta* England					
CDA	$16.1_{1.3}$	$5.8_{0.5}$	2.78	3.26	0.85	30.6
PDA	$15.5_{1.4}$	$5.4_{0.4}$	2.86	3.10	0.92	32.2
PCA	$14.7_{1.4}$	$4.0_{0.4}$	3.63	3.25	1.12	30.8
PC-10	$14.3_{1.1}$	$4.1_{0.3}$	3.49	3.25	1.07	30.8
15%V8	$14.6_{0.8}$	$4.0_{0.1}$	3.63	3.38	1.07	29.5
Mean	$15.0_{0.7}$	$4.7_{0.9}$	3.28	3.25	1.01	30.8

APPENDIX 3c: Influence of culture medium on conidial dimensions of *Marssonina* species following 10 days incubation at 20°C under a 12 hour white light photoperiod.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. Brunnea*	*Br 17*	*P. x Robusta*	Ireland		
CDA	15.3$_{1.4}$	5.3$_{0.4}$	2.88	3.09	0.93	32.3
PDA	15.8$_{1.3}$	5.4$_{0.3}$	2.89	3.14	0.92	31.9
PCA	15.1$_{1.3}$	4.1$_{0.3}$	3.68	3.11	1.18	32.1
PC-10	15.0$_{1.0}$	4.2$_{0.2}$	3.59	3.07	1.17	32.6
15%V8	15.0$_{1.0}$	4.3$_{0.3}$	3.46	3.08	1.12	32.5
Mean	15.2$_{0.3}$	4.7$_{0.6}$	3.30	3.10	1.06	32.3
Isolate	*M. brunnea*	*Br 18*	*P. x euramericana*	France		
CDA	16.0$_{1.2}$	4.8$_{0.3}$	3.30	2.98	1.10	33.5
PDA	16.1$_{1.2}$	4.9$_{0.2}$	3.30	3.00	1.09	33.3
PCA	14.3$_{0.9}$	3.8$_{0.2}$	3.71	2.86	1.30	34.9
PC-10	15.3$_{1.0}$	3.8$_{0.2}$	4.03	2.93	1.37	34.1
15%V8	15.4$_{1.1}$	4.2$_{0.2}$	4.03	3.01	1.34	33.2
Mean	15.4$_{0.7}$	4.2$_{0.6}$	3.67	2.95	1.24	33.8
Isolate	*M. brunnea*	*Br 19*	*P. deltoides*	Holland		
CDA	15.1$_{0.9}$	5.1$_{0.3}$	2.96	2.95	1.00	33.8
PDA	15.1$_{1.1}$	4.8$_{0.3}$	3.12	2.90	1.07	34.5
PCA	14.8$_{1.0}$	4.1$_{0.3}$	3.60	2.96	1.15	32.0
PC-10	14.3$_{0.8}$	4.0$_{0.2}$	3.65	3.10	1.17	32.2
15%V8	14.8$_{1.0}$	4.0$_{0.2}$	3.67	3.13	1.17	32.0
Mean	14.8$_{0.3}$	4.4$_{0.5}$	3.40	3.01	1.11	32.9
Isolate	*M. brunnea*	*Br 20*	*P. x euramericana*	cv. I214	Turkey	
CDA	14.8$_{1.1}$	4.0$_{0.2}$	3.69	3.30	1.12	30.3
PDA	15.2$_{0.9}$	5.2$_{0.4}$	3.00	3.00	1.00	33.2
PCA	14.8$_{0.9}$	4.2$_{0.3}$	3.57	3.23	1.10	31.0
PC-10	15.3$_{0.8}$	4.1$_{0.2}$	3.72	3.25	1.14	30.7
15%V8	15.6$_{1.0}$	4.4$_{0.2}$	3.56	3.14	1.13	31.8
Mean	15.1$_{0.3}$	4.4$_{0.5}$	3.51	3.18	1.10	31.4

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea*	*Br 21*	*P. tremuloides*	USA		
CDA	16.6$_{1.0}$	4.6$_{0.2}$	3.57	2.74	1.30	34.2
PDA	16.4$_{1.0}$	4.7$_{0.3}$	3.43	2.80	1.32	34.0
PCA	15.4$_{1.2}$	3.9$_{0.2}$	3.94	3.09	1.28	32.4
PC-10	15.7$_{0.9}$	3.9$_{0.2}$	4.00	3.00	1.33	33.3
15%V8	15.8$_{1.2}$	3.9$_{0.2}$	4.03	3.05	1.32	32.7
Mean	16.0$_{0.5}$	4.2$_{0.4}$	3.79	2.93	1.31	33.3
Isolate	*M. brunnea*	*Br 22*	*P. tremuloides*	USA		
CDA	16.9$_{1.0}$	4.7$_{0.4}$	3.60	2.64	1.36	37.8
PDA	17.0$_{1.0}$	4.7$_{0.3}$	3.60	2.72	1.31	36.7
PCA	15.3$_{0.7}$	3.9$_{0.2}$	3.92	3.07	1.27	32.5
PC-10	15.5$_{1.0}$	4.0$_{0.2}$	3.90	3.00	1.29	33.2
15%V8	15.7$_{1.1}$	4.0$_{0.2}$	3.93	3.12	1.26	32.0
Mean	15.8$_{1.1}$	4.2$_{0.4}$	3.79	2.91	1.30	34.4
Isolate	*M. brunnea*	*Br 23*	*P. tremuloides*	Alaska		
CDA	16.3$_{1.0}$	4.6$_{0.3}$	3.52	2.77	1.27	36.0
PDA	16.1$_{0.9}$	4.4$_{0.2}$	3.66	2.90	1.26	34.6
PCA	15.6$_{1.3}$	3.8$_{0.2}$	4.05	3.35	1.21	29.8
PC-10	16.0$_{1.2}$	3.9$_{0.2}$	4.03	3.07	1.31	32.6
15%V8	15.6$_{1.1}$	3.9$_{0.2}$	3.95	2.93	1.34	34.0
Mean	15.9$_{0.3}$	4.1$_{0.3}$	3.84	3.00	1.28	33.4
Isolate	*M. brunnea*	*Br 24*	*P. tremuloides*	USA		
CDA	15.9$_{1.0}$	4.9$_{0.3}$	3.26	2.99	1.09	33.4
PDA	15.4$_{0.9}$	4.6$_{0.3}$	3.33	2.97	1.12	33.6
PCA	15.2$_{0.5}$	4.2$_{0.2}$	3.65	3.14	1.16	31.8
PC-10	16.1$_{0.8}$	4.2$_{0.2}$	3.79	3.11	1.20	32.0
15%V8	15.4$_{0.8}$	4.1$_{0.2}$	3.70	3.06	1.22	32.6
Mean	15.6$_{0.4}$	4.4$_{0.3}$	3.54	3.05	1.16	32.7

APPENDIX 3d: Influence of culture medium on conidial dimensions of *Marssonina* species following 10 days incubation at 20°C under a 12 hour white light photoperiod.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. castagnei Cs 1a P. alba* Switzerland					
CDA	27.2±1.6	7.5±0.5	3.63	2.80	1.30	35.6
PDA	27.6±1.5	7.7±0.7	3.50	2.42	1.45	41.2
PCA	22.3±1.4	6.0±0.8	3.67	2.12	1.80	47.0
PC-10	23.7±1.6	6.6±0.7	3.60	2.30	1.56	43.6
15%V8	25.6±1.5	6.3±0.5	4.06	2.24	1.80	44.6
Mean	25.1±2.0	6.8±0.7	3.69	2.38	1.58	42.4
Isolate	*M. castagnei Cs 1b P. alba* Switzerland					
CDA	23.0±1.5	7.1±0.5	3.21	2.42	1.32	41.2
PDA	22.2±1.4	7.0±0.7	3.15	2.36	1.34	42.4
PCA	20.6±1.6	6.0±0.7	3.40	2.24	1.51	44.6
PC-10	20.8±1.5	5.8±0.6	3.60	2.26	1.59	44.2
15%V8	23.0±1.5	6.4±0.7	3.62	2.34	1.54	42.7
Mean	22.0±1.1	6.5±0.5	3.40	2.32	1.46	43.0
Isolate	*M. castagnei Cs 2 P. alba* Ireland					
CDA	18.0±1.4	6.2±0.5	2.90	2.88	1.12	37.0
PDA	17.4±1.2	6.0±0.5	2.87	2.64	1.09	37.8
PCA	18.2±1.3	6.1±0.4	3.00	2.86	1.05	34.9
PC-10	17.7±1.1	5.9±0.4	3.00	2.76	1.09	36.2
15%V8	17.6±1.2	6.0±0.3	2.91	2.63	1.10	37.9
Mean	17.8±0.3	6.0±0.1	2.94	2.75	1.09	36.8

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. castagnei Cs 3 P. alba* Turkey					
CDA	18.9±1.3	5.6±0.4	3.35	2.39	1.40	41.8
PDA	17.6±1.0	5.9±0.4	2.88	2.55	1.13	39.2
PCA	18.8±1.1	5.5±0.3	3.39	2.52	1.34	39.6
PC-10	19.3±1.1	5.4±0.3	3.56	2.55	1.40	39.2
15%V8	20.0±1.0	5.9±0.3	3.36	2.61	1.29	38.3
Mean	18.9±0.9	5.6±0.2	3.31	2.52	1.31	39.6
Isolate	*M. populi Po 1 M. canadensis* England					
CDA	18.0±1.3	6.0±0.4	2.97	3.04	0.98	32.9
PDA	18.0±1.2	6.0±0.4	3.00	3.10	0.97	32.3
PCA	18.2±1.4	6.0±0.4	3.10	3.44	0.90	29.0
PC-10	18.4±1.3	5.7±0.3	3.24	3.35	0.96	30.0
15%V8	18.6±1.0	6.0±0.4	3.14	3.31	0.95	30.2
Mean	18.2±0.3	5.9±0.1	3.09	3.25	0.95	30.9
Isolate	*M. populi Po 2 P. nigra* England					
CDA	19.5±1.0	6.9±0.4	2.81	3.04	0.92	32.8
PDA	20.0±1.1	7.1±0.4	2.80	3.33	0.83	30.3
PCA	19.1±1.3	5.9±0.3	3.24	3.50	0.93	28.6
PC-10	20.0±1.3	6.1±0.4	3.26	3.27	1.00	30.5
15%V8	19.3±1.0	6.0±0.3	3.21	3.54	0.90	28.2
Mean	19.6±0.4	6.4±0.5	3.06	3.34	0.92	30.1

APPENDIX 3e: Influence of culture medium on conidial dimensions of *Marssonina* species following 10 days incubation at 20°C under a 12 hour white light photoperiod.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. populi* *Po 3* *P. nigra* England					
CDA	$19.6_{1.2}$	$6.6_{0.4}$	2.97	3.48	0.85	28.7
PDA	$20.3_{1.3}$	$7.0_{0.3}$	2.91	3.63	0.80	27.5
PCA	$20.3_{1.2}$	$5.6_{0.3}$	3.60	3.71	0.96	26.9
PC-10	$20.7_{1.1}$	$5.8_{0.3}$	3.53	3.46	1.02	28.9
15%V8	$20.9_{1.2}$	$6.4_{0.3}$	3.27	3.84	0.85	26.1
Mean	$20.4_{0.5}$	$6.3_{0.6}$	3.25	3.62	0.89	27.6
Isolate	*M. populi* *Po 4* *P. nigra* Ireland					
CDA	$19.6_{1.1}$	$6.7_{0.3}$	2.91	3.06	0.95	32.6
PDA	$20.4_{1.2}$	$6.6_{0.3}$	3.05	3.38	0.90	29.6
PCA	$18.8_{1.2}$	$5.7_{0.3}$	3.27	3.34	0.98	29.9
PC-10	$20.2_{1.3}$	$6.0_{0.2}$	3.34	3.50	0.96	28.6
15%V8	$21.3_{1.2}$	$5.7_{0.3}$	3.70	3.74	0.98	26.7
Mean	$20.0_{0.9}$	$6.1_{0.5}$	3.25	3.40	0.95	29.5
Isolate	*M. populi* *Po 5* *P.x berolinensis* Germany					
CDA	$24.7_{1.2}$	$6.3_{0.3}$	3.92	3.22	1.21	31.0
PDA	$25.7_{1.4}$	$6.7_{0.4}$	3.85	2.94	1.31	34.0
PCA	$28.3_{1.3}$	$6.5_{0.4}$	4.37	3.18	1.37	31.4
PC-10	$24.4_{0.9}$	$6.2_{0.3}$	3.94	3.00	1.31	33.4
15%V8	$24.0_{1.4}$	$6.2_{0.4}$	3.87	2.93	1.36	34.0
Mean	$25.4_{1.7}$	$6.2_{0.4}$	3.99	3.05	1.31	32.8

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. populi* *Po 6* *P. nigra* Switzerland					
CDA	$21.3_{1.5}$	$7.1_{0.7}$	3.00	3.10	1.00	32.2
PDA	$21.5_{1.4}$	$6.8_{0.6}$	3.15	3.10	1.03	32.2
PCA	$22.2_{1.5}$	$6.5_{0.5}$	3.54	3.62	1.04	27.6
PC-10	$20.3_{1.7}$	$5.1_{0.6}$	3.95	3.71	1.07	27.0
15%V8	$20.0_{1.6}$	$5.4_{0.5}$	3.71	3.63	1.02	27.5
Mean	$21.0_{0.9}$	$6.2_{0.9}$	3.47	3.43	1.03	29.3
Isolate	*M. populi* *Po 7* *P. nigra* Switzerland					
CDA	$21.1_{1.3}$	$6.4_{0.4}$	3.30	3.05	1.07	32.8
PDA	$23.3_{1.4}$	$6.6_{0.5}$	3.33	2.96	1.14	33.7
PCA	$21.0_{1.5}$	$5.7_{0.3}$	3.67	3.58	1.02	27.8
PC-10	$21.0_{1.4}$	$5.5_{0.4}$	3.83	3.48	1.10	28.7
15%V8	$21.0_{1.4}$	$6.0_{0.3}$	3.52	3.42	1.03	29.2
Mean	$21.5_{1.0}$	$6.0_{0.4}$	3.54	3.30	1.07	30.4

APPENDIX 4a: Influence of media pH on conidial dimensions of *Mareaenina* species following 10 days incubation on 15%V8 at 20°C under a 12 hour white light photoperiod.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 1 P. yunnanensis* NZ					
pH 5.5	$16.0_{1.1}$	$4.1_{0.3}$	3.90	2.86	1.35	34.9
6.0	$15.3_{1.3}$	$4.1_{0.2}$	3.72	2.82	1.31	35.4
6.5	$15.9_{1.4}$	$3.9_{0.3}$	4.05	2.86	1.40	34.8
7.0	$15.3_{1.3}$	$4.0_{0.3}$	3.80	2.90	1.30	34.4
7.5	$14.8_{1.0}$	$4.1_{0.3}$	3.60	2.86	1.26	34.9
Mean	$15.5_{0.5}$	$4.0_{0.09}$	3.81	2.86	1.32	34.9
Isolate	*M. brunnea Br 2 P.* x *euramericana* NZ					
pH 5.5	$14.0_{1.3}$	$4.3_{0.4}$	3.25	2.80	1.18	35.0
6.0	$16.0_{1.1}$	$4.3_{0.4}$	3.72	2.90	1.25	34.4
6.5	$14.9_{1.3}$	$4.0_{0.5}$	3.70	2.90	0.98	31.4
7.0	$14.4_{1.0}$	$4.3_{0.6}$	3.40	2.80	1.22	35.0
7.5	$14.6_{1.1}$	$4.3_{0.4}$	3.35	3.10	1.08	32.0
Mean	$14.8_{0.7}$	$4.2_{0.1}$	3.48	2.90	1.14	33.6
Isolate	*M. brunnea Br 3 P. fremontii* cv. 61/48 NZ					
pH 5.5	$14.9_{1.2}$	$4.0_{0.2}$	3.80	3.10	1.22	32.2
6.0	$15.4_{1.0}$	$4.0_{0.2}$	3.80	3.00	1.22	33.3
6.5	$14.2_{0.9}$	$3.8_{0.2}$	3.67	3.20	1.16	31.2
7.0	$14.6_{0.8}$	$4.0_{0.2}$	3.65	2.80	1.30	35.6
7.5	$14.8_{0.9}$	$4.0_{0.2}$	3.70	3.00	1.22	33.3
Mean	$14.8_{0.4}$	$4.0_{0.1}$	3.72	3.02	1.22	33.1
Isolate	*M. brunnea Br 4 P. fremontii* x *P. nigra* cv. 66/9 NZ					
pH 5.5	$15.7_{1.0}$	$4.0_{0.2}$	3.96	3.03	1.33	32.9
6.0	$15.6_{1.0}$	$3.9_{0.2}$	3.96	2.95	1.35	34.0
6.5	$15.8_{1.0}$	$3.9_{0.2}$	4.00	2.93	1.38	34.0
7.0	$15.7_{0.9}$	$3.9_{0.1}$	3.97	2.96	1.38	33.7
7.5	$15.0_{1.0}$	$3.9_{0.1}$	3.80	2.98	1.28	33.5
Mean	$15.5_{0.3}$	$3.9_{0.04}$	3.94	2.97	1.34	33.6

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 5 P.* x *euramericana* cv. I-214 NZ					
pH 5.5	$15.6_{1.0}$	$4.3_{0.3}$	3.66	2.92	1.25	34.3
6.0	$15.5_{1.2}$	$4.2_{0.3}$	3.69	2.92	1.24	34.2
6.5	$15.4_{1.1}$	$4.2_{0.2}$	3.65	2.94	1.24	34.0
7.0	$15.6_{1.3}$	$4.2_{0.2}$	3.72	2.87	1.24	33.6
7.5	$15.3_{1.0}$	$4.2_{0.3}$	3.64	2.99	1.21	33.3
Mean	$15.5_{0.1}$	$4.2_{0.04}$	3.67	2.93	1.24	33.9
Isolate	*M. brunnea Br 6 P. alba* NZ					
pH 5.5	$17.4_{1.3}$	$4.2_{0.2}$	4.13	3.07	1.34	32.5
6.0	$17.3_{1.2}$	$4.1_{0.2}$	4.21	3.14	1.33	32.0
6.5	$17.2_{1.3}$	$4.1_{0.1}$	4.15	3.05	6.35	32.7
7.0	$17.0_{1.1}$	$4.2_{0.2}$	4.06	3.06	1.32	32.7
7.5	$17.0_{1.3}$	$4.1_{0.1}$	4.13	2.96	1.39	33.7
Mean	$17.2_{0.2}$	$4.1_{0.05}$	4.14	3.06	1.35	32.7
Isolate	*M. brunnea Br P.* x *euramericana* cv. Flevo NZ					
pH 5.5	$15.2_{0.7}$	$4.0_{0.2}$	3.77	3.13	1.20	32.0
6.0	$15.2_{1.1}$	$4.0_{0.2}$	3.74	3.04	1.23	33.0
6.5	$15.0_{0.7}$	$4.1_{0.2}$	3.63	3.01	1.20	33.2
7.0	$15.1_{1.0}$	$4.0_{0.2}$	3.74	3.04	1.23	32.8
7.5	$15.3_{0.9}$	$4.0_{0.2}$	3.77	3.04	1.24	32.8
Mean	$15.2_{0.1}$	$4.0_{0.04}$	3.73	3.05	1.22	32.8
Isolate	*M. brunnea Br 8 P.* x *euramericana* cv. NL 2194 NZ					
pH 5.5	$17.2_{1.6}$	$4.0_{0.2}$	4.32	3.06	1.41	32.6
6.0	$17.0_{1.5}$	$4.0_{0.2}$	4.20	2.96	1.42	33.7
6.5	$17.0_{1.5}$	$4.0_{0.2}$	4.22	2.97	1.42	33.6
7.0	$17.5_{1.5}$	$4.1_{0.2}$	4.30	3.01	1.42	33.2
7.5	$17.0_{1.6}$	$4.0_{0.1}$	4.20	2.91	1.44	34.3
Mean	$17.1_{0.2}$	$4.0_{0.04}$	4.25	2.98	1.42	33.5

APPENDIX 4b: Influence of media pH on conidial dimensions of *Marssonina* species following 10 days incubation on 15%V8 at 20°C under a 12 hour white light photoperiod.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 9 P. delt.* x *P. maxi.* cv. I83/58 NZ					
pH 5.5	15.0±0.9	4.0±0.2	3.37	2.76	1.22	36.2
6.0	15.4±1.2	4.0±0.2	3.90	3.05	1.27	32.7
6.5	15.3±1.0	4.0±0.2	3.81	3.07	1.24	32.5
7.0	15.6±1.0	4.0±0.1	3.90	3.10	1.26	32.2
7.5	15.3±1.0	4.0±0.2	3.80	3.08	1.23	32.4
Mean	15.3±0.2	4.0±0.0	3.76	3.01	1.24	33.2
Isolate	*M. brunnea Br 10 P. deltoides* USA					
pH 5.5	14.0±0.5	4.2±0.2	3.36	3.02	1.13	33.1
6.0	14.0±0.6	4.2±0.3	3.36	3.00	1.14	33.0
6.5	14.5±1.0	4.3±0.2	3.40	3.01	1.12	33.2
7.0	14.3±0.7	4.0±0.3	3.70	3.00	1.21	33.0
7.5	14.0±0.6	4.1±0.2	3.41	3.00	1.12	33.0
Mean	14.2±0.2	4.2±0.1	3.45	3.01	1.14	33.1
Isolate	*M. brunnea Br 11 P. deltoides* USA					
pH 5.5	14.1±1.0	4.2±0.3	3.36	3.00	1.14	33.3
6.0	14.5±0.7	4.0±0.3	3.60	3.24	1.10	30.8
6.5	14.1±0.9	4.0±0.2	3.50	2.95	1.18	33.8
7.0	14.3±0.7	4.0±0.3	3.60	3.06	1.16	32.4
7.5	14.2±0.9	4.0±0.3	3.50	2.83	1.23	35.2
Mean	14.2±0.1	4.0±0.1	3.51	3.02	1.16	33.1
Isolate	*M. brunnea Br 12 P. deltoides* USA					
pH 5.5	14.5±1.1	4.0±0.2	3.62	3.12	1.15	32.0
6.0	15.0±1.0	4.1±0.3	3.65	3.16	1.16	31.6
6.5	15.0±1.0	4.1±0.2	3.65	3.23	1.13	31.0
7.0	14.8±1.0	4.0±0.2	3.70	3.06	1.20	33.6
7.5	14.7±0.8	4.1±0.2	3.60	3.13	1.15	32.0
Mean	14.8±0.2	4.1±0.05	3.64	3.14	1.16	32.0

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 13 P. deltoides* USA					
pH 5.5	15.3±0.8	4.0±0.2	3.75	3.14	1.19	31.9
6.0	15.0±0.9	4.0±0.2	3.70	3.04	1.21	32.8
6.5	14.7±0.9	4.0±0.2	3.70	3.06	1.20	32.7
7.0	15.0±0.7	4.0±0.2	3.72	3.11	1.19	32.1
7.5	14.8±0.6	4.0±0.3	3.70	3.14	1.17	31.8
Mean	14.9±0.2	4.0±0.0	3.71	3.10	1.19	32.3
Isolate	*M. brunnea Br 14 P. trichocarpa* USA					
pH 5.5	15.2±1.0	3.9±0.2	3.90	2.96	1.31	33.8
6.0	15.3±0.7	3.8±0.2	4.08	3.37	1.20	29.6
6.5	15.3±0.8	3.9±0.3	3.90	3.07	1.26	32.5
7.0	15.5±0.8	4.0±0.1	3.90	3.14	1.24	31.8
7.5	15.4±0.7	4.0±0.2	3.92	3.03	1.29	33.0
Mean	15.3±0.1	3.9±0.1	3.94	3.11	1.26	32.1
Isolate	*M. brunnea Br 15 P. deltoides* USA					
pH 5.5	15.0±0.9	4.2±0.2	3.52	3.00	1.17	33.3
6.0	14.9±0.8	4.3±0.2	3.47	2.98	1.16	33.5
6.5	14.7±0.9	4.2±0.2	3.45	3.12	1.10	32.0
7.0	14.5±0.8	4.2±0.2	3.45	2.95	1.17	33.9
7.5	14.9±0.8	4.3±0.2	3.48	2.99	1.16	33.4
Mean	14.8±0.2	4.2±0.05	3.47	3.00	1.15	33.2
Isolate	*M. brunnea Br 16 P. Robusta* England					
pH 5.5	14.5±0.6	4.0±0.2	3.58	3.19	1.12	31.3
6.0	14.7±0.9	4.1±0.2	3.59	3.18	1.13	31.3
6.5	14.6±0.7	4.0±0.1	3.63	3.38	1.07	29.5
7.0	15.0±0.8	4.1±0.2	3.67	3.17	1.15	31.5
7.5	14.8±0.7	3.9±0.2	3.75	3.24	1.15	30.8
Mean	14.7±0.2	4.0±0.08	3.64	3.23	1.12	30.9

APPENDIX 4c: Influence of media pH on conidial dimensions of *Marssonina* species following 10 days incubation on 15%V8 at 20°C under a 12 hour white light photoperiod.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 17 P. Robusta* Ireland					
pH 5.5	$14.6_{0.7}$	$4.3_{0.2}$	3.40	3.19	1.06	31.3
6.0	$14.4_{0.8}$	$4.2_{0.3}$	3.44	3.08	1.12	32.5
6.5	$15.0_{1.0}$	$4.3_{0.3}$	3.46	3.08	1.12	32.5
7.0	$14.9_{1.0}$	$4.3_{0.3}$	3.30	3.01	1.09	33.2
7.5	$14.8_{0.8}$	$4.4_{0.3}$	3.30	3.04	1.09	32.8
Mean	$14.7_{0.2}$	$4.3_{0.07}$	3.38	3.08	1.10	32.5
Isolate	*M. brunnea Br 18 P.* x *euramericana* France					
pH 5.5	15.4	$3.8_{0.2}$	4.06	2.96	1.37	33.7
6.0	15.1	$3.8_{0.2}$	3.97	3.10	1.28	32.2
6.5	15.3	$3.8_{0.2}$	4.03	3.00	1.34	33.4
7.0	15.4	$3.8_{0.2}$	4.08	3.03	1.34	33.0
7.5	15.4	$3.8_{0.2}$	4.03	3.03	1.33	33.0
Mean	$15.3_{0.1}$	$3.8_{0.0}$	4.03	3.02	1.33	33.1
Isolate	*M. brunnea Br 19 P. deltoides* Holland					
pH 5.5	$14.9_{1.0}$	$4.0_{0.2}$	3.68	3.18	1.15	31.4
6.0	$14.8_{0.8}$	$4.0_{0.1}$	3.73	3.10	1.21	32.4
6.5	$14.4_{0.7}$	$4.1_{0.2}$	3.54	3.10	1.14	32.4
7.0	$15.0_{1.0}$	$4.0_{0.2}$	3.71	3.14	1.18	32.0
7.5	$14.8_{0.8}$	$4.0_{0.2}$	3.70	3.14	1.17	31.8
Mean	$14.8_{0.2}$	$4.0_{0.04}$	3.67	3.13	1.17	32.0
Isolate	*M. brunnea Br 20 P.* x *euramericana* cv. I214 Turkey					
pH 5.5	$14.9_{0.7}$	$4.2_{0.2}$	3.53	3.13	1.13	31.9
6.0	$15.3_{0.8}$	$4.3_{0.2}$	3.57	3.08	1.16	32.4
6.5	$15.6_{1.0}$	$4.4_{0.2}$	3.56	3.14	1.13	31.8
7.0	$15.5_{0.9}$	$4.4_{0.2}$	3.56	3.06	1.16	32.6
7.5	$15.5_{0.8}$	$4.4_{0.2}$	3.55	3.12	1.13	32.0
Mean	$15.4_{0.28}$	$4.3_{0.1}$	3.55	3.10	1.14	32.1

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 21 P. tremuloides* USA					
pH 5.5	$15.9_{1.0}$	$3.9_{0.2}$	4.04	2.98	1.35	33.6
6.0	$15.9_{1.0}$	$3.9_{0.1}$	4.04	2.97	1.36	33.6
6.5	$15.8_{1.0}$	$3.9_{0.1}$	4.00	3.00	1.33	33.3
7.0	$16.0_{0.9}$	$3.9_{0.2}$	4.09	3.06	1.33	32.7
7.5	$15.9_{1.0}$	$3.9_{0.1}$	4.08	3.01	1.35	33.2
Mean	$15.9_{0.07}$	$3.9_{0.0}$	4.05	3.00	1.34	33.3
Isolate	*M. brunnea Br 22 P. tremuloides* USA					
pH 5.5	$15.8_{1.1}$	$4.0_{0.3}$	3.94	3.09	1.27	32.4
6.0	$16.0_{0.8}$	$3.9_{0.2}$	4.06	2.92	1.40	34.2
6.5	$15.4_{1.0}$	$3.9_{0.3}$	3.85	3.09	1.24	32.3
7.0	$15.4_{0.8}$	$3.9_{0.2}$	3.95	3.04	1.30	32.8
7.5	$15.4_{1.0}$	$3.9_{0.2}$	3.94	3.08	1.28	32.5
Mean	$15.6_{0.3}$	$3.9_{0.04}$	3.95	3.04	1.30	32.8
Isolate	*M. brunnea Br 23 P. tremuloides* Alaska					
pH 5.5	$15.9_{1.2}$	$3.9_{0.2}$	4.03	3.00	1.35	33.4
6.0	$15.7_{1.3}$	$3.9_{0.2}$	4.04	2.92	1.38	34.2
6.5	$15.3_{1.0}$	$4.0_{0.2}$	3.80	3.03	1.25	33.0
7.0	$15.3_{1.1}$	$3.9_{0.2}$	3.90	3.02	1.28	33.0
7.5	$15.5_{1.0}$	$3.9_{0.1}$	3.95	2.93	1.34	34.1
Mean	$15.5_{0.3}$	$3.9_{0.04}$	3.94	2.98	1.32	33.5
Isolate	*M. brunnea Br 24 P. tremuloides* USA					
pH 5.5	$15.8_{1.0}$	$4.2_{0.2}$	3.83	3.08	1.23	32.4
6.0	$16.1_{1.0}$	$4.1_{0.2}$	3.91	3.16	1.24	31.6
6.5	$16.3_{0.8}$	$4.1_{0.2}$	3.96	2.97	1.33	33.6
7.0	$16.4_{0.8}$	$4.1_{0.2}$	4.00	3.14	1.27	31.8
7.5	$16.0_{1.1}$	$4.1_{0.2}$	3.90	3.07	1.26	32.5
Mean	$16.1_{0.24}$	$4.1_{0.0}$	3.92	3.08	1.27	32.4

APPENDIX 4d: Influence of media pH on conidial dimensions of *Marssonina* species following 10 days incubation on 15%V8 at 20°C under a 12 hour white light photoperiod.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. castagnei* *Cs 1a* *P. alba* Switzerland					
pH 5.5	$23.0_{1.4}$	$6.5_{0.8}$	3.54	2.30	1.46	43.4
6.0	$24.5_{1.4}$	$6.4_{0.6}$	3.82	2.30	1.70	43.8
6.5	$25.0_{1.5}$	$6.2_{0.7}$	4.00	2.21	1.81	45.1
7.0	$24.7_{1.4}$	$6.3_{0.5}$	3.92	2.31	1.68	43.2
7.5	$24.1_{1.5}$	$6.6_{0.5}$	3.63	2.35	1.54	45.5
Mean	$24.2_{0.8}$	$6.4_{0.16}$	3.78	2.29	1.64	44.2
Isolate	*M. castagnei* *Cs 1b* *P. alba* Switzerland					
pH 5.5	$22.8_{1.4}$	$6.2_{0.7}$	3.67	2.27	1.62	44.0
6.0	$22.9_{1.5}$	$6.4_{0.6}$	3.58	2.29	1.56	43.6
6.5	$22.8_{1.4}$	$6.4_{0.5}$	3.56	2.37	1.50	42.2
7.0	$23.0_{1.4}$	$6.3_{0.5}$	3.63	2.26	1.60	44.3
7.5	$23.0_{1.5}$	$6.5_{0.6}$	3.52	2.26	1.56	44.2
Mean	$22.9_{0.1}$	$6.3_{0.1}$	3.59	2.29	1.57	43.6
Isolate	*M. castagnei* *Cs 2* *P. alba* Ireland					
pH 5.5	$18.5_{1.0}$	$6.1_{0.4}$	3.01	2.44	1.23	40.9
6.0	$18.5_{1.2}$	$5.9_{0.4}$	3.14	2.56	1.22	39.0
6.5	$18.0_{1.2}$	$5.8_{0.5}$	3.11	2.52	1.23	39.6
7.0	$18.6_{1.2}$	$5.7_{0.4}$	3.24	2.50	1.30	40.0
7.5	$17.5_{1.1}$	$6.0_{0.4}$	2.91	2.61	1.11	38.3
Mean	$18.2_{0.46}$	$5.9_{0.16}$	3.08	2.53	1.22	39.6

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. castagnei* *Cs 3* *P. alba* Turkey					
pH 5.5	$19.6_{1.1}$	$5.9_{0.3}$	3.32	2.66	1.25	37.5
6.0	$19.4_{1.2}$	$5.8_{0.3}$	3.31	2.73	1.29	36.3
6.5	$20.0_{1.0}$	$5.9_{0.3}$	3.36	2.61	1.29	38.3
7.0	$20.2_{1.2}$	$5.8_{0.2}$	3.50	2.47	1.41	40.5
7.5	$20.1_{1.0}$	$5.9_{0.3}$	3.39	2.64	1.28	37.8
Mean	$19.9_{0.3}$	$5.8_{0.05}$	3.38	2.62	1.30	38.1
Isolate	*M. populi* *Po 1* *P. canadensis* England					
pH 5.5	$18.4_{1.1}$	$6.0_{0.4}$	3.10	3.23	0.95	31.0
6.0	$18.6_{1.3}$	$6.0_{0.4}$	3.12	3.33	0.93	30.0
6.5	$18.3_{1.1}$	$6.0_{0.4}$	3.10	3.30	0.92	30.2
7.0	$18.6_{1.2}$	$6.0_{0.3}$	3.10	3.22	0.95	31.0
7.5	$18.6_{1.1}$	$6.0_{0.3}$	3.10	3.30	0.94	30.2
Mean	$18.5_{0.1}$	$6.0_{0.0}$	3.10	3.28	0.94	30.5
Isolate	*M. populi* *Po 2* *P. nigra* England					
pH 5.5	$20.0_{1.1}$	$6.3_{0.3}$	3.15	3.40	0.92	29.4
6.0	$19.6_{0.9}$	$6.0_{0.3}$	3.15	3.57	0.88	28.0
6.5	$19.3_{0.9}$	$6.0_{0.2}$	3.21	3.54	0.90	28.2
7.0	$19.6_{1.1}$	$6.0_{0.3}$	3.26	3.48	0.94	28.7
7.5	$19.3_{1.1}$	$6.0_{0.3}$	3.19	3.36	0.95	29.7
Mean	$19.5_{0.29}$	$6.1_{0.1}$	3.19	3.47	0.92	28.8

APPENDIX 4e: Influence of media pH on conidial dimensions of *Marssonina* species following 10 days incubation on 15%V8 at 20°C under a 12 hour white light photoperiod.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. populi* Po 3 *P. nigra* England					
pH 5.5	$20.8_{1.0}$	$6.2_{0.3}$	3.34	3.97	0.84	25.2
6.0	$20.7_{1.2}$	$6.3_{0.3}$	3.24	3.78	0.86	26.4
6.5	$20.9_{1.3}$	$6.4_{0.3}$	3.27	3.84	0.85	26.1
7.0	$21.5_{1.0}$	$6.3_{0.4}$	3.40	3.81	0.90	26.2
7.5	$21.2_{1.0}$	$6.4_{0.3}$	3.31	4.00	0.83	25.0
Mean	$21.0_{0.3}$	$6.3_{0.08}$	3.31	3.88	0.85	25.8
Isolate	*M. populi* Po 4 *P. nigra* Ireland					
pH 5.5	$21.0_{1.1}$	$5.7_{0.3}$	3.65	3.53	1.03	28.3
6.0	$21.5_{1.2}$	$5.7_{0.3}$	3.76	3.60	1.04	27.8
6.5	$21.3_{1.2}$	$5.7_{0.3}$	3.70	3.74	1.00	26.7
7.0	$21.2_{1.2}$	$5.7_{0.3}$	3.73	3.70	1.01	27.0
7.5	$21.4_{1.1}$	$5.5_{0.3}$	3.85	3.56	1.08	28.0
Mean	$21.3_{0.2}$	$5.7_{0.09}$	3.74	3.62	1.03	27.6
Isolate	*M. populi* Po 5 *P.* x *berolinensis* Germany					
pH 5.5	$22.3_{1.4}$	$5.8_{0.3}$	4.15	2.93	1.42	34.0
6.0	$23.9_{1.2}$	$6.2_{0.3}$	3.85	2.85	1.35	35.0
6.5	$24.1_{1.3}$	$6.1_{0.4}$	3.93	2.85	1.38	35.0
7.0	$24.0_{1.2}$	$6.2_{0.4}$	3.87	2.93	1.32	34.0
7.5	$23.9_{1.1}$	$6.0_{0.3}$	3.94	3.04	1.30	32.9
Mean	$23.6_{0.7}$	$6.0_{0.2}$	3.95	2.92	1.35	34.2

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. populi* Po 6 *P. nigra* Switzerland					
pH 5.5	$20.1_{1.5}$	$5.9_{0.5}$	3.40	3.83	0.92	26.1
6.0	$20.6_{1.5}$	$5.7_{0.4}$	3.61	3.53	1.01	28.3
6.5	$19.7_{1.0}$	$5.8_{0.5}$	3.40	3.52	0.96	28.3
7.0	$20.7_{1.5}$	$5.5_{0.5}$	3.75	3.52	1.09	28.4
7.5	$20.1_{1.4}$	$5.5_{0.5}$	3.61	3.41	1.06	29.3
Mean	$20.2_{0.4}$	$5.7_{0.2}$	3.55	3.56	1.01	28.1
Isolate	*M. populi* Po 7 *P. nigra* Switzerland					
pH 5.5	$20.4_{1.3}$	$5.8_{0.5}$	3.52	3.51	1.00	28.5
6.0	$20.8_{1.3}$	$5.8_{0.4}$	3.60	3.57	1.00	28.0
6.5	$20.6_{1.3}$	$5.7_{0.4}$	3.62	3.53	1.03	28.3
7.0	$20.3_{1.2}$	$5.7_{0.3}$	3.60	3.37	1.06	29.6
7.5	$20.2_{1.2}$	$5.6_{0.3}$	3.60	3.33	1.08	30.0
Mean	$20.4_{0.2}$	$5.7_{0.1}$	3.59	3.46	1.03	28.9

APPENDIX 5a: Influence of incubation temperature on conidial dimensions of *Marssonina* species following 10 days incubation on 15%V8, pH 6.5 under a 12 hour white light photoperiod.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea* Br 1 *P. yunnanensis* NZ					
12 °C	$17.3_{1.3}$	$3.9_{0.3}$	4.44	2.86	1.55	34.9
16 °C	$16.3_{1.4}$	$4.3_{0.2}$	3.80	2.86	1.30	34.9
20 °C	$15.4_{1.3}$	$4.1_{0.3}$	3.80	2.90	1.30	34.4
24 °C	$15.5_{1.3}$	$4.2_{0.3}$	3.70	2.82	1.31	35.4
28 °C	$14.1_{1.5}$	$4.2_{0.3}$	3.45	2.93	1.19	3.40
Mean	$15.7_{1.2}$	$4.1_{0.1}$	3.84	2.87	1.33	34.7
Isolate	*M. brunnea* Br 2 *P.* x *euramericana* NZ					
12 °C	$15.1_{1.0}$	$4.0_{0.4}$	3.77	2.90	1.25	34.4
16 °C	$15.3_{1.4}$	$4.0_{0.4}$	3.82	3.00	1.26	33.5
20 °C	$14.4_{1.3}$	$4.0_{0.3}$	3.60	3.00	1.20	33.2
24 °C	$14.9_{1.3}$	$4.0_{0.4}$	3.72	2.90	1.25	34.4
28 °C	$13.8_{1.0}$	$4.3_{0.4}$	3.20	2.84	1.13	35.1
Mean	$14.7_{0.6}$	$4.1_{0.1}$	3.62	2.93	1.22	34.1
Isolate	*M. brunnea* Br 3 *P. fremontii* cv. 61/93NZ					
12 °C	$16.4_{1.1}$	$4.1_{0.3}$	3.98	3.00	1.33	33.3
16 °C	$16.5_{1.0}$	$3.9_{0.2}$	4.20	3.06	1.37	32.6
20 °C	$15.7_{0.7}$	$4.0_{0.2}$	3.90	3.03	1.24	32.9
24 °C	$15.8_{0.8}$	$4.1_{0.2}$	3.85	2.94	1.31	34.0
28 °C	$15.3_{1.0}$	$3.9_{0.2}$	3.95	3.02	1.30	33.0
Mean	$15.9_{0.5}$	$4.0_{0.1}$	3.97	3.01	1.31	33.2
Isolate	*M. brunnea* Br 4 *P. fremontii* x *P. nigra* cv.66/9 NZ					
12 °C	$15.6_{1.1}$	$4.0_{0.2}$	3.93	2.95	1.33	33.9
16 °C	$15.4_{0.8}$	$4.0_{0.2}$	3.80	3.05	1.27	32.7
20 °C	$15.4_{0.8}$	$4.0_{0.2}$	3.87	3.05	1.25	32.7
24 °C	$15.8_{1.0}$	$3.9_{0.1}$	4.00	2.93	1.38	34.0
28 °C	$15.7_{1.0}$	$3.8_{0.2}$	4.10	2.87	1.42	34.7
Mean	$15.6_{0.2}$	$3.9_{0.1}$	3.94	2.97	1.33	33.6

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea* Br 5 *P.* x *euramericana* cv. I-214 NZ					
12 °C	$15.2_{1.4}$	$4.2_{0.2}$	3.62	3.02	1.20	33.1
16 °C	$15.1_{0.8}$	$4.1_{0.2}$	3.64	2.97	1.22	33.6
20 °C	$15.2_{1.3}$	$4.2_{0.2}$	3.60	3.07	1.17	32.5
24 °C	$15.3_{1.5}$	$4.2_{0.2}$	3.64	2.94	1.23	34.0
28 °C	$15.3_{1.0}$	$4.2_{0.2}$	3.64	2.95	1.24	33.8
Mean	$15.2_{0.03}$	$4.2_{0.04}$	3.63	2.99	1.21	33.4
Isolate	*M. brunnea* Br 6 *P. alba* NZ					
12 °C	$17.4_{1.0}$	$4.1_{0.2}$	4.20	3.00	1.40	33.3
16 °C	$17.4_{1.5}$	$4.1_{0.2}$	4.21	2.94	1.43	33.9
20 °C	$17.0_{1.0}$	$4.1_{0.2}$	4.24	3.00	1.41	33.3
24 °C	$17.0_{1.3}$	$4.0_{0.2}$	4.15	2.98	1.39	33.5
28 °C	$16.8_{1.3}$	$4.1_{0.2}$	4.09	2.93	1.39	34.1
Mean	$17.1_{0.3}$	$4.1_{0.2}$	4.18	2.97	1.40	33.6
Isolate	*M. brunnea* Br 7 *P.* x *euramericana* cv. Flevo NZ					
12 °C	$14.9_{0.8}$	$4.0_{0.2}$	3.67	3.11	1.20	32.2
16 °C	$15.4_{1.0}$	$4.0_{0.2}$	3.80	3.00	1.27	33.4
20 °C	$15.1_{0.7}$	$4.0_{0.2}$	3.77	2.75	1.22	36.3
24 °C	$14.7_{1.1}$	$4.0_{0.3}$	3.68	3.11	1.18	32.2
28 °C	$14.4_{1.0}$	$4.0_{0.2}$	3.61	3.13	1.15	32.0
Mean	$14.9_{0.4}$	$4.0_{0.0}$	3.70	3.02	1.20	33.2
Isolate	*M. brunnea* Br 8 *P.* x *euramericana* cv. NL 2194 NZ					
12 C	$17.3_{1.5}$	$4.0_{0.2}$	4.25	3.10	1.37	32.2
16 C	$17.6_{1.6}$	$4.1_{0.2}$	4.25	3.02	1.41	33.1
20 C	$17.4_{1.5}$	$4.1_{0.3}$	4.20	2.95	1.42	33.9
24 C	$17.4_{1.2}$	$4.1_{0.2}$	4.24	3.00	1.41	33.4
28 C	$17.4_{1.1}$	$4.2_{0.2}$	4.14	3.00	1.38	33.3
Mean	$17.4_{0.1}$	$4.1_{0.07}$	4.22	3.01	1.40	33.2

APPENDIX 5b: Influence of incubation temperature on conidial dimensions of *Marssonina* species following 10 days incubation on 15%V8, pH 6.5 under a 12 hour white light photoperiod.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 9 P. delt.* x *P. mazi.* I83/58 NZ					
12°C	$15.3_{1.3}$	$4.0_{0.2}$	3.81	3.02	1.26	33.0
16°C	$15.3_{1.2}$	$4.0_{0.2}$	3.80	3.02	1.26	33.1
20°C	$15.5_{1.2}$	$4.0_{0.2}$	3.81	3.01	1.26	33.2
24°C	$15.2_{1.4}$	$4.1_{0.2}$	3.72	2.95	1.26	33.9
28°C	$14.8_{1.5}$	$4.0_{0.2}$	3.70	2.96	1.25	33.8
Mean	$15.2_{0.2}$	$4.0_{0.04}$	3.77	2.99	1.26	33.4
Isolate	*M. brunnea Br 10 P. deltoides* USA					
12°C	$14.5_{0.9}$	$4.2_{0.3}$	3.45	3.10	1.12	32.2
16°C	$14.1_{0.8}$	$4.2_{0.2}$	3.35	3.10	1.09	32.2
20°C	$14.0_{0.9}$	$4.1_{0.3}$	3.41	3.00	1.09	33.0
24°C	$14.0_{0.9}$	$4.1_{0.3}$	3.41	3.00	1.11	33.0
28°C	$14.2_{0.8}$	$4.2_{0.3}$	3.40	2.95	1.14	33.8
Mean	$14.2_{0.2}$	$4.2_{0.05}$	3.40	3.03	1.11	32.8
Isolate	*M. brunnea Br 11 P. deltoides* USA					
12°C	$14.5_{0.6}$	$4.2_{0.3}$	3.42	3.03	1.12	32.9
16°C	$14.4_{0.7}$	$4.3_{0.3}$	3.36	2.90	1.12	34.6
20°C	$14.1_{1.0}$	$4.0_{0.2}$	3.48	2.95	1.17	33.8
24°C	$14.6_{0.8}$	$4.1_{0.3}$	3.55	3.00	1.18	33.2
28°C	$14.4_{0.7}$	$4.3_{0.2}$	3.34	2.98	1.12	33.5
Mean	$14.4_{0.2}$	$4.2_{0.1}$	3.43	2.97	1.14	33.6
Isolate	*M. brunnea Br 12 P. deltoides* USA					
12°C	$14.7_{1.0}$	$4.3_{0.3}$	3.44	3.10	1.09	32.2
16°C	$15.6_{1.1}$	$4.3_{0.2}$	3.63	3.25	1.11	30.7
20°C	$15.6_{0.8}$	$4.4_{0.3}$	3.54	2.94	1.22	33.9
24°C	$14.2_{0.7}$	$4.2_{0.2}$	3.36	3.11	1.08	32.1
28°C	$14.6_{0.9}$	$4.4_{0.2}$	3.33	3.12	1.06	32.0
Mean	$14.9_{0.6}$	$4.3_{0.08}$	3.46	3.10	1.11	32.2

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 13 P. deltoides* USA					
12°C	$15.1_{0.8}$	$4.1_{0.2}$	3.70	3.12	1.18	32.0
16°C	$15.0_{1.0}$	$4.1_{0.2}$	3.67	3.08	1.19	32.5
20°C	$14.7_{0.8}$	$4.0_{0.2}$	3.74	3.21	1.16	31.2
24°C	$14.6_{0.8}$	$4.0_{0.1}$	3.70	3.14	1.17	31.8
28°C	$14.7_{0.8}$	$4.1_{0.1}$	3.62	3.02	1.19	33.1
Mean	$14.8_{0.2}$	$4.0_{0.05}$	3.68	3.11	1.18	32.1
Isolate	*M. brunnea Br 14 P. trichocarpa* USA					
12°C	$15.8_{0.8}$	$4.0_{0.2}$	3.90	3.06	1.26	32.6
16°C	$15.4_{1.0}$	$4.0_{0.2}$	3.84	3.10	1.24	32.3
20°C	$15.3_{0.9}$	$4.0_{0.1}$	3.87	3.13	1.23	32.0
24°C	$15.6_{0.7}$	$4.0_{0.2}$	3.84	3.10	1.24	32.2
28°C	$15.4_{0.8}$	$4.0_{0.2}$	3.86	3.82	1.37	35.5
Mean	$15.5_{0.2}$	$4.0_{0.0}$	3.86	3.04	1.27	32.9
Isolate	*M. brunnea Br 15 P. deltoides*					
12°C	$14.7_{0.8}$	$4.2_{0.2}$	3.46	2.96	1.17	33.7
16°C	$15.1_{0.8}$	$4.2_{0.3}$	3.60	3.02	1.19	33.0
20°C	$14.9_{0.8}$	$4.2_{0.2}$	3.52	3.04	1.16	33.1
24°C	$14.9_{0.9}$	$4.3_{0.3}$	3.48	2.92	1.19	34.3
28°C	$14.1_{0.9}$	$4.3_{0.3}$	3.27	3.06	1.07	32.6
Mean	$14.7_{0.4}$	$4.2_{0.05}$	3.47	3.00	1.15	33.3
Isolate	*M. brunnea Br 16 P. Robusta* England					
12°C	$15.6_{0.9}$	$4.1_{0.2}$	3.75	3.25	1.15	30.7
16°C	$16.1_{0.8}$	$4.2_{0.3}$	3.82	3.17	1.20	31.4
20°C	$16.0_{1.0}$	$4.1_{0.2}$	3.88	3.25	1.19	30.8
24°C	$16.2_{0.9}$	$4.1_{0.2}$	3.94	3.32	1.18	30.1
28°C	$15.0_{0.7}$	$4.2_{0.2}$	3.61	3.06	1.18	32.7
Mean	$15.8_{0.5}$	$4.1_{0.05}$	3.80	3.21	1.18	31.1

APPENDIX 5c: Influence of incubation temperature on conidial dimensions of *Marssonina* species following 10 days incubation on 15%V8, pH 6.5 under 12 hour white light photoperiod.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 17 P. Robusta* Ireland					
12°C	$15.2_{1.1}$	$4.3_{0.3}$	3.54	3.12	1.13	32.0
16°C	$15.3_{0.9}$	$4.4_{0.3}$	3.43	3.08	1.11	32.4
20°C	$15.2_{0.9}$	$4.4_{0.3}$	3.35	3.15	1.06	31.7
24°C	$15.1_{0.8}$	$4.4_{0.2}$	3.46	3.15	1.09	31.7
28°C	$15.1_{0.9}$	$4.4_{0.2}$	3.46	3.20	1.08	31.2
Mean	$15.2_{0.08}$	$4.4_{0.04}$	3.45	3.14	1.09	31.8
Isolate	*M. brunnea Br 18 P. x euramericana* France					
12°C	16.1	3.8	4.20	2.81	1.46	35.0
16°C	15.7	3.8	4.15	3.06	1.35	32.6
20°C	15.3	3.7	4.12	3.08	1.34	32.5
24°C	15.6	3.8	4.09	2.93	1.39	34.1
28°C	15.9	3.8	4.17	2.83	1.47	35.2
Mean	$15.7_{0.3}$	$3.8_{0.04}$	4.15	2.94	1.40	33.9
Isolate	*M. brunnea Br 19 P. deltoides* Holland					
12°C	14.8	$4.0_{0.2}$	3.65	3.13	1.17	32.0
16°C	14.6	$4.0_{0.2}$	3.63	3.10	1.17	32.2
20°C	14.5	$4.0_{0.2}$	3.60	3.17	1.13	31.5
24°C	14.6	$4.0_{0.2}$	3.60	3.13	1.15	31.9
28°C	14.8	$4.4_{0.3}$	3.30	3.02	1.09	33.1
Mean	$14.7_{0.2}$	$4.1_{0.2}$	3.55	3.11	1.14	32.1
Isolate	*M. brunnea Br 20 P. x euramericana* I-214 Turkey					
12°C	$15.7_{1.1}$	$4.5_{0.2}$	3.49	3.02	1.16	33.1
16°C	$15.7_{0.9}$	$4.4_{0.2}$	3.57	3.04	1.17	32.8
20°C	$15.8_{1.1}$	$4.5_{0.2}$	3.50	3.04	1.14	32.8
24°C	$15.5_{0.9}$	$4.4_{0.2}$	3.51	2.97	1.18	33.7
28°C	$15.6_{0.8}$	$4.4_{0.2}$	3.55	3.20	1.10	31.2
Mean	$15.7_{0.11}$	$4.4_{0.05}$	3.52	3.05	1.15	32.7

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 21 P. tremuloides* USA					
12°C	$15.7_{0.8}$	$3.9_{0.2}$	3.98	2.95	1.35	33.9
16°C	$15.8_{1.1}$	$3.9_{0.1}$	4.08	3.05	1.34	32.8
20°C	$16.1_{1.0}$	$3.9_{0.1}$	4.15	2.90	1.44	34.8
24°C	$16.1_{1.1}$	$3.9_{0.2}$	4.11	3.00	1.38	33.4
28°C	Irregular conidia					
Mean	$15.9_{0.2}$	$3.9_{0.0}$	4.08	2.97	1.38	33.7
Isolate	*M. brunnea Br 22 P. tremuloides* USA					
12°C	$15.8_{1.0}$	$3.9_{0.2}$	4.04	3.09	1.31	32.4
16°C	$16.0_{0.8}$	$3.8_{0.2}$	4.14	2.93	1.41	34.1
20°C	$16.1_{1.0}$	$3.9_{0.2}$	4.16	2.93	1.42	34.1
24°C	$16.1_{0.9}$	$3.9_{0.2}$	4.08	2.98	1.37	33.5
28°C	Irregular conidia					
Mean	$16.0_{0.1}$	$3.9_{0.05}$	4.10	2.98	1.38	33.5
Isolate	*M. brunnea Br 23 P. tremuloides* Alaska					
12°C	$15.5_{1.0}$	$3.9_{0.2}$	3.90	3.04	1.28	32.9
16°C	$15.7_{1.0}$	$3.9_{0.2}$	3.95	2.93	1.35	34.1
20°C	$15.9_{1.0}$	$4.0_{0.2}$	3.00	2.90	1.38	34.5
24°C	$15.6_{1.2}$	$3.9_{0.2}$	3.94	3.00	1.31	33.3
28°C	$15.2_{1.2}$	$3.9_{0.2}$	3.86	3.03	1.27	33.0
Mean	$15.6_{0.6}$	$3.9_{0.04}$	3.93	2.98	1.32	33.5
Isolate	*M. brunnea Br 24 P. tremuloides* USA					
12°C	$15.9_{1.1}$	$4.0_{0.2}$	4.00	3.06	1.30	32.6
16°C	$16.0_{1.0}$	$4.0_{0.2}$	3.93	2.95	1.33	33.8
20°C	$16.3_{0.9}$	$4.1_{0.2}$	3.95	2.96	1.33	33.7
24°C	$16.4_{1.0}$	$4.0_{0.1}$	4.06	2.90	1.41	34.8
28°C	$15.1_{1.0}$	$4.2_{0.2}$	3.61	3.33	1.08	29.9
Mean	$15.9_{0.5}$	$4.0_{0.09}$	3.91	3.04	1.29	33.0

APPENDIX 5d: Influence of incubation temperature on conidial dimensions of *Marssonina* species following 10 days incubation on 15%V8, pH 6.5 under 12 hour white light photoperiod.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.	Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. castagnei* 1a *P. alba* Switzerland						Isolate	*M. castagnei* Cs 3 *P. alba* Turkey					
12°C	$24.4_{1.4}$	$6.3_{0.7}$	3.84	2.00	1.74	49.7	12°C	$19.2_{1.1}$	$5.9_{0.3}$	3.26	2.52	1.29	39.6
16°C	$23.5_{1.4}$	$6.0_{0.6}$	3.90	2.14	1.82	46.6	16°C	$18.9_{1.2}$	$5.8_{0.2}$	3.22	2.61	1.23	38.2
20°C	$25.1_{1.4}$	$6.2_{0.7}$	4.07	2.30	1.78	43.8	20°C	$19.9_{1.1}$	$5.9_{0.3}$	3.38	2.55	1.32	39.2
24°C	$22.5_{1.5}$	$6.3_{0.6}$	3.57	2.50	1.32	40.0	24°C	$20.0_{0.9}$	$6.0_{0.3}$	3.38	2.64	1.28	37.8
28°C	No growth						28°C	No growth					
Mean	$23.9_{1.1}$	$6.2_{0.1}$	3.85	2.23	1.66	45.0	Mean	$19.5_{0.5}$	$5.9_{0.08}$	3.31	2.58	1.24	38.7
Isolate	*M. castagnei Cs 1b P. alba* Switzerland						Isolate	*M. populi Po 1 P. canadensis* England					
12°C	$23.3_{1.5}$	$6.3_{0.5}$	3.70	2.40	1.54	41.7	12°C	$19.2_{1.3}$	$5.8_{0.4}$	3.30	3.12	1.05	32.0
16°C	$23.1_{1.4}$	$6.3_{0.6}$	3.66	2.26	1.62	44.2	16°C	$18.9_{1.2}$	$6.0_{0.3}$	3.20	3.10	1.03	32.3
20°C	$22.8_{1.4}$	$6.2_{0.5}$	3.67	2.24	1.63	44.5	20°C	$18.4_{1.1}$	$5.9_{0.3}$	3.13	3.31	0.94	30.1
24°C	$22.7_{1.2}$	$6.3_{0.5}$	3.62	2.31	1.56	43.3	24°C	$18.5_{1.0}$	$6.0_{0.3}$	3.00	3.40	0.90	29.5
28°C	No growth						28°C	No growth					
Mean	$23.0_{0.2}$	$6.3_{0.05}$	3.66	2.30	1.59	43.4	Mean	$18.7_{0.4}$	$5.9_{0.1}$	3.16	3.23	0.98	31.0
Isolate	*M. castagnei Cs 2 P. alba* Ireland						Isolate	*M. populi Po 2 P. nigra* England					
12°C	$19.0_{1.2}$	$6.0_{0.4}$	3.16	2.68	1.18	37.3	12°C	$19.7_{0.9}$	$6.2_{0.3}$	3.16	3.35	0.94	29.8
16°C	$18.5_{1.1}$	$6.0_{0.4}$	3.08	2.65	1.16	37.6	16°C	$19.5_{1.0}$	$6.0_{0.3}$	3.21	3.43	0.94	29.1
20°C	$18.0_{1.3}$	$6.0_{0.4}$	2.98	2.57	1.15	38.8	20°C	$19.7_{0.9}$	$6.0_{0.3}$	3.27	3.50	0.93	28.5
24°C	$18.9_{1.4}$	$5.7_{0.3}$	3.29	2.42	1.36	41.3	24°C	$19.7_{1.0}$	$6.0_{0.3}$	3.27	3.70	0.90	27.0
28°C	No growth						28°C	No growth					
Mean	$18.6_{0.4}$	$5.9_{0.1}$	3.13	2.58	1.21	38.7	Mean	$19.6_{0.1}$	$6.0_{0.1}$	3.23	3.49	0.93	28.6

APPENDIX 5e: Influence of incubation temperature on conidial dimensions of *Marssonina* species following 10 days incubation on 15%V8, pH 6.5 under 12 hour white light photoperiod.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. populi* *Po 3* *P. nigra* England					
12°C	$21.4_{1.0}$	$6.1_{0.3}$	3.50	3.48	1.00	28.7
16°C	$21.3_{1.0}$	$6.2_{0.3}$	3.43	3.54	0.97	28.2
20°C	$21.4_{0.9}$	$6.0_{0.3}$	3.55	3.80	0.93	26.3
24°C	$21.9_{0.9}$	$6.3_{0.2}$	3.40	3.90	0.98	25.7
28°C	No growth					
Mean	$21.5_{0.3}$	$6.1_{0.1}$	3.47	3.68	0.97	27.2
Isolate	*M. populi* *Po 4* *P. nigra* Ireland					
12°C	$21.6_{1.0}$	$5.6_{0.2}$	3.82	3.60	1.06	27.8
16°C	$21.3_{0.9}$	$5.7_{0.3}$	3.76	3.57	1.05	28.0
20°C	$21.5_{1.1}$	$5.8_{0.3}$	3.68	3.55	1.04	28.2
24°C	$21.4_{1.2}$	$5.8_{0.2}$	3.67	3.68	1.00	27.2
28°C	No growth					
Mean	$21.4_{0.1}$	$5.7_{0.09}$	3.73	3.60	1.04	27.8
Isolate	*M. populi* *Po 5* *P.* x *berolinensis* Germany					
12°C	$24.3_{1.4}$	$6.3_{0.4}$	3.85	2.88	1.33	34.6
16°C	$23.7_{1.2}$	$6.3_{0.3}$	3.78	2.90	1.30	34.9
20°C	$24.0_{1.5}$	$6.2_{0.3}$	3.89	3.06	1.27	32.6
24°C	$24.7_{1.4}$	$6.1_{0.3}$	4.06	3.00	1.35	33.2
28°C	No growth					
Mean	$24.1_{0.4}$	$6.2_{0.1}$	3.89	2.96	1.31	33.8

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. populi* *Po 6* *P. nigra* Switzerland					
12°C	$22.0_{1.5}$	$5.6_{0.5}$	3.86	3.00	1.27	33.3
16°C	$22.4_{1.4}$	$6.0_{0.4}$	3.72	3.36	1.10	29.7
20°C	$20.5_{1.4}$	$5.8_{0.6}$	3.50	3.10	1.15	32.3
24°C	$20.7_{1.4}$	$5.6_{0.5}$	3.70	3.50	1.05	28.5
28°C	No growth					
Mean	$21.4_{0.9}$	$5.7_{0.2}$	3.69	3.24	1.14	31.0
Isolate	*M. populi* *Po 7* *P. nigra* Switzerland					
12°C	$21.4_{1.4}$	$5.8_{0.4}$	3.66	3.33	1.10	30.0
16°C	$21.2_{1.3}$	$5.8_{0.3}$	3.66	3.36	1.09	29.7
20°C	$20.5_{1.2}$	$5.8_{0.3}$	3.54	3.51	1.01	28.5
24°C	$20.6_{1.2}$	$5.8_{0.4}$	3.54	3.46	1.02	28.8
28°C	No growth					
Mean	$21.0_{0.4}$	$5.8_{0.0}$	3.60	3.41	1.05	29.2

APPENDIX 5a: Influence of photoperiod on conidial dimensions of *Marssonina* species following 10 days incubation on 15%V8, pH 6.5, at 20°C.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 1 P. yunnanensis* NZ					
D	$16.0_{1.2}$	$4.0_{0.2}$	4.00	2.92	1.41	34.0
L/D	$16.6_{1.2}$	$4.0_{0.2}$	4.08	2.90	1.41	34.5
NUV/D	$17.0_{1.6}$	$4.1_{0.2}$	4.10	3.09	1.40	32.4
Mean	$16.5_{0.5}$	$4.0_{0.06}$	4.06	2.97	1.41	33.6
Isolate	*M. brunnea Br 2 P.x euramericana* NZ					
D	$14.9_{1.0}$	$4.2_{0.3}$	3.60	3.00	1.20	33.2
L/D	$14.4_{1.3}$	$4.0_{0.3}$	3.60	3.00	1.20	33.5
NUV/D	$14.2_{1.2}$	$4.4_{0.3}$	3.45	2.90	1.20	34.4
Mean	$14.5_{0.4}$	$4.2_{0.2}$	3.55	2.97	1.20	33.7
Isolate	*M. brunnea Br 3 P. fremontii* cv. 61/98 NZ					
D	$15.3_{1.0}$	$4.0_{0.2}$	3.76	3.00	1.24	33.4
L/D	$15.7_{0.7}$	$4.0_{0.2}$	3.90	3.03	1.24	32.9
NUV/D	$15.2_{1.0}$	$4.0_{0.2}$	3.73	2.95	1.27	33.8
Mean	$15.4_{0.2}$	$4.0_{0.0}$	3.80	3.00	1.25	33.4
Isolate	*M. brunnea Br 4 P. fremontii* x *P. nigra* cv.66/9 NZ					
D	$15.4_{0.8}$	$3.9_{0.2}$	3.90	3.05	1.27	32.7
L/D	$16.4_{0.8}$	$4.0_{0.1}$	4.10	3.11	1.32	32.0
NUV/D	$16.2_{1.0}$	$4.0_{0.2}$	4.06	3.00	1.34	33.3
Mean	$16.0_{0.5}$	$4.0_{0.06}$	4.02	3.05	1.31	32.7
Isolate	*M. brunnea Br 5 P. x euramericana* cv. I214 NZ					
D	$15.1_{1.0}$	$4.2_{0.2}$	3.62	2.90	1.25	34.5
L/D	$15.1_{0.8}$	$4.2_{0.2}$	3.62	3.07	1.17	32.5
NUV/D	$15.5_{1.2}$	$4.2_{0.2}$	3.69	2.94	1.26	34.0
Mean	$15.2_{0.2}$	$4.2_{0.0}$	3.64	2.97	1.23	33.7

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 6 P. alba* NZ					
D	$16.8_{1.2}$	$4.2_{0.2}$	4.04	2.99	1.35	33.4
L/D	$17.0_{1.0}$	$4.1_{0.2}$	4.14	2.98	1.43	33.5
NUV/D	$17.2_{1.2}$	$4.2_{0.2}$	4.13	3.01	1.37	33.2
Mean	$17.0_{0.2}$	$4.2_{0.06}$	4.10	2.99	1.38	33.4
Isolate	*M. brunnea Br 7 P. x euramericana* cv. Flevo NZ					
D	15.0	$4.0_{0.3}$	3.76	3.00	1.26	33.6
L/D	15.1	$4.0_{0.2}$	3.77	3.04	1.22	32.8
NUV/D	14.8	$4.0_{0.2}$	3.67	3.01	1.22	33.2
Mean	$14.9_{0.1}$	$4.0_{0.0}$	3.73	3.02	1.23	33.2
Isolate	*M. brunnea Br 8 P. x euramericana* cv. NL 2194 NZ.					
D	$17.3_{1.5}$	$4.1_{0.2}$	4.12	2.90	1.44	34.4
L/D	$17.3_{1.5}$	$4.1_{0.3}$	4.22	2.90	1.42	34.4
NUV/D	$16.6_{1.6}$	$4.1_{0.2}$	4.04	2.98	1.36	33.5
Mean	$17.0_{0.4}$	$4.1_{0.0}$	4.13	2.93	1.41	34.1
Isolate	*M. brunnea Br 9 P. delt.* x *P. maxi.* cv. I83/58 NZ					
D	$15.5_{1.2}$	$4.1_{0.2}$	3.80	3.00	1.26	33.3
L/D	$15.5_{1.2}$	$4.0_{0.2}$	3.81	3.01	1.26	33.2
NUV/D	$15.4_{1.3}$	$4.0_{0.2}$	3.82	3.03	1.26	33.0
Mean	$15.4_{0.06}$	$4.0_{0.06}$	3.81	3.01	1.26	33.2
Isolate	*M. brunnea Br 10 P. deltoides* USA					
D	$14.0_{0.9}$	$4.1_{0.3}$	3.41	3.10	1.12	32.2
L/D	$14.2_{0.8}$	$4.2_{0.3}$	3.40	3.20	1.05	31.4
NUV/D	$14.0_{0.8}$	$4.0_{0.3}$	3.40	3.00	1.13	33.0
Mean	$14.1_{0.1}$	$4.1_{0.1}$	3.40	3.10	1.10	32.2

APPENDIX 6b: Influence of photoperiod on conidial dimensions of *Marssonina* species following 10 days incubation on 15%V8, pH 6.5, at 20°C.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 11 P. deltoides* USA					
D	$14.2_{0.9}$	$4.0_{0.3}$	3.55	2.96	1.17	33.7
L/D	$14.3_{1.0}$	$4.4_{0.3}$	3.23	3.13	1.03	31.9
NUV/D	$14.3_{1.1}$	$4.3_{0.3}$	3.35	3.15	1.06	32.0
Mean	$14.3_{0.06}$	$4.2_{0.2}$	3.38	3.08	1.09	32.5
Isolate	*M. brunnea Br 12 P. deltoides* USA					
D	$15.3_{1.2}$	$4.1_{0.2}$	3.76	3.32	1.13	30.1
L/D	$15.5_{1.2}$	$4.2_{0.2}$	3.70	3.10	1.20	32.3
NUV/D	$15.3_{1.1}$	$4.1_{0.2}$	3.73	3.10	1.22	32.3
Mean	$15.4_{0.1}$	$4.1_{0.06}$	3.73	3.17	1.18	31.6
Isolate	*M. brunnea Br 13 P. deltoides* USA					
D	$15.4_{1.0}$	$4.0_{0.2}$	3.83	3.11	1.22	32.1
L/D	$14.7_{0.8}$	$4.0_{0.2}$	3.74	3.21	1.16	31.2
NUV/D	$15.2_{0.8}$	$4.0_{0.01}$	3.80	3.10	1.22	32.3
Mean	$15.1_{0.4}$	$4.0_{0.0}$	3.79	3.14	1.20	31.8
Isolate	*M. brunnea Br 14 P. trichocarpa* USA					
D	$15.5_{0.7}$	$4.0_{0.2}$	3.93	3.00	1.31	33.3
L/D	$15.4_{0.2}$	$4.0_{0.2}$	3.88	3.07	1.26	32.5
NUV/D	$15.4_{0.8}$	$4.0_{0.2}$	3.90	3.14	1.24	31.9
Mean	$15.4_{0.05}$	$4.0_{0.0}$	3.90	3.07	1.27	32.6
Isolate	*M. brunnea Br 15 P. deltoides* USA					
D	$14.8_{0.8}$	$4.3_{0.2}$	3.44	3.02	1.14	33.1
L/D	$14.9_{0.8}$	$4.2_{0.2}$	3.52	3.04	1.16	33.3
NUV/D	$14.7_{0.9}$	$4.2_{0.2}$	3.45	3.12	1.10	32.0
Mean	$14.8_{0.1}$	$4.2_{0.06}$	3.47	3.06	1.13	32.8

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 16 P. Robusta* England					
D	$15.6_{0.8}$	$4.0_{0.1}$	3.94	3.16	1.24	31.6
L/D	$14.8_{0.7}$	$3.9_{0.2}$	3.75	3.20	1.18	31.0
NUV/D	$16.1_{0.6}$	$4.2_{0.2}$	3.83	3.19	1.20	31.4
Mean	$15.5_{0.6}$	$4.0_{0.15}$	3.83	3.18	1.21	31.3
Isolate	*M. brunnea Br 17 P. Robusta* Ireland					
D	$15.3_{0.7}$	$4.3_{0.2}$	3.52	3.16	1.11	31.6
L/D	$15.2_{0.9}$	$4.4_{0.3}$	3.35	3.15	1.06	31.7
NUV/D	$15.0_{1.0}$	$4.3_{0.3}$	3.46	3.08	1.12	32.5
Mean	$15.2_{0.15}$	$4.3_{0.06}$	3.44	3.13	1.10	31.9
Isolate	*M. brunnea Br 18 P.* x *euramericana* France					
D	$15.4_{0.7}$	$3.8_{0.2}$	4.07	3.13	1.30	32.0
L/D	$15.3_{1.2}$	$3.8_{0.2}$	4.03	3.08	1.34	32.5
NUV/D	$15.5_{1.3}$	$3.8_{0.2}$	4.14	3.04	1.35	32.8
Mean	$15.5_{0.2}$	$3.8_{0.0}$	4.08	3.08	1.33	32.4
Isolate	*M. brunnea Br 19 P. deltoides* Holland					
D	14.6	$4.0_{0.1}$	3.60	3.23	1.11	31.0
L/D	14.6	$4.0_{0.1}$	3.62	3.15	1.15	31.7
NUV/D	14.8	$4.0_{0.2}$	3.67	3.11	1.18	32.1
Mean	$14.7_{0.1}$	$4.0_{0.0}$	3.63	3.16	1.15	31.6
Isolate	*M. brunnea Br 20 P.* x *euramericana* cv. I214 Turkey					
D	$15.4_{0.9}$	$4.4_{0.2}$	3.50	3.25	1.07	30.7
L/D	$15.6_{0.9}$	$4.4_{0.2}$	3.56	3.14	1.13	31.8
NUV/D	$15.5_{0.8}$	$4.3_{0.2}$	3.58	3.09	1.16	32.3
Mean	$15.5_{0.1}$	$4.4_{0.05}$	3.55	3.16	1.12	31.6

APPENDIX 6c: Influence of photoperiod on conidial dimensions of *Marssonina* species following incubation on 15%V8, pH 6.5, at 20°C.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. brunnea Br 21 P. tremuloides* USA					
D	$16.1_{0.9}$	$3.9_{0.2}$	4.08	2.97	1.37	33.7
L/D	$16.1_{1.0}$	$3.9_{0.1}$	4.15	2.90	1.44	34.8
NUV/D	$15.8_{1.0}$	$3.9_{0.1}$	4.03	3.05	1.32	32.7
Mean	$16.0_{0.2}$	$3.9_{0.0}$	4.09	2.97	1.37	33.7
Isolate	*M. brunnea Br 22 P. tremuloides* USA					
D	$16.0_{0.9}$	$3.9_{0.2}$	4.10	2.97	1.38	33.6
L/D	$16.1_{0.8}$	$3.9_{0.2}$	4.16	2.93	1.42	34.1
NUV/D	$15.7_{1.0}$	$4.0_{0.2}$	3.93	3.12	1.26	32.0
Mean	$15.9_{0.2}$	$3.9_{0.06}$	4.06	3.00	1.35	33.2
Isolate	*M. brunnea Br 23 P. tremuloides* Alaska					
D	$15.7_{1.0}$	$3.9_{0.1}$	3.97	3.00	1.32	33.3
L/D	$15.9_{1.0}$	$4.0_{0.2}$	4.00	2.90	1.38	34.5
NUV/D	$15.6_{1.1}$	$3.9_{0.2}$	3.95	2.93	1.34	34.0
Mean	$15.7_{0.2}$	$3.9_{0.06}$	3.97	2.94	1.34	33.8
Isolate	*M. brunnea Br 24 P. tremuloides* USA					
D	$16.3_{0.9}$	$4.1_{0.2}$	3.95	2.96	1.33	33.7
L/D	$16.0_{0.8}$	$4.1_{0.2}$	3.90	3.02	1.29	33.1
NUV/D	$16.3_{0.8}$	$4.1_{0.2}$	3.96	2.97	1.33	33.6
Mean	$16.2_{0.2}$	$4.1_{0.0}$	3.94	2.98	1.32	33.4
Isolate	*M. castagnei Ca 1a P. alba* Switzerland					
D	$24.9_{1.5}$	$6.3_{0.7}$	3.95	2.30	1.60	43.3
L/D	$24.0_{1.6}$	$5.9_{0.1}$	4.08	2.32	1.75	43.1
NUV/D	$24.5_{1.4}$	$6.4_{0.7}$	3.82	2.30	1.69	43.3
Mean	$24.4_{0.4}$	$6.2_{0.2}$	3.95	2.31	1.68	43.2

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. castagnei Ca 1b P. alba* Switzerland					
D	$23.3_{1.4}$	$6.3_{0.5}$	3.70	2.30	1.61	43.6
L/D	$22.8_{1.4}$	$6.2_{0.6}$	3.67	2.24	1.63	44.5
NUV/D	$22.7_{1.4}$	$6.4_{0.5}$	3.56	2.30	1.55	43.5
Mean	$22.9_{0.3}$	$6.3_{0.1}$	3.64	2.28	1.59	43.8
Isolate	*M. castagnei Cs 2 P. alba* Ireland					
D	$19.2_{1.2}$	$6.0_{0.4}$	3.17	2.48	1.28	40.3
L/D	$19.0_{1.0}$	$6.0_{0.3}$	3.16	2.68	1.18	37.3
NUV/D	$19.3_{1.2}$	$6.2_{0.3}$	3.13	2.35	1.33	42.4
Mean	$19.2_{0.15}$	$6.1_{0.1}$	3.15	2.50	1.26	40.0
Isolate	*M. castagnei Cs 3 P. alba* Turkey					
D	$20.0_{0.8}$	$5.8_{0.3}$	3.41	2.54	1.34	39.4
L/D	$19.9_{0.9}$	$5.9_{0.3}$	3.38	2.55	1.32	39.2
NUV/D	$20.0_{1.0}$	$5.9_{0.3}$	3.38	2.47	1.36	40.4
Mean	$20.0_{0.06}$	$5.9_{0.06}$	3.39	2.52	1.34	39.7
Isolate	*M. populi Po 1 P. canadensis* England					
D	$18.3_{1.0}$	$6.0_{0.3}$	3.10	3.20	0.96	31.2
L/D	$18.4_{1.0}$	$5.7_{0.3}$	3.23	3.25	0.99	30.7
NUV/D	$18.0_{1.0}$	$5.8_{0.2}$	3.10	3.20	0.96	31.2
Mean	$18.2_{0.2}$	$5.8_{0.1}$	3.14	3.22	0.97	31.0
Isolate	*M. populi Po 2 P. nigra* England					
D	$19.5_{1.0}$	$6.1_{0.3}$	3.22	3.45	0.93	28.9
L/D	$19.7_{0.9}$	$6.0_{0.3}$	3.27	3.50	0.93	28.5
NUV/D	$19.3_{0.9}$	$6.0_{0.3}$	3.21	3.54	0.90	28.2
Mean	$19.5_{0.2}$	$6.0_{0.06}$	3.23	3.50	0.92	28.5

APPENDIX 6d: Influence of photoperiod on conidial dimensions of *Marssonina* species following incubation on 15%V8, pH 6.5, at 20°C.

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. populi* *Po 3* *P. nigra* England					
D	$21.2_{0.9}$	$6.2_{0.3}$	3.41	3.78	0.90	26.4
L/D	$21.4_{0.9}$	$6.0_{0.3}$	3.55	3.80	0.93	26.3
NUV/D	$20.9_{1.2}$	$6.4_{0.3}$	3.27	3.84	0.85	26.1
Mean	$21.2_{0.2}$	$6.2_{0.2}$	3.41	3.81	0.89	26.3
Isolate	*M. populi* *Po 4* *P. nigra* Ireland					
D	$21.2_{0.9}$	$5.9_{0.3}$	3.56	3.39	1.05	29.5
L/D	$21.5_{1.1}$	$5.8_{0.3}$	3.68	3.55	1.04	28.2
NUV/D	$21.7_{1.0}$	$5.6_{0.3}$	3.86	3.73	1.03	26.8
Mean	$21.5_{0.2}$	$5.8_{0.1}$	3.70	3.56	1.04	28.2
Isolate	*M. populi* *Po 5* *P.x berolinensis* Germany					
D	$24.0_{1.4}$	$6.2_{0.4}$	3.87	2.93	1.36	34.0
L/D	$24.0_{1.5}$	$6.2_{0.3}$	3.89	3.06	1.27	32.6
NUV/D	$24.2_{1.4}$	$6.2_{0.3}$	3.86	2.94	1.31	34.0
Mean	$24.0_{0.1}$	$6.2_{0.6}$	3.87	2.97	1.31	33.5

Treatment	Length (u)	Breadth (u)	L:B	TL:S	LS:B	% Sept.
Isolate	*M. populi* *Po 6* *P. nigra* Switzerland					
D	$20.5_{1.6}$	$5.9_{0.6}$	3.47	3.10	1.15	32.3
L/D	$22.0_{1.7}$	$5.5_{0.5}$	4.00	3.43	1.17	29.1
NUV/D	$20.5_{1.6}$	$5.5_{0.5}$	3.70	3.66	1.00	27.3
Mean	$21.0_{0.8}$	$5.6_{0.2}$	3.72	3.40	1.11	29.6
Isolate	*M. populi* *Po 7* *P. nigra* Switzerland					
D	$20.8_{1.4}$	$5.7_{0.3}$	3.67	3.54	1.04	28.3
L/D	$20.5_{1.2}$	$5.8_{0.3}$	3.54	3.51	1.01	28.5
NUV/D	$20.3_{1.1}$	$5.8_{0.3}$	3.50	3.60	0.97	27.8
Mean	$20.5_{0.2}$	$5.7_{0.06}$	3.57	3.55	1.01	28.2

APPENDIX 7a: Field collections of *M. castagnei* examined for morphology of microconidiophores and microconidia

Specimen Number	Host Species	Origin	Collector	Date
1	*P. alba*	Dane Co., Wisconsin, USA	R.F. Cain	6.9.1953
2	*P. alba*	Ankara, Turkey	H. Bremer	25.9.1941
3	*P. alba*	Zurich, Switzerland	A. Volkart	7.9.1907
4	*P. alba*	Germany	H. Sydow	.8.1919
5	*P. alba*	Zurich, Switzerland	R.H. Rimpau	3.8.1960
6	*P. alba*	Miramas, France	J.L. Castagne	-
7	*P. alba*	Vidzeme, Latvia	K. Starcs	.10.1934
8	*P. alba*	Italy	Prof. C. Pollini	.1888
9	*P. alba*	Norway	Klapp	8.10.1941
10	*P. alba*	Frankfurt, Germany	P. Magnus	17.9.1873
11	*P. alba*	Temesvar, Hungary	G. Moesz	.9.1925
12	*P. alba*	Temesvar, Hungary	G. Moesz	.9.1925
13	*P. alba*	Temesvar, Hungary	G. Moesz	27.9.1923
14	*P. alba*	Brandenburg, Germany	H. Sydow	18.8.1919
15	*P. alba*	Kesthely, Hungaria	G. Moesz	9.1.1927
16	*P. alba*	Frankfurt, Germany	P. Magnus	17.9.1873
17	*P. alba*	Italy	Prof. Pollini	.1888
18	*P. alba*	Parma, Italy	Prof. Passerini	.1874
19	*P. alba*	Weisskirchen, Czechoslovakia	Dr. F. Petrak	27.8.1924
20	*P. alba*	Dublin, Ireland	F. O'Riordain	31.10.1979

APPENDIX 7b: Field collections of *M. populi* examined for morphology of microconidiophores and microconidia

Specimen Number	Host	Origin	Collector	Date
21	*P. berolinensis*	Hann. Munden, Germany	H. Butin	12.5.1956
22	*P. regenerata*	Kassel, Germany	H. Butin	15.10.1956
23	*P. marilandica*	Hann. Munden, Germany	H. Butin	20.8.1955
24	*P. nigra*	Zurich, Switzerland	R.H. Rimpau	17.8.1960
25	*P. nigra*	Zurich, Switzerland	R.H. Rimpau	7.9.1960
26	*P. nigra*	Niederdonau, Germany	F. Petrak	.9.1939
27	*P. balsamifera*	Harpenden, England	J.S. Dickens	.1979
28*	*P. nigra*	France	M.A. Libert	.1834
29	*P. angustifolia*	Wyoming, USA	W.G. Solheim	30.9.1956
30	*P. angustifolia*	Wyoming, USA	W.G. Solheim	4.10.1952
31	*P. nigra*	Surrey, England	D.A. Burdekin	27.7.1979
32	*P. marilandica*	Liempde, Holland	J. Gremmen	24.4.1963
33*	*P. nigra*	France	M.A. Libert	.1834
34	*P. nigra*	Belgium	Eosquinet	28.9.1855
35	*P. nigra*	Ypres, Belgium	Eosquinet	9.10.1857
36	*P. nigra*	Dublin, Ireland	F. O'Riordain	31.10.1979
37	*P. nigra*	Milton, England	A.G. Bailey	10.11.1979
38	*P. nigra*	Surrey, England	A.G. Bailey	12.11.1979

* Type specimens

APPENDIX 7c: Field collections of *M. brunnea* examined for morphology of microconidiophores and microconidia

Specimen Number	Host	Origin	Collector	Date
39	*P. tremula*	Zurich, Switzerland	R.H. Rimpau	20.10.1960
40	*P. tremula*	Zurich, Switzerland	R.H. Rimpau	23.10.1960
41	*P. tremula*	Ontario, Canada	J. Dearness	.9.1923
42	*P. tremula*	Pavia, Italy	F. Cavara	.1890
43	*P. tremula*	Westfalen, Germany	A. Ludwig	20.9.1923
44	*P. tremula*	Westfalen, Germany	A. Ludwig	19.9.1923
45	*P. grandidentata*	Ontario, Canada	J. Dearness	8.8.1913
46	*P. tremuloides*	Wisconsin, USA	J.J. Davis	25.9.1894
47	*P. tremuloides*	Utah, USA	A.O. Garrett	9.8.1928
48	*P. tremuloides*	Wisconsin, USA	J.J. Davis	31.8.1916
49	*P. tremuloides*	Washington, USA	C.V. Piper	31.8.1894
50	*P. deltoides*	Colorado, USA	C.F. Baker	30.8.1892
51	*P.x euramericana* cv. Robusta	Dublin, Ireland	F. O'Riordain	31.10.1979
52	*P. deltoides*	Colorado, USA	T.E. Hinds	30.10.1979
53-57	*P. species*	Palmerston North, NZ	Author	25.5.1978

APPENDIX 8

PREPARATION OF MATERIAL FOR SCANNING AND TRANSMISSION ELECTRON MICROSCOPY

A. SCANNING ELECTRON MICROSCOPY

Small blocks of mycelium were fixed in 3% glutaraldehyde & 2% formaldehyde in 0.1M phosphate buffer (pH 7.2) (Karnovsky, 1965) for three hours. The blocks were then washed in three changes of distilled water. Excess moisture was removed with blotting paper and the specimen quick-frozen in liquid freon, followed by liquid nitrogen. The blocks were then transferred to super-cooled brass discs (liquid nitrogen) and placed in a vacuum overnight. Freeze-dried specimens were glued to metal stubs and coated with gold (approx. 150A^0). Observations were made with a Cwikscan 100 field emission scanning electron microscope.

B. TRANSMISSION ELECTRON MICROSCOPY

Small blocks of agar, squares of leaf tissue, were fixed in 3% glutaraldehyde + 2% formaldehyde in 0.1M phosphate buffer (pH 7.2) (Karnovsky, 1965), vacuum infiltrated and stored in the primary fixative at 4^{0}C. Specimens were then transferred to fresh buffer (three buffer washes in 30 mins.) and post-fixed for 3-5 hours in 1% osmium tetroxide (OsO_4) at 4^{0}C. Following three buffer washes, the fixed material was dehydrated in an ethanol series (25%, 50%, 75%, 95%, 100%), treated with propylene oxide (15 mins.), infiltrated and embedded in Fluka epoxy resin (cured 25 hours at 60^{0}C). Sections were cut with a diamond knife on a LKB ultramicrotome and mounted on supported grids. Grid mounted sections were stained 5-7 minutes with saturated uranylacetate in 50% ethanol, washed in 50% ethanol, then in distilled water and stained 5-7 min. with lead citrate (Venable & Coggeshall, 1965), washed with distilled water and examined with Philips EM 200 electron microscope.

APPENDIX 9a: *M. castagnei* - dimensions of microconidia from field collections

Specimen Number	Length (u) Min.	Mean	Max.	Breadth (u) Min.	Mean	Max.	L:B Ratio
1 [a]	3.6	4.4	5.4	1.3	1.5	1.8	2.9
2	3.6	4.4	6.0	1.4	1.6	1.8	2.7
3	3.6	4.6	6.0	1.4	1.6	1.8	2.9
4	4.0	4.9	6.0	1.2	1.5	1.8	3.3
5	4.0	4.9	6.4	1.1	1.4	1.8	3.5
6	4.0	5.3	6.4	1.0	1.3	1.6	4.1
7	4.0	4.6	6.4	1.0	1.3	1.6	3.5
8	4.0	4.8	6.0	1.2	1.5	1.8	3.2
9	4.0	5.4	6.4	1.2	1.4	1.8	3.8
10	3.6	4.5	6.0	1.3	1.5	2.2	3.0
11	3.6	4.7	6.0	1.2	1.4	2.2	3.3
12	3.4	4.9	6.0	1.2	1.4	1.8	3.5
13	3.6	5.2	6.4	1.2	1.5	2.0	3.5
14	4.0	5.7	8.0	1.2	1.6	1.8	3.6
15	4.0	4.8	6.8	1.2	1.4	1.6	3.4
16	4.4	5.1	6.4	1.2	1.4	2.0	3.6
17	3.2	4.6	6.0	1.2	1.4	1.7	3.3
18	4.0	5.0	7.2	1.2	1.4	2.0	3.6
19	4.0	4.8	6.2	1.2	1.9	2.5	2.5
20	3.6	5.1	8.0	1.1	1.4	1.8	3.6
Mean	3.2	$4.9_{0.3}$ [b]	8.0	1.0	$1.5_{0.3}$	2.5	$3.3_{0.4}$

[a] For identification of isolates see Appendix 7a
[b] Standard deviation

APPENDIX 9b: *M. populi* - dimensions of microconidia from field collections

Specimen Number	Length (u) Min.	Mean	Max.	Breadth (u) Min.	Mean	Max.	L:B Ratio
21[a]	3.4	5.1	6.8	1.0	1.3	1.8	3.9
22	3.6	5.0	6.8	1.1	1.3	1.8	3.8
23	4.0	5.4	7.6	1.1	1.3	1.8	4.1
24	4.0	4.8	6.8	1.1	1.4	1.8	3.4
25	4.0	5.2	6.8	1.2	1.4	1.8	3.7
26	5.2	6.8	8.8	1.1	1.3	1.6	5.2
27	4.0	5.4	7.0	1.0	1.2	1.6	4.5
28	3.6	6.1	8.0	1.0	1.2	1.6	5.1
29	3.6	5.5	6.8	1.2	1.4	1.6	3.9
30	3.6	5.8	8.0	1.2	1.4	1.8	4.1
31	4.0	6.1	8.0	1.2	1.6	2.0	3.8
32	4.0	5.9	8.0	1.1	1.3	1.6	4.5
33	3.6	5.6	7.0	1.2	1.4	1.7	4.0
34	3.6	5.4	6.4	1.2	1.3	1.8	4.1
35	4.0	5.7	8.0	1.2	1.4	2.0	4.1
36	4.0	6.3	9.0	1.1	1.5	2.0	4.2
37	4.0	5.9	8.0	1.1	1.3	1.8	4.5
38	4.0	4.8	6.6	1.1	1.3	1.6	3.7
Mean	3.4	$5.6_{0.5}$ [b]	9.0	1.0	$1.3_{0.1}$	2.0	$4.1_{0.4}$

[a] For identification of isolates see Appendix 7b
[b] Standard deviation

APPENDIX 9c: *M. brunnea* - dimensions of microconidia from field collections

Specimen Number	Length (u) Min.	Mean	Max.	Breadth (u) Min.	Mean	Max.	L:B Ratio
39[a]	3.6	4.4	5.2	1.1	1.3	1.8	3.4
40	3.4	4.3	5.6	1.0	1.3	1.6	3.3
41	3.4	4.1	6.0	1.0	1.3	2.0	3.1
42	3.2	4.5	6.0	1.0	1.2	1.6	3.7
43	3.2	4.2	5.6	1.0	1.3	1.8	3.2
44	3.6	4.6	5.6	1.2	1.3	1.6	3.5
45	3.4	4.5	6.0	1.0	1.2	1.6	3.7
46	3.6	4.8	8.0	1.0	1.3	1.6	3.7
47	3.6	4.6	6.0	1.0	1.3	1.6	3.5
48	3.4	4.6	6.0	1.1	1.3	1.6	3.5
49	3.2	4.6	6.0	1.1	1.4	2.0	3.3
50	3.4	4.6	6.0	1.1	1.2	1.6	3.8
51	3.2	4.6	8.0	1.0	1.3	1.6	3.5
52	3.2	4.1	6.0	1.1	1.2	1.6	3.4
53	3.6	5.4	6.8	1.1	1.3	1.6	4.5
54	3.5	4.0	5.6	1.1	1.3	1.6	3.1
55	3.2	3.8	5.0	1.1	1.2	1.6	3.2
56	3.4	5.2	6.4	1.2	1.4	2.0	3.7
57	3.4	5.4	7.6	1.1	1.3	1.8	4.1
Mean	3.2	$4.5_{0.4}$[b]	8.0	1.0	$1.3_{0.06}$	2.0	$3.5_{0.3}$

[a] For identification of isolates see Appendix 7c

[b] Standard deviation

APPENDIX 10

TAXONOMY OF SPECIES WITHIN THE GENUS *POPULUS*

Much of this review is based on the article by Schreiner in 'Seeds of Woody Plants in the United States', United States Department of Agriculture, 1974.

The genus *Populus* includes approximately 40 species of large deciduous trees represented throughout Europe, Asia and America. As most species are readily propagated from stem or root cuttings, new clones may be multiplied quickly with the assurance that each tree will be the same.

Species of poplar are grouped into four sections:

(i) Section Leuce - the silver and aspen poplars.
(ii) Section Aigeiros - the black poplars.
(iii) Section Tacamahaca - the balsam poplars.
(iv) Section Leucoides - a small section comprising only two species.

A. SECTION LEUCE:

Includes the silver poplars *(P. alba)* and the aspen poplars *(P. tremula, P. tremuloides)* which are grouped under sub-sections *Albidae* and *Trepidae* respectively.

(1) Sub-section *Albidae* - *P. alba* L. or silver (white, abele) poplar. These species are endemic to central and southern Europe to western Siberia and central Asia.

(2) Sub-section *Trepidae* - The aspen poplars comprising the following species:

(i) *P. tremuloides* Michx. (*P. aurea* Tidestr.) commonly known as the quaking aspen, trembling aspen, or golden aspen. This species is endemic to the American continent.

(ii) *P. grandidentata* Michx. the big tooth aspen; endemic to North America.

(iii) *P. sieboldiana* Miq. (*P. tremula* var. villosa French & Sav.). The siebold aspen; endemic to Japan.

(iv) *P. tremula* L. the European aspen; found in Europe, northern Africa and north eastern Asia. First generation hybrids between European *P. tremula* and American *P. tremuloides* have been produced commercially by controlled hybridization in Europe. Such hybrids show superior growth to either parent species (Schreiner, 1959).

Natural hybrids of the subsections *Albidae* and *Trepidae* occur, for example *P.* x *canescens* (Ait.) Sm. (the grey poplar) which is a hybrid of *P. alba* x *P. tremula*. This hybrid species is found in Europe and western Asia.

B. SECTION AIGEIROS:

The largest section of poplars which is comprised of two main groups; the American and European black poplars.

(1) The American black poplars (cottonwood poplars). This group is quite diverse and includes the following species, all of which are endemic to the American continent.

(i) *P. acuminata* Rybd. - the lance leaf cottonwood.

(ii) *P. angustifolia* James - the narrow leaf cottonwood.

(iii) *P. deltoides* Bartr. - eastern cottonwood, Carolina poplar or necklace poplar. Included in this species are numerous varieties. For instance; *P. deltoides* var. deltoides; var. missouriensis; var. virginiana and var. occidentalis.

(iv) *P. fremontii* S. Wats. var. fremontii - the Fremont cottonwood.

P. fremontii var. Wislizenii S. Wats. - the Rio Grande cottonwood.

(2) The European black poplars *(P. nigra)* consist of a single species *(P. nigra* L.*)* occurring throughout Europe and western Asia.

The American black poplar *(P. deltoides)* was introduced to Europe before 1750AD and hybridised naturally with *P. nigra*.

The resulting 'euramerican' hybrid seedlings showed remarkable hybrid vigour and many were selected and extensively cultivated, often supplanting the parent species. To these new hybrid species were applied names such as Robusta, Serotina and Marilandica (Wilkinson, 1975). Since then many new selections of *P.* x *euramericana* (Dode) Guinier poplars have been bred and released under names such as; Giorgione, Longhi, Veneziano, or numbers; NL 2195, OP 66, NZ 5001, I78, ANU 65-27, depending on the research institute responsible for the hybridization and selection.

C. SECTION TACAMAHACA:

Species of this section are commonly known as 'balsam poplars' and are characterized by leaves with a dark green adaxial surface and white to light green abaxial surface. Leaf buds commonly exude amounts of an amber, sticky, aromatic substance 'balsam'. The Section *Tacamahaca* is the second largest section of the genus, comprising the following main species;

(i) *P. balsamifera* L. (= *P. tacamahaca* Mill., *P. candicans* Ait.) - the balsam or tacamahaca poplar which is endemic to the United States.

(ii) *P. trichocarpa* Torr. & Gray (= *P. hastata* Dode) - the black cottonwood or California poplar which is endemic to the United States.

(iii) *P. maximowiczii* Henry (= *P. Koreana* Rehd.) - the Japanese poplar occurring throughout N.E. Asia and Japan.

(iv) *P. simonii* Carr. - the simon poplar which is endemic to N.W. China and Korea.

(v) *P. laurifolia* Ledeb. (*P. yunnanensis* Dode) - the laurel poplar which is endemic to Siberia and China.

(vi) *P. szechuanica* Schneid. - the Himalayan or Tibetian poplar which is endemic to central Asia.

In addition to the above species there are numerous hybrids between these species. For example, *P.* x *androscoggin* is a hybrid of *P. maximowiczii* and *P. trichocarpa*. There are also hybrids of *P. koreana* x *P. trichocarpa* and *P. tacamahaca* x *P. trichocarpa*.

D. SECTION LEUCOIDES:

This is the smallest section of the genus comprising two commercially insignificant species, *P. lasiocarpa* Oliv. and *P. wilsonii* Schneid.

In addition to species derived from intrasectional crosses, there are also numerous cultivars derived from intersectional crosses between species of the Sections; *Leuce* x *Aigeiros*, *Aigeiros* x *Tacamahaca* and *Leuce* x *Tacamahaca*. During the last 20 years an extensive interchange of seeds, pollen, cuttings and flowering branches has occurred between research stations throughout the world and new selections bred for resistance to *Melampsora* rusts and *Marssonina* species are continually being produced. There are thousands of such poplar clones under test and hundreds in commercial use throughout the world (Schreiner, 1959).

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