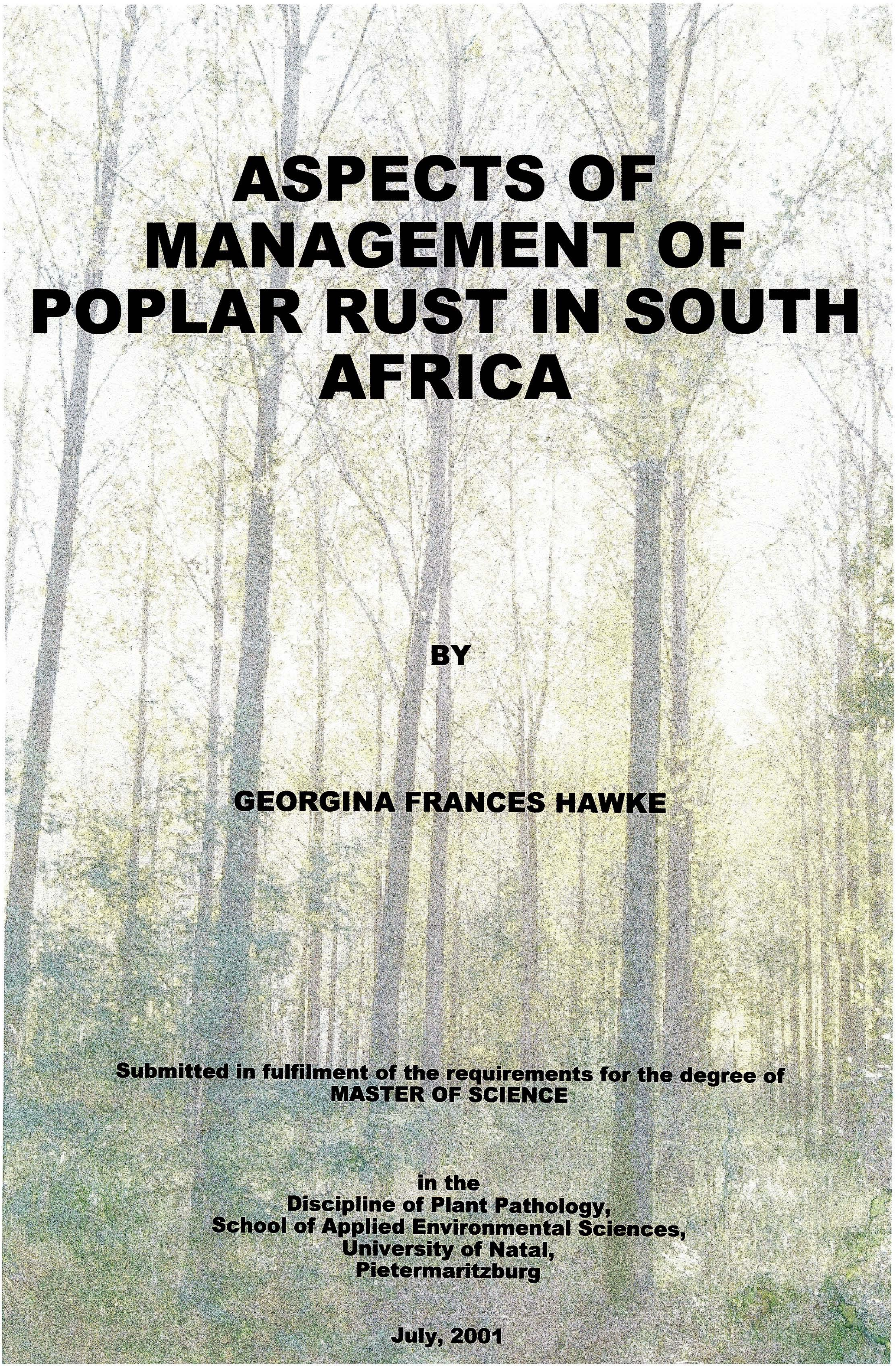

ASPECTS OF MANAGEMENT OF POPLAR RUST IN SOUTH AFRICA

BY

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DECLARATION

The experimental work described in this thesis was conducted at the University of Natal, Pietermaritzburg and the Lion Match Company Plantations, Seven Oaks, Kwa-Zulu Natal, from 1998 to 2000 under the supervision of Professor Mark D. Laing.

The results have not been submitted in any other form to another University and except where the work of others is acknowledged in the text, are the results of my own investigation.

Hawke

GEORGINA FRANCES HAWKE
March, 2002

I certify that the above statement is correct.

PROF. M. D. LAING
(SUPERVISOR)

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ABSTRACT

An assessment of infection on poplar clones grown at the Lion Match Company Redclyff Nursery in Seven Oaks, KwaZulu-Natal was made to ascertain the nature of infection of the three common species of *Melampsora* infecting poplars in South Africa. These three species are *M. larici-populina, M. medusae* and the hybrid, *M. medusae-populina.* Their contrasting wall echinulations were used to differentiate these species using a scanning electron microscope. A visual rating scale measuring percentage leaf area infected (LAI) was used to determine disease severity. Rust development was slow in new material obtained from New Zealand, indicating rust resistance. This new material was not infected with *M. larici-populina.* Plant material from Europe showed severe susceptibility to *M. larici-populina. Melampsora medusae-populina* was the most prevalent species found at Lion Match Company plantations, Seven Oaks, KwaZulu-Natal, South Africa in the survey conducted from January to April, 1998.

A once-off survey of rust infected poplar leaves from ten different locations (over 1 500km apart) was conducted to ascertain the effect of geographical and meteorological conditions on the presence and severity of rust on poplars. The most popularly grown clones in South Africa are Clone 65/29, Clone 65/31, Clone I488, Clone 129 and the old clone *Populus deltoides* var. *missouriensis.* Clone 65/31 had the greatest severity of disease (10.4%) closely followed by Clone I488 (9.5%). Clone 129 had the least amount of disease (1.8%). The most common rust species occurring in South Africa was M. larici-populina. The hybrid, *M. medusae-populina*, was the least prevalent, although race studies found this species to be the most virulent.

Four trials were conducted to determine the potential of fungicides to control rust infections on poplars. Sixteen fungicides were tested. Naturally infected poplar trees of the clone 65/31, grown in pots, were used as test material. The first trial had 16 fungicide treatments and an untreated control. Four treatments were significantly more effective than others: Alto (cyproconazole) (at 0.3ml/L) with and without the adjuvant Armoblen 600 (at 0.75ml/L), Anvil (hexaconazole) (at 0.2ml/L) and Early Impact (flutriafol + carbendazim) (at 0.6ml/L), respectively. Oxycarboxin appeared to have enhanced disease progression. Two experimental strobilurins, Stroby WG and Quadris FL (kresoxim-methyl, BASF and azoxystrobin, Zeneca) (at 0.12ml/L, and 0.4ml/L, respectively) and a new class of fungicide, Astor WG40 (experimental, Novartis) (at 2g/L) controlled rust poorly. Four treatments were used in the second trial: Quadris as a foliar spray (0.4ml/L), Impact applied on superphosphate granules ((1ml + 5g)/tree), and two controls; superphosphate alone (5g/tree) and untreated. The key finding of the second trial was that Impact gave complete control as a granular application over a 56 day period. Superphosphate alone enhanced rust development slightly. A third trial was conducted which corroborated results obtained in the first two trials: Alto plus Armoblen 600 was the best treatment, Early Impact the next best, then Alto, Anvil, superphosphate coated with Impact, Impact alone, the untreated control, Duett and lastly, superphosphate alone. The superphosphate treatment again slightly increased the disease percentages. A fourth trial was conducted with different rates of Alto (0.1, 0.2, 0.3, 0.5, 0.7ml/L, and an untreated control), applied with the standard rate of Armoblen 600. All rates of Alto gave control of the disease, the highest rate being the most effective.

A 3 x 3 x 3 factorial design was used to determine the effect of nitrogen (N), phosphorus (P) and potassium (K) on the growth of poplar trees and development of rust infection. Nitrogen (limestone ammonium nitrate (LAN) at 28% N) was applied at 0, 15.5 and 31kg/ha, K (KCl at 50% K) was applied at 0, 16.7 and 33.3 kg/ha and P (single superphosphate at 10% P) at 0, 5.3 and 10.6kg/ha. Over one year the single best tree grew 4.1 m, having received a treatment of 31kg N/ha, 10.6kg P/ha and 16.7kg K/ha. This same treatment gave the best mean growth of 3.1m. The poorest treatment was 15.5kg N/ha, 5.3kg P/ha and 33.3kg K/ha, with a mean of 1.7m in growth. The treatment of 15.5kg N/ha, 0kg P/ha and 33.3kg K/ha resulted in the lowest disease level with a mean of 23.5%

leaf area infected (LAI). Treatment with 31kg N/ha, 0kg P/ha and 33.3kg K/ha resulted in the highest disease level with 39.2% LAI. The results suggested that higher N applications increased disease susceptibility, although this trend was not significant.

TABLE OF CONTENTS

Page No.

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Leaf rust, caused by a number of *Melampsora* species, is probably the most widely distributed and serious foliar disease of the *Ageiros* and *Tacamahaca* poplars and their hybrids. There are at least eight *Melampsora* species capable of infecting these poplars, but most damage in plantation cultures may be attributed to the following three species:

i) *M. allii-populina* Kleb. (Europe, North Africa)
ii) *M. larici-populina* Kleb. (Europe, Japan, South America, Australia, New Zealand, South Africa)
iii) *M. medusae* Thüm (North America, Southern Europe, Australia, New Zealand, South Africa) (FAO, 1985; Hubbes *et al.*, 1983).

Poplars were brought into South Africa by the Lion Match Company for the purpose of making matches. One rust pathogen, *M. larici-populina*, was introduced to South Africa with plant material and has been resident here since then. In 1985, abnormally high levels of disease were observed on match poplar (*P. deltoides* var. *missouriensis*). Thelma Trench, a University of Natal Plant Pathologist, was approached to investigate the disease. *Melampsora medusae* was discovered for the first time, and found to be the cause of the new rust epidemic, as it is pathogenic on many poplar clones that have excellent resistance to *M. larici-populina* (Trench and Churchill, 1987). Poplar clones now needed to be resistant to both these species, whereas the extant clones were susceptible to one or other of the rust species. Procuring resistant poplar plant material has since been complicated by this, as many of the sources of poplar plant material do not have this same problem. The Lion Match Company made funding available to sponsor an honours project investigating poplar decline in South Africa, which was taken up by Christy Fleming. The objectives of the project were to survey five plantations in KwaZulu-Natal, determine the species infecting the plantations; investigate the ultrastructure of the causal organism of rust; develop a rust rating scale and conduct inoculation studies with *Melampsora* spores on poplar cuttings (Fleming, 1996). Further funding was committed to another honours and masters project to fulfill these goals.

The research was developed by the University of Natal and the Lion Match Company and conducted for the benefit of all poplar growers in South Africa. With current production levels the Lion Match Company have estimated that 6 000ha of poplars are required to supply the factory with all the necessary wood for match production. The hectarage in South Africa sits at little more that 2 000ha and this is declining. Current research included improving yields of existing plantations as well as improving knowledge of water use by poplars.

Poplar cultivation has been restricted as a result of the Water Policy which has inhibited timber cultivation in riparian zones. This situation is worsening due to The National Water Bill, 1998 (Gildenhuys, 1999). This is to preserve South Africa's dwindling water sources. Traditionally, poplars have been planted in vleis and wetlands as they are well known as riparian varieties/plants. Hence, the reduction of potential sites for growing poplars. Marginal areas with good soils can be supplemented with water through irrigation. A drip fertigation trial has been implemented to determine the performance of poplars under this type of intensive silviculture in the Natal Midlands. The purpose of this exercise was to maximise yields on lands that are normally considered unsustainable for growth of poplars, through lack of water. Amending the land with lime (to reduce high levels of acid saturation) was also deemed necessary for suitability of poplar production. An additional trial was planned, exploring various levels of lime. However, this trial was not carried out.

One of the most obvious methods of controlling fungal plant pathogens is with the use of fungicides. Fungicide trials were conducted to evaluate the efficacy of various chemicals in combatting poplar rust in South Africa. The discovery of a particularly effective fungicide lead to trials exploring the efficacy of different levels of this fungicide. The nature of infection of the different species of *Melampsora* occurring in South Africa was also examined in a survey of various poplar clones grown in the country. The comprehension of this survey will enable further selection and screening of material brought into this country.

This project has been industry driven and all decisions made have been based on economic and commercial suitability. In order to conform to this, frequent consultation with Harold Churchill and Charles Couchman (foresters for the Lion Match Company) took place. Most of the trials were conducted at the Lion Match Company plantations, Seven Oaks, KwaZulu-Natal. These plantations were managed by Harold Churchill and Charles Couchman, who made themselves available whenever assistance was needed.

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Chapter 1 THE GENUS *POPULUS*

1.1 INTRODUCTION

Poplars are amongst the oldest contemporary angiosperm genera, with over 125 species recorded as fossils and 30 - 40 species existing today (FAO, 1985). Trees of the genus *Populus* (poplars, cottonwoods and aspens) are widely distributed over the northern hemisphere and planted in many other parts of the world. In their native ecosystems they play a major role in the recolonisation of sites after disturbances and provide important habitats for fish and wildlife. Poplars have been closely associated with agriculture due to the fact that they are fast growing, are used as a source of fuel, fibre, lumber and animal feed, and are easily propagated vegetatively. In recent years, poplars have received increasing attention as a renewable source of biomass for energy and short-fibre finish for paper making. Managed in short rotations and under intensive culture, they have shown impressive productivity and have become a highly promising crop option, especially for marginal agricultural land. With the systematic shrinkage of forest land worldwide and an expanding human population, poplars are likely to gain importance in providing needed wood products, while at the same time contributing to a more favourable carbon balance (Stettler *et al.,* 1996a).

1.2 DEFINITION OF THE GENUS *POPULUS*

Species of the genus *Populus* are all single trunked, deciduous (or semi-evergreen) trees and most spread clonally by means of root-borne sucker shoots (soboliferous), a feature uncommon among trees. They are among the fastest-growing temperate trees, a quality tied ecologically to their role as vegetational pioneers as well as functionally to their heterophyllous growth habit. In contrast to northern oaks (*Quercus* spp.), whose entire growth consists solely of a spring flush of leaves that overwinter as well-formed primordia in the buds (preformed or early leaves), poplar shoots continue to grow after bud burst, by initiating, expanding, and maturing leaves (neoformed or late leaves) throughout the growing season (Eckenwalder, 1996).

Poplars have long been recognised as a group and have a unique combination of characteristics that distinguish them from all other genera of trees. The defining features are primarily in the reproductive structures. Like many other wind-pollinated trees, the flowers (small and closely packed into flask-shaped capsules containing a number of cottony-tufted seeds) are borne in pendant racemes (catkins) that vary in flower number and density among poplar species and sections, but are generally similar for males and females of the same species (see Fig. 1.1). Many trees with male catkins, like oaks, have different arrangements of the female flowers. Among temperate trees with female catkins, only the poplars and willows (*Salix* spp.) have seeds with a coma of cottony hairs on parietal placentas in thin-walled capsules (Eckenwalder, 1996). The flowers are wind-pollinated, and the seeds are dispersed by wind or water (Grolier Encyclopaedia, 1996). The leaves are usually long-stalked and broad, with coarsely toothed or nearly smooth margins. The overwintering leaf buds are covered by several overlapping scales, which are larger toward the tip of the bud (Grolier Encyclopaedia, 1996).

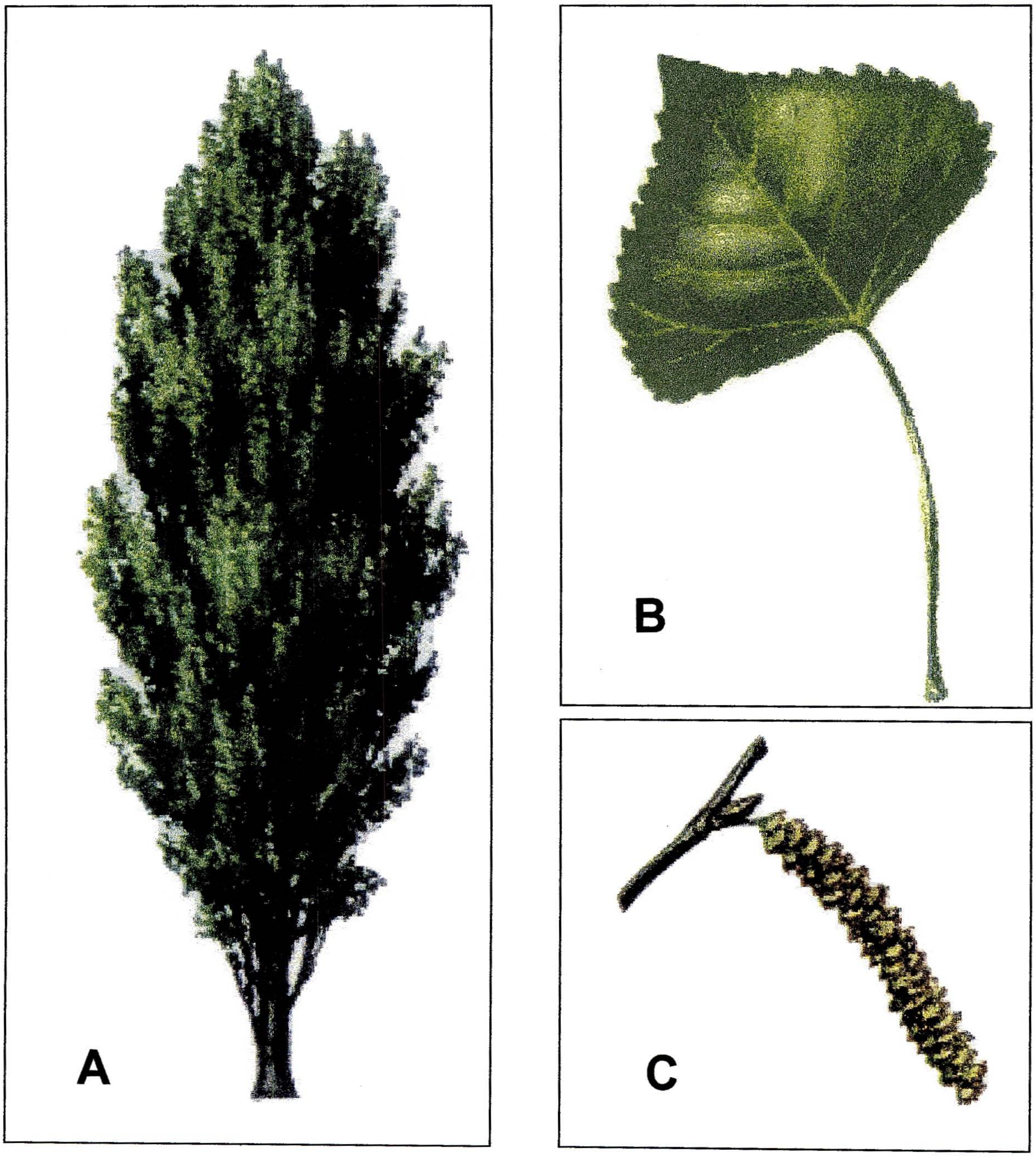


Fig. 1.1 Some key features of a typical poplar tree (Grolier Encyclopaedia, 1996) A-Gross morphology of a poplar tree, B-A poplar leaf, C-Flower, either male or female

Compared to the willows, there are relatively few species of poplars but each clearly falls into a number of morphologically and ecologically distinct groups, traditionally recognised as taxonomic Sections (Table 1.1).

Table 1.1 Species of each section with their generalised distributions (Eckenwalder, 1996)

Section	W. Eurasia & N. Africa	E. Eurasia	E. Africa	N. America
Abaso	-	-	-	*mexicana*
Turanga	*euphratica*	*euphratica* *pruinosa*	*ilicifolia*	-
Leucoides	-	*lasiocarpa* *glauca,* s.l.	-	*heterophylla*
Aigeiros*	*nigra*	*nigra*, s.l.	-	*deltoides* *fremontii*
Tacamahaca	*laurifolia*	*ciliata* *szechuanica* *yunnanensis* *suaveolens,* s.l. *simonii*, s.l. *laurifolia*	-	*balsamifera* *trichocarpa* *augustifolia*
Populus	*alba* *tremula*	*alba* *adenopoda* *gamblei* *sieboldii* *tremula,* s.l.	-	*simaroa* *guzmanantlensis* *monticola* *grandidentata* *tremuloides*

* Includes the 'true' poplars and is represented by the two species, Lombardy poplar (*P. nigra* L.) and eastern cottonwood (*P. deltoides* Bartr.), which together with their hybrids make up over 90% of all cultivated poplars in the world (Heilman, 1999).

1.3 CLIMATIC REQUIREMENTS

Most poplars are native to the northern and temperate regions of the northern hemisphere down to a 30°N latitude. However, poplars have been widely planted beyond this latitude in both the northern and southern hemispheres (Heilman, 1999). The mean annual temperatures at Pietermaritzburg and Vicksburg (a natural poplar growing area in the USA) are almost equal. The summer temperatures of the KwaZulu-Natal mist-belt compare well with Bordeaux (one of the poplar growing areas in France). However, in France, the winter temperatures are considerably lower. Poplars tolerate high summer temperatures (a mean of 27.8°C) but require low temperatures (a mean of 5.6°C) during the winter months (Tingle, 1966).

Optimal climatic conditions for growing *P. deltoides* in South Africa are the temperate summer rainfall areas of KwaZulu-Natal and Mpumalanga. Within this area, poplar growing is primarily concentrated in the mist belts of Tzaneen, Graskop, Sabie and Piet Retief in Mpumalanga and Kranskop, Greytown, Howick, Richmond, Ixopo and Harding in KwaZulu-Natal (Tingle, 1966). The mist belt, the main poplar growing area in KwaZulu-Natal, has the following characteristics:

i) an altitude 750 - 1 400m,
ii) rainfall 760mm per annum and over,
iii) mean annual temperature between 17°C and 19°C,
iv) wet and humid summers with rains from early spring,
v) winters which vary from mild to cold and with winter frosts occurring in lower altitudes.

(Kotze, 1937)

Climatic influences on plant material grown naturally has been found to affect its performance when grown in areas with a different climate. In a trial assessing material collected from natural stands, Dunlap and Stettler (1996) found that clones from cooler areas had shorter growing seasons (their spring flush occurred later, and budset earlier) than clones from warmer areas.

1.4 SOIL REQUIREMENTS

Populus deltoides grows on a wide variety of soils and gives the highest volume production on medium-textured soils which are inherently moist but well drained. In a trial conducted on two different soil types, Zivanov and Ivanisevic (1985) found that at five years of age the mean stand volume on soils classified as loamy was nearly twice that on sandy soils. Soils should have an effective rooting depth of 900mm, should be moist but well drained, friable and with a water table during summer occurring within 3m of the surface (Herbert, 1993). Alluvial soils along rivers and streams satisfy these conditions. South Africa does not have large areas of alluvial soils and flood plains. The rivers in KwaZulu-Natal fall sharply from the 3 000m high Drakensberg escarpment to the sea over a distance of approximately 160km. Therefore, poplar plantations are confined to small areas and consequently plantations are small (Tingle, 1966).

The most favourable pH range lies between 5.5 and 7.5. Alluvial soils which have an abundance of nutrients and oxygen but above all have a constant supply of moving water rank as the best sites for poplars, provided that in summer the water-table remains at about 1.5 to 3m beneath the surface. The clone chosen should be specific for the site in terms of water and soil type. Rotovation is preferred to deeper cultivation and poplars should not be planted in soils that dry out in summer (Baule and Fricker, 1970).

Some points to be noted about soils:

i) Sufficiently deep clay and clay-loam soils with a 610mm layer of loam above heavy clay are suitable for growing poplar (Herbert, 1993)
ii) Sandy-loam soils are suitable, provided they are deep and well-drained (Herbert, 1993)
iii) Coarse sandy soils in areas where the watertable may drop below 1.8m in winter and which are subject to summer drought are not suitable, e.g., sand bars in river areas of the Thornveld

iv) Soils with the top-layer removed by erosion cannot be used for growing poplar

v) A site with laterite occurring within 600mm of the surface is not suitable

vi) On areas sloping towards a river or stream the species should not be planted higher than 2.4m above the normal water level in the stream.

1.5 FERTILIZATION

Plants must obtain from the environment the specific raw materials required in the complex biochemical reactions necessary for the maintenance of their cells and for growth. In addition to light, plants require water and certain chemical elements for metabolism and growth. Under favourable environmental conditions, most green plants can use light energy to transform CO_2 and H_2O into organic compounds for their energy source. They can also synthesize all of their required amino acids and vitamins, using inorganic nutrients drawn from the environment. At least 10 of the chemical elements present in plants are necessary for normal growth. In the absence of any one of these elements, plants display characteristic abnormalities of growth or deficiency symptoms, and often such plants do not reproduce normally. Sixteen elements (carbon, hydrogen, oxygen, potassium, calcium, magnesium, nitrogen, phosphorous, sulfur, iron, manganese, zinc, copper, chlorine, boron and molybdenum) have been designated as essential chemical elements for plant growth (Raven *et al.*, 1992). Table 1.2 lists their concentration in healthy plants, the principal form in which each element is absorbed and the important functions of these 16 elements.

Table 1.2 A summary of the functions of inorganic nutrients in plants (adapted from Raven *et al.*, 1992)

Element	**Principal Form in Which Element is Absorbed**	**Usual Concentration in Healthy Plants (% or ppm of dry weight)**	**Important Functions**
Macronutrients			
Carbon	CO_2	~44%	Component of organic compounds
Oxygen	H_2O or O_2	~44%	Component of organic compounds
Hydrogen	H_2O	~6%	Component of organic compounds
Nitrogen	NO_3^- or NH_4^+	1-4%	Component of amino acids, proteins, nucleotides, nucleic acids, chlorophylls and coenzymes
Potassium	K^+	0.5-6%	Involved in osmosis and ionic balance and in opening and closing of stomata; activator of many enzymes
Calcium	Ca^{2+}	0.2-3.5%	Component of cell walls; enzyme cofactor; involved in cellular membrane permeability; component of calmodulin, a regulator of membrane and enzyme activities
Phosphorous	$H_2PO_4^-$ or HPO_4^{2-}	0.1-0.8%	Component of energy-carrying phosphate compounds (ATP and ADP), nucleic acids, several essential coenzymes, phospholipids
Magnesium	Mg^{2+}	0.1-0.8%	Part of the chlorophyll molecule; activator of many enzymes
Sulfur	SO_4^{2-}	0.05-1%	Component of some amino acids and proteins of coenzyme A
Micronutrients			
Iron	Fe^{2+} or Fe^{3+}	25-300 ppm	Required for chlorophyll synthesis; component of cytochromes and nitrogenase
Chlorine	Cl^-	100-10000 ppm	Involved in osmosis and ionic balance; probably essential in photosynthetic reactions that produce oxygen
Copper	Cu^{2+}	4-30 ppm	Activator or component of some enzymes
Manganese	Mn^{2+}	15-800 ppm	Activator of some enzymes; required for integrity of chloroplast membrane and for oxygen release in photosynthesis
Zinc	Zn^{2+}	15-100 ppm	Activator or component of many enzymes
Molybdenum	MoO_4^{2-}	0.1-5.0 ppm	Required for nitrogen fixation and nitrate reduction
Boron	$B(OH)_3$ or $B(OH)_4^-$	5-75 ppm	Influences Ca^{2+} utilization, nucleic acid synthesis, and membrane integrity

Table 1.3 Nutrient accumulations in poplars grown in western Mississippi, USA (Baker and Blackmon, 1977), Po Valley, Italy (Bisoffi, 1997) and Germany (Jug *et al.*, 1999)

	Mississippi, USA		Germany	Po Valley, Italy
	Proportion of element returning to soil via abscised leaves	Nutrient accumulation after the first season of growth of *Populus deltoides*	total bioelement removals via shoot biomass during a five year rotation	Nutrients, absorbed by a ton of poplar dry woody biomass (stem and branches) in Clone I-214 after 13 years (stand density - 280 trees/ha)
	(%)	(kg/ha)	(kg/ha)	(kg/ton)
N	26	43.5	90-270	5.6
P	33	4.7	15-45	0.7
K	28	34.7	30-180	5.1
Ca	62	36.5	55-350	11.8
Mg	51	6.4	5-25	

From Table 1.3 similar trends can be seen in the proportion of nutrients removed from each of the different sites. Application of nutrients for an optimal yield would depend on soil fertility status, population density of the stand, type of minerals being utilised for fertilizer application and method of application. Monitoring of fertilizer application rates should be supplemented with analysis of foliar nutrient levels. Table 1.4 depicts the range of foliar nutrient levels of some poplar species.

Table 1.4 Range of foliar nutrient levels adapted from Van den Berg (1990), covering the tree species (Species), elements that were analysed (El), type of stand (Type), visual deficiency symptoms (Observed), visual deficiency (Threshold), low range (Low), intermediate range (Intermediate), optimum range (Optimum) and high range (High). All macronutrients have been expressed as percent (g/100g dry matter), micronutrients as mg/kg and SO_4^{2-} as mg S/kg

Species	El	Type	Observed	Threshold	Low	Intermediate	Optimum	High
Populus deltoides Marsh.	N	Plantations			1.33-1.9	2.09-2.32		
	P				<0.17	0.13-0.18		
	K				<1.3	1.12-1.13		
	Ca					1.06-2.15		
	Mg				<0.18	0.37-0.52		
	S					0.7		
	SO_4^{2-}					6000		
Populus nigra L.	N	Amenity Trees	1.53	2.0	2.0-2.4	2.4-2.8	>2.8	
	P			0.10	0.1-0.15	0.16-0.21	>0.20	
	K			0.5	0.5-0.8	0.8-1.67	>1.5	
	Ca					2.41		
	Mg			0.10	0.1-0.18	0.18-0.28	>0.28	
	Fe		65-74			64-346		
	Mn				15-17	20-198		737
Overall	N		1.53	1.4-2	1.33-2.4	1.5-3.4	2.0- > 2.8	
	P			0.1	0.1- < 0.17	0.13-0.6	0.15- >0.2	
	K			0.4-0.5	0.4- < 1.3	0.7-2.2	0.8- >1.6	
	Ca				0.2	0.3-2.6	0.5-0.8	
	Mg			0.08-0.1	0.08- < 0.18	0.14-0.54	0.2- >0.28	
	Cu					5.1-20		
	Zn					36-1411		
	Fe		65-74			60-587	90	
	Mn			17	61	16-934	>100	481-737
	S					0.24-0.7		
	SO_4^{2-}					6000		
	B						30-70	

1.6 IRRIGATION AND WATER REQUIREMENTS

According to Bisoffi (1997), in the Po Valley, Italy, 350L of water are required to produce 1kg of dry matter. In a study initiated by the Department of Water Affairs and Forestry (DWAF), South Africa and conducted by the Council for Scientific and Industrial Research (CSIR) on transpiration of species in riparian zones, it was found that the leaf area index for poplars ranged from 0.93 - 1.45, which is considerably less than other commercially grown timber (*Eucalyptus grandis'* leaf area indices range from 2.00 - 4.60). Because of the low 'leaf area index', rainfall interception by poplar foliage is much less than other commercially grown species. During summer there is no layer of leaf mulch to inhibit rainfall penetration (DWAF report, 1999).

Table 1.5 Water usage of individual trees, poplar stands and other crops, modified from DWAF report (1999)

	Stand usage		**Individual tree usage (litres/day)**		
Season	**Water loss/day (litres)**	**Equivalent rain/day (mm)**	**Min.**	**Mid-range**	**Max.**
Spring	33 000 - 70 000	3.3 - 7.0	53	82	112
Summer	16 000 - 31 000	1.6 - 3.1	26	38	50
Autumn	10 000 - 20 000	1.0 - 2.0	16	24	32
Winter	0	0	0	0	0
Comparison of annual water use of different crops (the mid-range to maximum tree usage has been used to calculate poplar annual water requirements)					
Poplars			629-871mm		
Maize			820mm		
Citrus			1 485mm		
Sugar			1 740mm		
Wheat			620mm		
Veld			850mm		

Due to the comparatively high percentage of light filtering through to the poplar stand floor, the undergrowth development in these stands was markedly higher than under similar aged pine and eucalypt stands. The transpiration rate of a poplar tree is greater than that for eucalypts (which are known to be one of the highest water consumers) because the leaf area index of poplar is much less than eucalypts

(Table 1.5), the water usage of a stand of poplar is also much less than that of eucalypts. Furthermore, eucalypts are in general planted at a higher density than poplars (1 200 - 2 000 trees/ha for eucalypts and 625 trees/ha for poplars) (DWAF report, 1999).

Baker (1977) subjected cuttings of eastern cottonwood (*Populus deltoides*) to spring flooding for four weeks (after foliation). *Populus deltoides* was intolerant to the excess water application and the cuttings lost their leaves and died back to the root collar during flooding. However, regeneration from the root collar did take place. In another study also including flooding for four weeks, a 50% reduction in photosynthesis was recorded (Regehr *et al.*, 1975). Pereira and Kozlowski (1977) found subsequent responses to flooding to include inhibition of root growth, alterations in root and stem morphology, formation of adventitious roots, and leaf senescence. *Populus deltoides* flooding rapidly induced stomatal closure on the adaxial leaf surface as well as significantly inducing stomatal closure on the abaxial epidermis. In both long- and short-term experiments, flooding did not, however, significantly increase plant water stress (Pereira and Kozlowski, 1977). Cuttings with a water table maintained at the soil surface continuously performed worse than cuttings grown without a water table. It was concluded that growth of young *P. deltoides* would be improved with the water table raised to the lower part of the normal root zone (Broadfoot, 1973). Maintaining the soil moisture capacity above 60% field capacity was more effective than irrigating to full field capacity (Zivanov *et al.*, 1985).

Irrigation has been used successfully to increase production in poplar stands. Liu *et al.* (1988) found mean height, diameter and volume increments to be 22.0-42.6% greater after the third year in irrigated trees than those receiving only rain. Irrigation also increased stem and branch dry weight, leaf number and area, encouraging the accumulation of assimilates and their transport to branches. The increase in crown leaf area promoted volume growth (Liu *et al.*, 1988).

1.7 POPLAR BREEDING AND THE ROLE OF HYBRIDIZATION

Twelve countries have poplar breeding programs. These countries are Australia, Belgium, Canada, China, France, Germany, Italy, the Netherlands, New Zealand, Portugal, Turkey and the United States. Part of the reason for such interest in breeding is the ease of vegetative propagation of poplar. Superior hybrids can be easily and quickly cloned using stem cuttings. Therefore, within a very short time these clones can be made available for planting on a large scale (Heilman, 1999).

Domestication of poplar trees has been largely a process of interspecific hybridization and clonal selection. Hybrids have given poplars their prominence and it is believed that in no other planted forest tree have hybrids played such a pivotal role as in *Populus* spp., especially because of the high potential to cross between the 30-odd species (Zsuffa, 1975, In Stettler *et al.*, 1996b). The ease with which most poplars can be propagated vegetatively allows the bulk of a varied hybrid progeny to be ignored for the benefit of a select few that can be multiplied and perpetuated at will (Stettler *et al.*, 1996b). Rapid juvenile growth has made poplars a tree of choice to farmers who for centuries have planted them for a wide variety of uses (FAO, 1980, In Stettler *et al.*, 1996b). Emphasis on the early part of their life cycle and on favourable cultural growing conditions (i.e., high nutrient levels, control of weeds and herbivores, and irrigation) have tended to selectively enhance rapidly growing hybrid clones and to compensate for their shortcomings in sexual reproduction and allocation to defence. Thus, whereas hybrids might face considerable fitness challenges from their parental species in the wild, they can display their commercially attractive features under artificial conditions to full advantage (Stettler *et al.*, 1996b).

In a trial assessing material collected from natural stands, Dunlap and Stettler (1996) found that clones selected from an area with lower disease pressure were more susceptible to rust than clones from areas with greater disease pressure. The higher rust incidence resulted in earlier budset and leaf fall. Heritabilities indicated that spring flush, leaf fall and rust incidence were under moderately to very strong genetic control.

The main criteria selected for in breeding programmes of poplar clones in Belgium are disease resistance, adaptation to climate and max. growth. Recently, wood quality has also been included, testing the effect of various poplar pathogens on the physical and mechanical quality wood (Steenackers *et al.*, 1995).The French institute for agricultural research (INRA) has two objectives in its poplar breeding programmes: constitution of breeding populations of *P. nigra, P. deltoides* and *P. trichocarpa* and clonal selection. Breeding populations are enlarged using factorial mating designs with these three species. This is to broaden the narrow genetic base that exists in poplar cultivation in France. The Association Forest-Cellulose (AFOCEL) breeding programme focuses on short term selection with release of new clones within five years. AFOCEL also uses genetic engineering to improve poplars (Pâques *et al.*, 1995).

The targeted reconstitution of genomes through hybridization, recombination and selection is an attractive and efficient way to combine desirable traits in poplars, with the hope of seeing these new genetic constructs functioning well in typical production environments. The three main objectives for hybridization in this context are to:

i) combine desirable traits from different species
ii) capture heterosis, or hybrid vigour
iii) obtain increased developmental homeostasis, i.e., greater phenotypic stability in varied environments

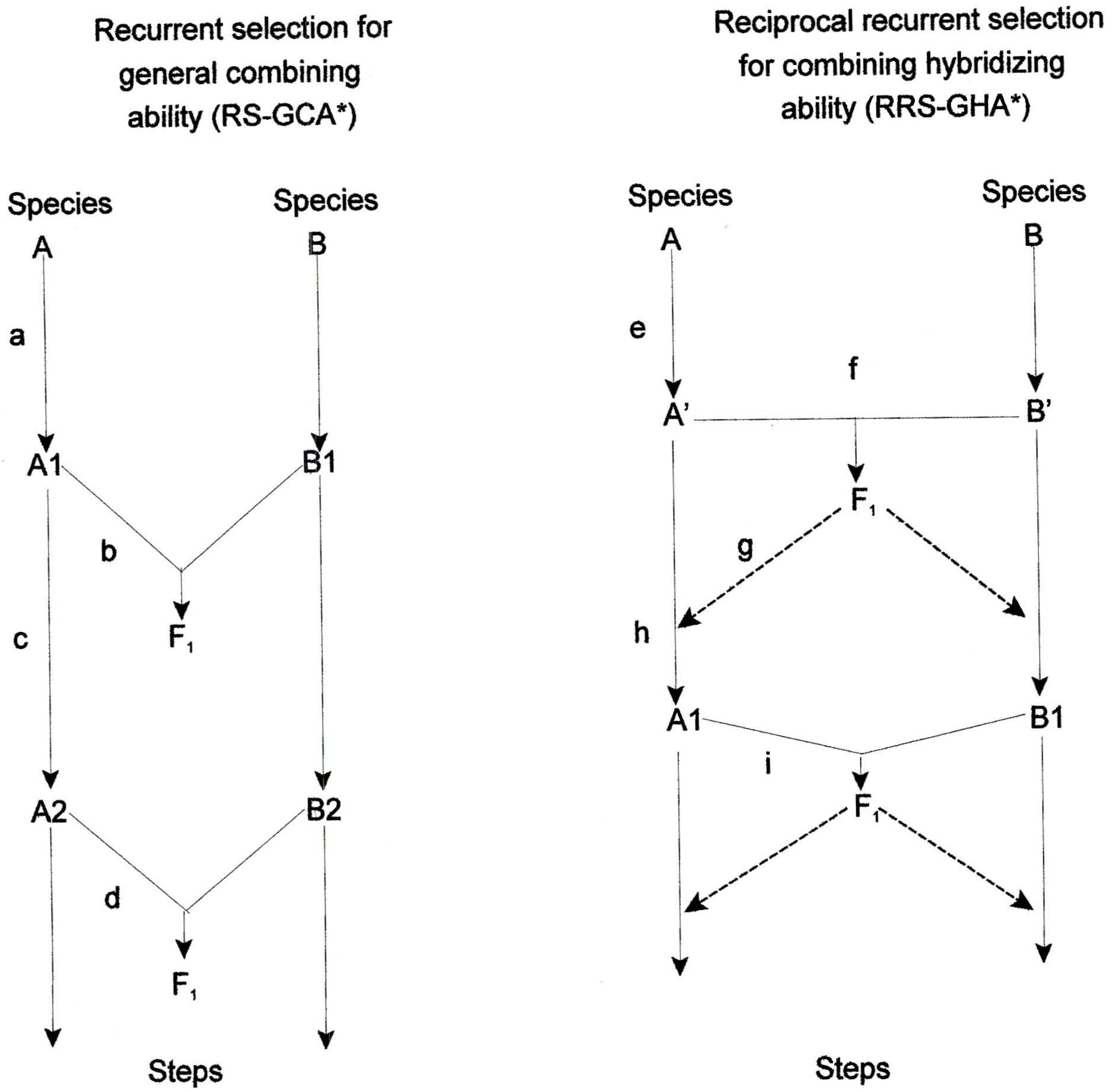
(Stettler *et al.*, 1996b).

Recurrent F_1 breeding is aimed at capturing both additive and non-addative genetic variance. It maximises heterozygosity and, therefore, captures heterosis due to dominance and overdominance. On the other hand it cannot take advantage of favourable recessives present at low frequency in one of the parental populations which may be important, e.g., in disease resistance. It sets progressively moving standards against which to measure genetic gain through hybridization. Two pathways can be distinguished:

i) recurrent selection for general combining ability (RS-GCA) (Fig. 1.2a-d)
ii) reciprocal recurrent selection for general hybridizing ability (RRS-GHA) (Fig. 1.3e-i) (Stettler *et al.*, 1996b).

RS-GCA is initiated with within-species breeding in both species to identify parents with high GCA. These are then crossed to give rise to F_1 families, within which cloned testing will identify selections for mass propagation. At the same time, intraspecific matings among high GCA parents will produce the next improved generation from which to recruit parents for the second F_1 hybrids. By contrast, RRS-GHA is initiated by crossing nonprogeny-tested (but possibly clonally tested) parents from the two species (Fig. 1.3e). Family testing of F_1 hybrids will identify parents with high GHA (Nikles, 1993, In Stettler *et al.*, 1996b). Intraspecific mating among these parents will give rise to the next improved generation in each species from which parents will be chosen for the second F_1 crosses. Gains through RRS-GHA will be greater than through RS-GCA, the greater the ratio of nonadditive to additive genetic variance, and the lower the GCA/GHA correlation. But foundation populations for RRS-GHA need to be large if crossabilities and GCA/GHA correlations are low (Nikles, 1993, In Stettler *et al.*, 1996b).

In contrast to F_1 breeding (Fig. 1.2 and Fig. 1.3), where species-specific linkages are left intact, the breeding program illustrated in Fig. 1.3 emphasizes recombination and maximizes genetic variance. It captures transgressive variation which is especially attractive in a clonable tree. It also allows for the combination of heterozygosity at some loci with homozygosity at others and, therefore, to the maximization of hybrid superiority by tapping dominance and overdominance without eliminating or masking favourable recessives. However, for this to be done efficiently, molecular analysis of an adequate number of progenies is necessary. Backcrossing permits introgression of selected genomic portions of a donor species into the genetic background of a recipient species. This procedure, too, has become remarkably streamlined through molecular tools, reducing the process of eliminating the undesirable bulk of the donor genome from eight to three generations (Tranksley *et al.*, 1989, In Stettler *et al.*, 1996b).



a. Mating and progeny testing within species

b. Selection of parents with high GCA and interspecific crossing

c. Intraspecific mating among parents with high GCA and testing of progenies

d. Selection of progenies to serve as parents for next round of interspecific crossing

e. Phenotypic selection of parents

f. Interspecific crossing of selected parents

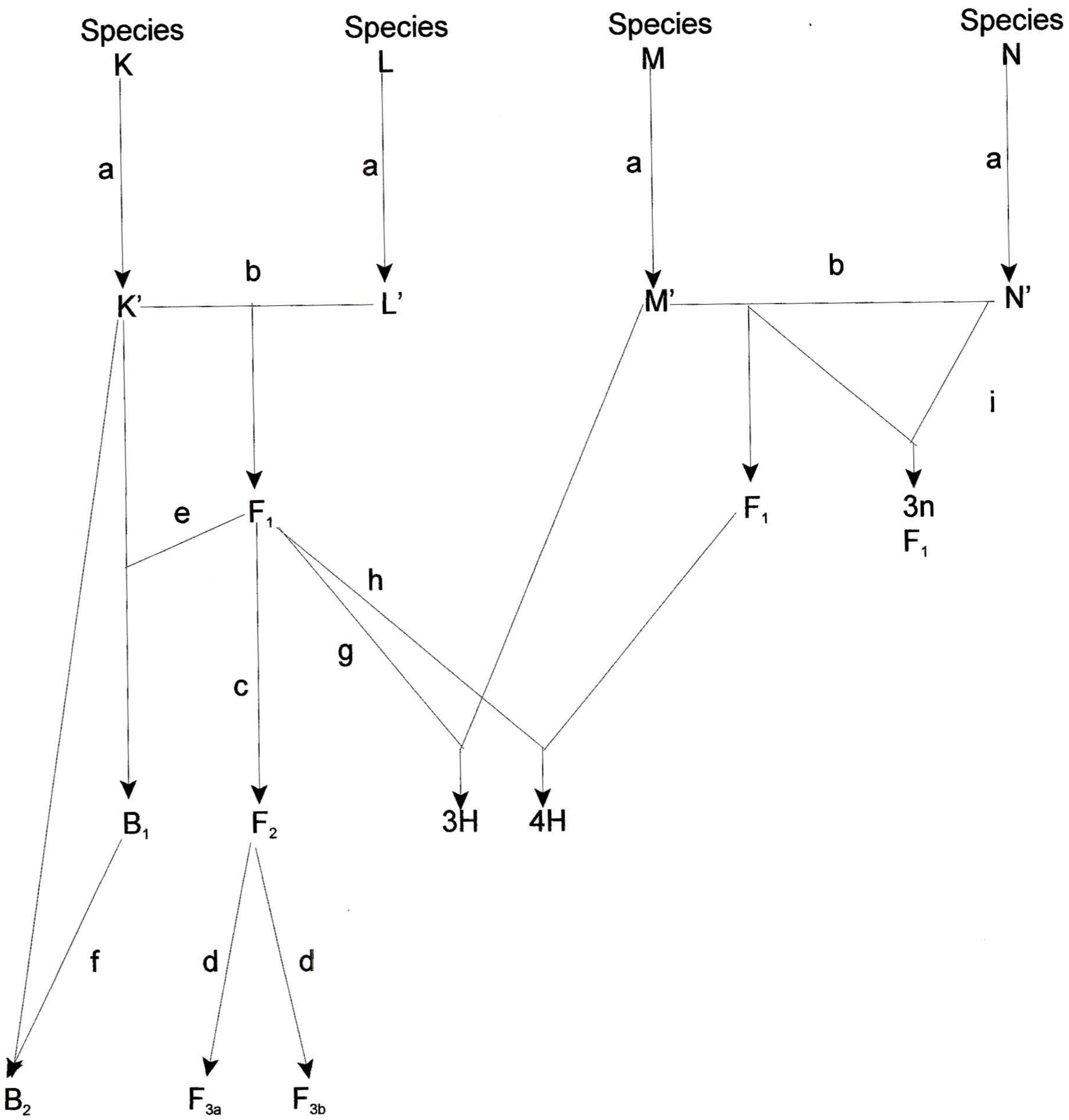
g. Testing of F_1 families and identification of parents with high GHA

h. Intraspecific mating among parents with high GHA

I. Selection of progenies to serve as parents for next round of interspecific crossing

* GCA vs. GHA: The relative ability of an individual to transmit genetic superiority to its offspring when crossed with a number of individuals of the same species (GCA), vs. with those of another species (GHA).

Fig. 1.2 The main steps in two different recurrent F_1 breeding schemes (adapted from Stettler *et al.* 1996b)



Pathways:

a.	Phenotypic selection of parents	f.	Selection and continued back-crossing to parental species
b.	Interspecific crossing among selected parents	g.	Selection and crossing with third species, resulting in 3-way hybrids
c.	Selection and mating among F_1 individuals	h.	Crossing among selected F_1 to generate 4-way hybrids
d.	Selection and mating among F_2 individuals, possible sub-lining	I.	Unreduced gametes from parent N' result in triploid F_1 hybrids (MNN)
e.	Selection and backcrossing to parental species		

Fig. 1.3 Possible pathways of advanced generation hybridization (adapted from Stettler *et al.*, 1996b).

1.8 FOLIAR DISEASES OF POPLARS

Diseases have been the major limiting factor in growing hybrid poplars and aspen (*P. tremuloides*) for biomass production in the north-central USA. One of the most critical needs for the development of poplar biomass production systems is the availability of high yielding clones that have greater disease resistance than the ones now being planted. Recent research has concentrated on screening clones for disease resistance, increasing disease resistance in poplar clones through the use of tissue culture and somaclonal selection technologies, and studying pathogen variation. Understanding of pathogen populations affecting poplars is incomplete and there is uncertainty about how much variation in pathogenicity may exist among species, races, or pathotypes. This complicates disease resistance screening and can jeopardize the success of biomass plantations (Ostry and Dietrichson, 1994). Table 1.6 lists foliar diseases that have been reported around the world.

Table 1.6 Reported incidences of foliar diseases of poplars worldwide

Disease	**Common name/ symptom**	**Regional occurrence**	**Reference**
FUNGAL			
Alternaria alternata			Singh *et al.*, 1991b; Khan, 1991;
Astrodochium coloradense		Canada, USA (Colorado)	Harrison, 1993
Bipolaris maydis (Cochliobolus heterostrophus)		India	Chauhan and Pandy, 1992
Cercospora populina	leaf blotch	India	Chauhan and Pandy, 1991
Cladosporium humile	leaf spot	India (Kashmir Valley)	Rehill *et al.*, 1988
Colletotrichum gloeosporioides (Glomerella cingulata)	leaf spot	India	Bhat and Hegde, 1990
Coryneum populinum	leaf spot	China (Jin County)	Su *et al.*, 1993
Cytospora chrysosperma (Valsa sordida)	die back	USA (Oklahoma)	Filer and Randall, 1977; Conway and Morrison, 1983
Dendropthoe falcata		India (Uttar Pradesh)	Singh *et al.*, 1991a
Diplodia gossypina		USA	Jones and Rowan, 1974
Discosporium populeum (Drepanopeziza punctiformi)		France	Pinon and Valadon, 1997
Dothiorella gregaria		Japan/China	Wang *et al.*, 1996
Fusicladium spp.		India (Kashmir Valley)	Khan, 1991
Hypoxylon mammatum	leaf necrosis	USA	Schipper, 1973; Kruger and Manson, 1993; Ostry and Dietrichson, 1994
Marssonina brunnea *M. brunnea* f.sp. *brunnea* *M. brunnea* f.sp. *trepidae* *M. castagnei* *M. populi*	anthracnose leaf spot	E. Slovakia, India (Kashmir Valley), Canada, New Zealand, USA, Germany, France	Castellani, 1970; Schipper, 1973; Heather *et al.*, 1975; Jokela *et al.*, 1976; Pinon, 1979; Gergacz, 1981; Melchoir *et al.*, 1983; Spiers and Wenham, 1983a and b; Spiers, 1984; Spiers and Hopcroft, 1984; Coleman *et al.*, 1988; Khan, 1991; Kruger and Manson, 1993; Ostry and Dietrichson, 1994; Kohan, 1996; Pinon and Valadon, 1997

Melampsora abietis-canadensis *M. aecidioides* *M. alli-populina* *M. cilliata* *M. larici-populina* *M. medusae* *M. occidentalis* *M. populnea*	leaf rust	America, India, South Africa, Europe, China, Australia	Shain, 1975; Jokela *at al.*, 1976; Rehill *et al.*, 1988; Khan, 1991; Hsiang and Chastagner, 1993; Pinon and Valadon, 1997; Harrison, 2000
Myrothecium roridum		India	Singh *et al.*, 1991b
Phoma macrostroma		India	Chauhan and Pandy, 1991
Phyllactinia guttata		S. Korea	Lee *et al.*, 1982
Phyllosticta adjuncta		Australia	Singh and Heather, 1981; Singh *et al.*, 1991b
Pollacia elegans	leaf scab	France, India (Kashmir Valley)	Taris, 1980; Rehill *et al.*, 1988
Sclerotium rolfsii (Corticium rolfsii)	leaf spot	India (Uttar Pradesh)	Singh *et al.*, 1991b; Misra and Khan, 1991
Septoria musiva *S. populi* *S. populicola*	leaf spot	USA (Mississipi) British Columbia, India	Filer and McCracken, 1971; Shain, 1975; Cooper and Filer, 1976; Zalasky, 1978; Rehill *et al.*, 1988; Khan, 1991; Mottet *et al.*, 1991; Ostry and Dietrichson, 1994; Woodbury *et al.*, 1994; Newcombe *et al.*, 1995; Newcombe and Bradshaw, 1996
Sphaceloma populi	leaf spot	India (Kashmir Valley)	Rehill *et al.*, 1988; Singh *et al.*, 1991b
Uncinula salicis	powdery mildew	India (Kashmir Valley)	Rehill *et al.*, 1988
Venturia populina		India (Kashmir Valley)	Koul *et al.*, 1989
BACTERIAL			
Aplanobacter populi		Netherlands	Gremmen and Koster, 1972; Ride *et al.*, 1977
Micrococcus populi		Slovakia	Kohan, 1996
VIRAL			
Poplar mosaic virus		E. Germany, Poland	Boccardo *et al.*, 1973; Edwards *et al.*, 1986; Kontzog, 1991; Pinon and Valadon, 1997

1.9 POPLARS IN SOUTH AFRICA

In South Africa poplar is grown primarily for the manufacturing of matches. The match industry absorbs the total local production of logs with a diameter of more than 200mm over bark. The timber between 120-200mm is converted to wood wool for fruit and industrial packing material (Tingle, 1966).

Figures 1.4 - 1.10 are a compilation of some of the promising clones grown by the Lion Match Company. Some of the clones are still being evaluated. The photographs were taken on the 1st of April, 1997 and key identifying features (the bark morphology, gross morphology, leaf shape and apical area) were taken to compare the different clones. A hailstorm occurred prior to taking the pictures and this has caused the extensive leaf damage that appears in the pictures. Figure 1.4 is of Clone 65/29. This hybrid was formed from *P. deltoides* 60/164 (Stoneville) x *P. nigra* cv. *chile* and is one of the fastest growing clones in South Africa. Strong apical dominance has results in a tall, straight trunk (Edwards, 1998). This clone also has a smooth bark.

Figure 1.5 is of Clone 65/31. This hybrid has the same parentage as Clone 65/29. However, it appears to be more susceptible to rust infection (Fig. 1.6). The appearance of this clone to Clone 65/29 is very similar due to their parentage. The bark may be smoother than Clone 65/29. Figure 1.6 shows a stand of poplars of two different genotypes, on the right is the more susceptible Clone 65/31 (the susceptibility is shown by the less dense foliage which has a yellow colour), and on the left is the more resistant Clone 65/29.

Figure 1.7 is of Clone I 488. The parentage of this clone was unknown to the author at the time of writing. The bark is slightly more rough than the two previous clones. A distinguishing feature of this clone is the lower level of apical dominance (Edwards, 1998), causing extensive branching among the canopy of the individuals. In some cases (Fig. 1.7B), apical dominance has been completely lost, and the upper part of the tree is comprised of many clustered branches.

Another means of distinguishing one clone from another is by their time of flushing. At the time these pictures were taken, Clones 65/29 and 65/31 were dormant, whereas Clone I 488 was flushing (Fig. 1.7C).

Figure 1.8 shows the crooked tree form of Clone 70/51. This is not desirable for match making as the machines are designed only to carry straight pieces of wood. In this nursery, this clone has been found to be slow growing (Churchill, 1998) and is therefore not grown commercially in South Africa. The bark of this clone is slightly rough.

Figure 1.9 shows the Clone 60/112. This is not a commercially grown clone due to its slow growth and thin trunk. In some trees the bark is smoother than in others and from Fig. 1.9C, it can be seen that the shoot is just beginning to flush. This picture also shows that the abscission zone may be weaker than in other clones due to the fact that some of the leaves have already dropped from the shoot. The parentage of either of these two clones was not known by the author at the time of writing.

Figure 1.10 is of Clone 129. This clone is grown commercially and is one of the older clones in South Africa. Although this clone is slow growing, it has the reputation of being relatively resistant to the *Melampsora* rust (Churchill, 1998). The bark appears to be rough, with vertical striations (Fig. 1.10B). Note that the flushing is quite advanced, although not as advanced as I 488 (Fig. 1.7).

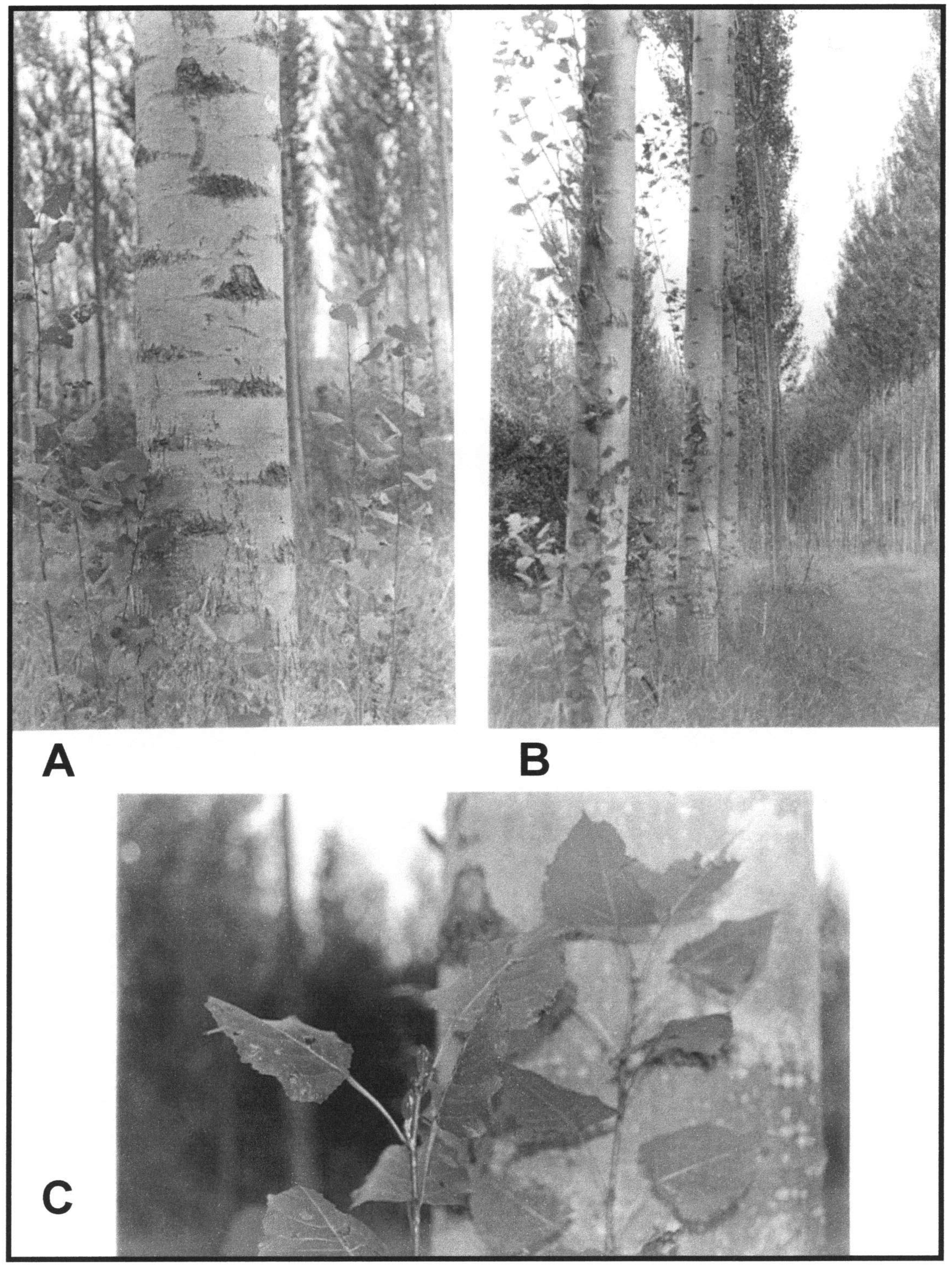


Fig. 1.4 Distinguishing features of the Clone 65/29 grown at the Lion Match Company Ltd, plantations, Seven Oaks, KwaZulu-Natal. A - bark, B - gross tree morphology, C - shoot tip

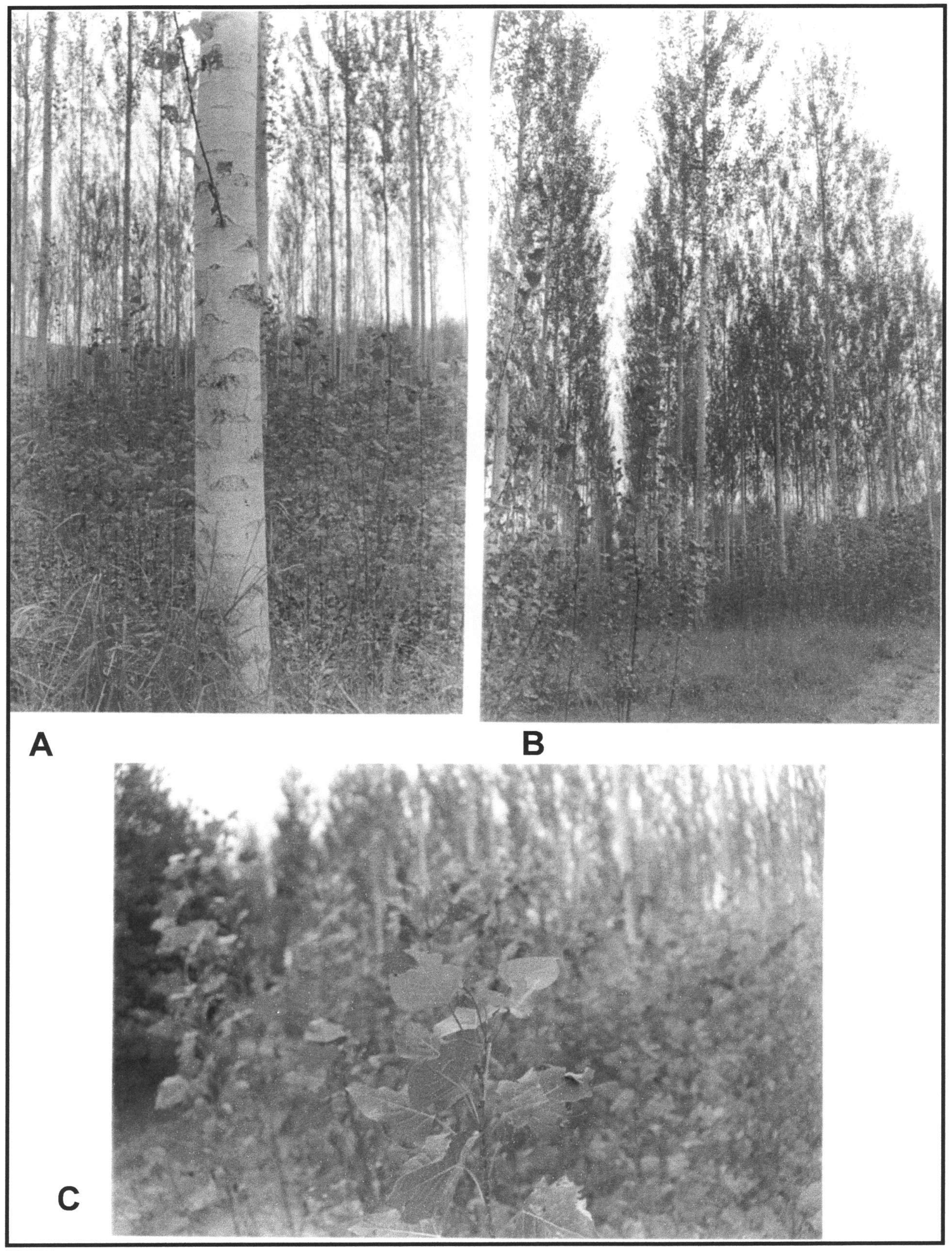


Fig. 1.5 Distinguishing features of the Clone 65/31 grown at the Lion Match Company Ltd, plantations, Seven Oaks, KwaZulu-Natal. A - bark, B - gross tree morphology, C - shoot tip

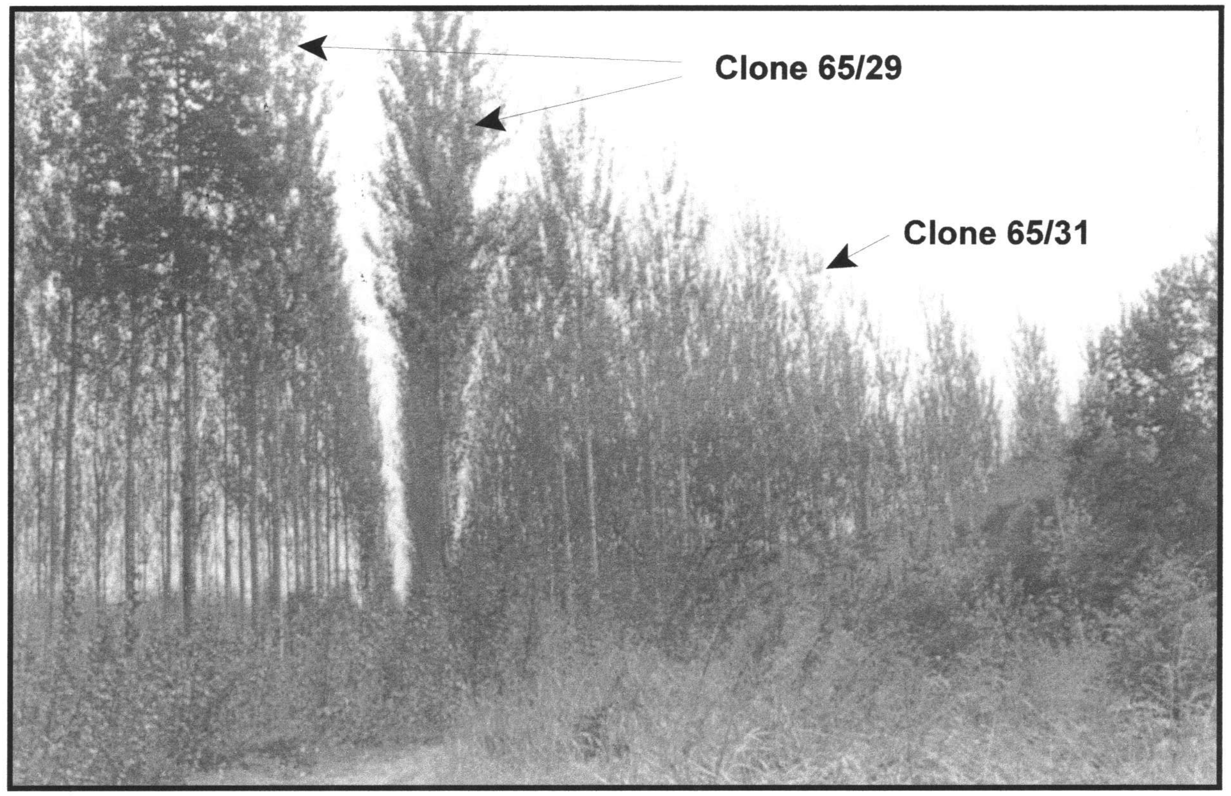


Fig. 1.6 Two stands, Clone 65/29 (left) and Clone 65/31 (right), grown at the Lion Match Company Ltd, plantations, Seven Oaks, KwaZulu-Natal. The susceptibility of Clone 65/31 is apparent due to its lighter colour when comparing with the more resistant Clone 65/29 which has a darker green appearance

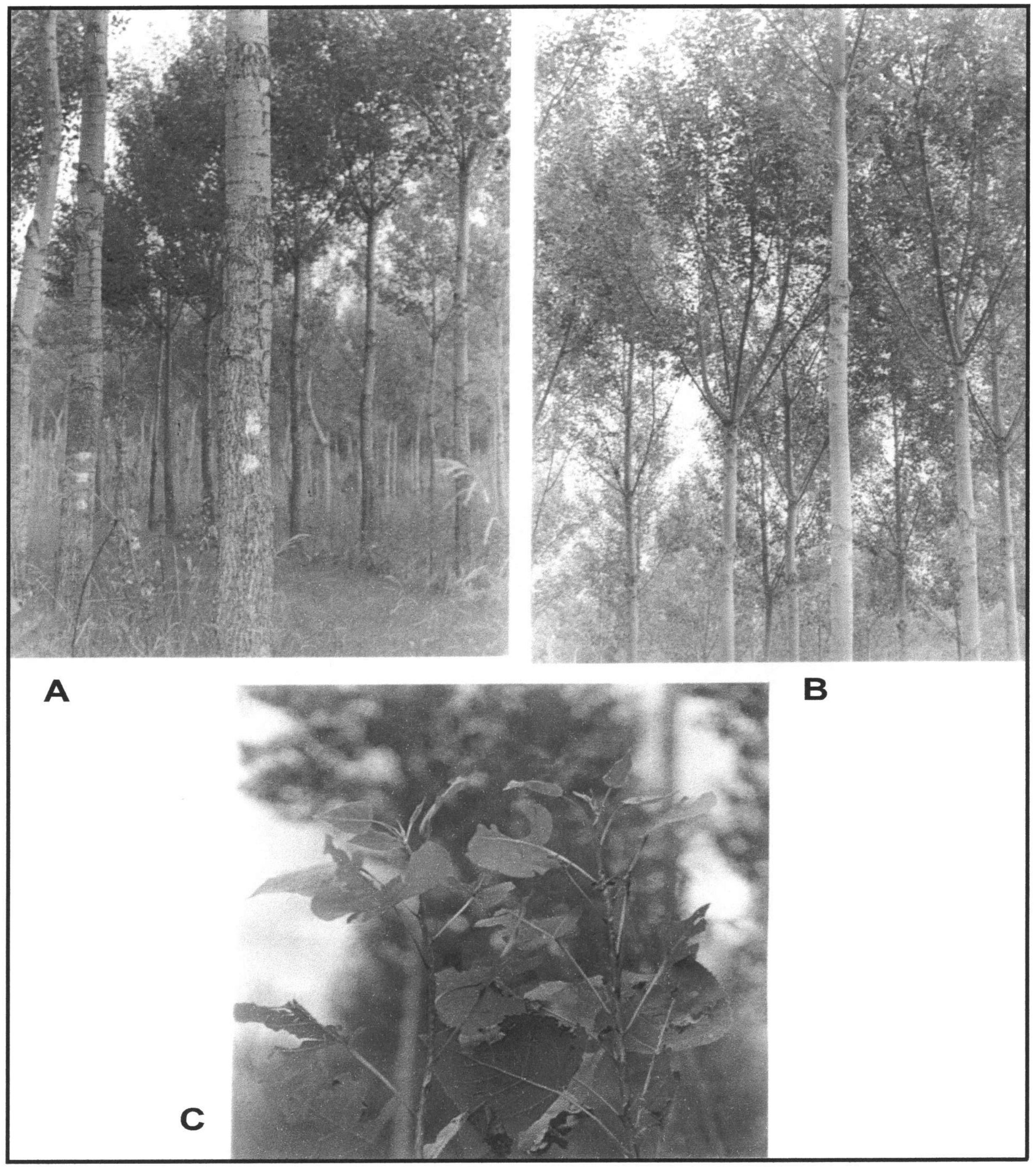


Fig. 1.7 Distinguishing features of the Clone I488 grown at the Lion Match Company Ltd, plantations, Seven Oaks, KwaZulu-Natal. A - bark, B - gross tree morphology, C - shoot tip

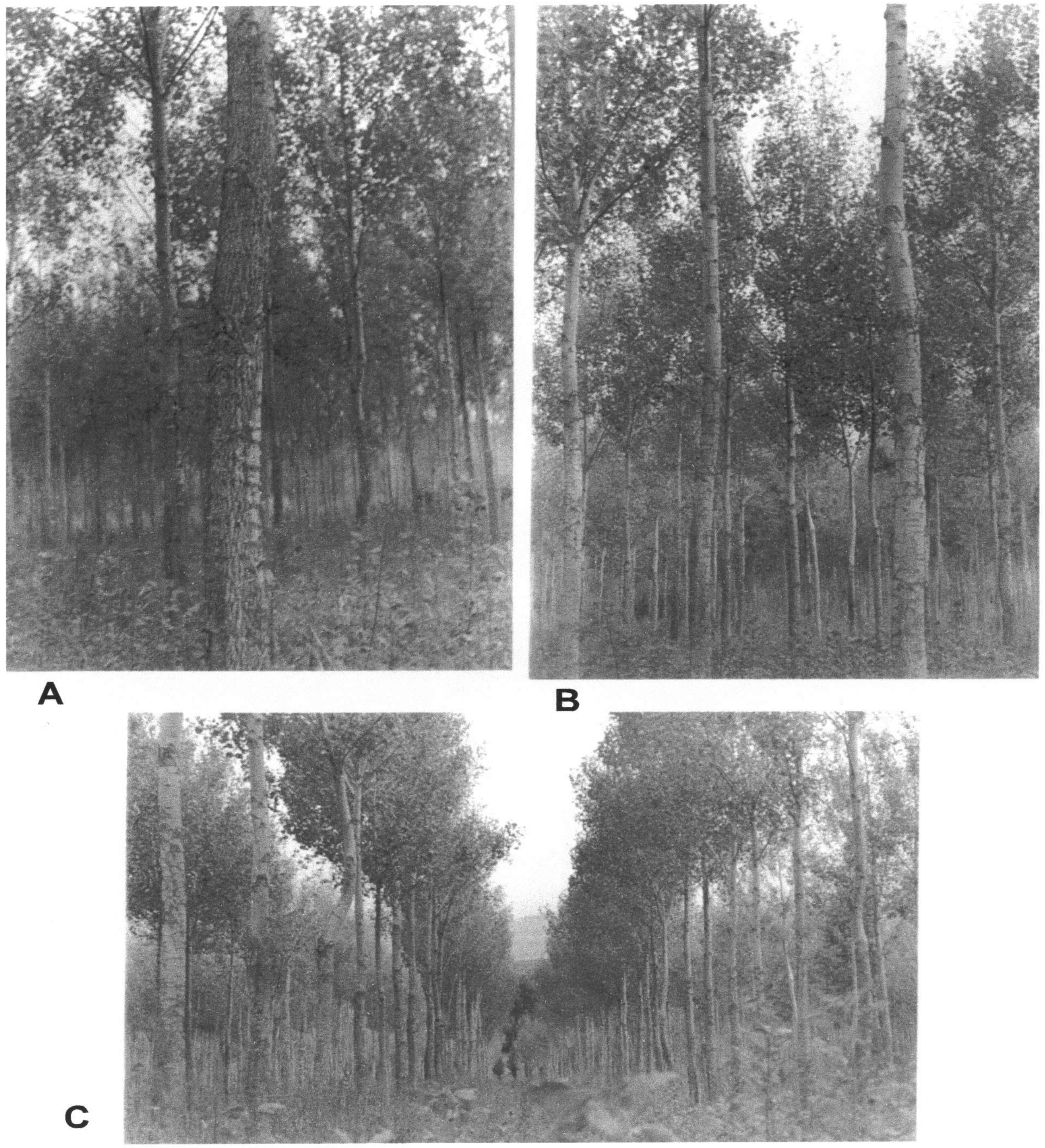


Fig. 1.8 Distinguishing features of the Clone 70/51 grown at the Lion Match Company Ltd, plantations, Seven Oaks, KwaZulu-Natal. A - bark, B - gross tree morphology, C - shoot tip

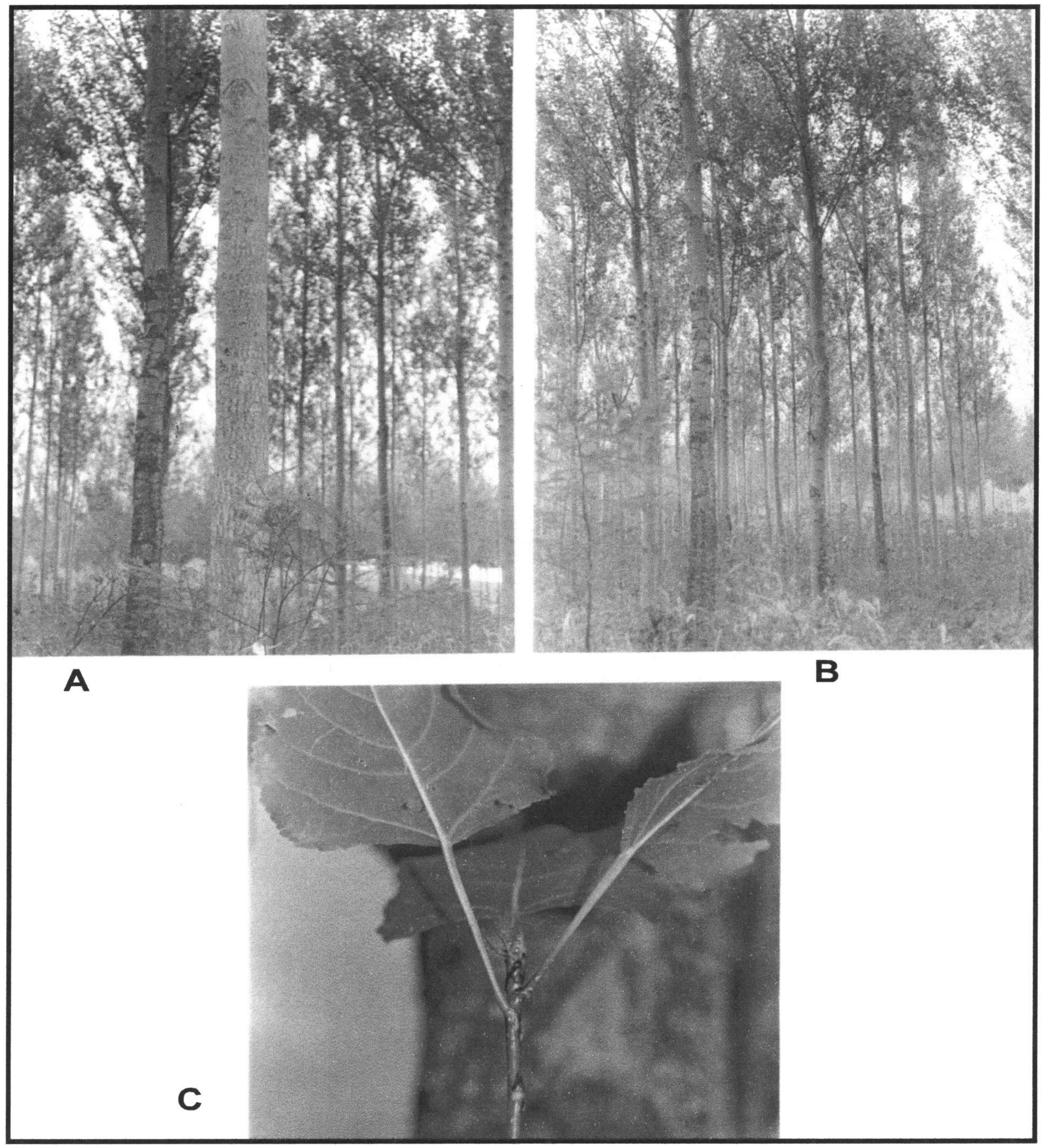


Fig. 1.9 Distinguishing features of the Clone 60/112 grown at the Lion Match Company Ltd, plantations, Seven Oaks, KwaZulu-Natal. A - bark, B - gross tree morphology, C - shoot tip

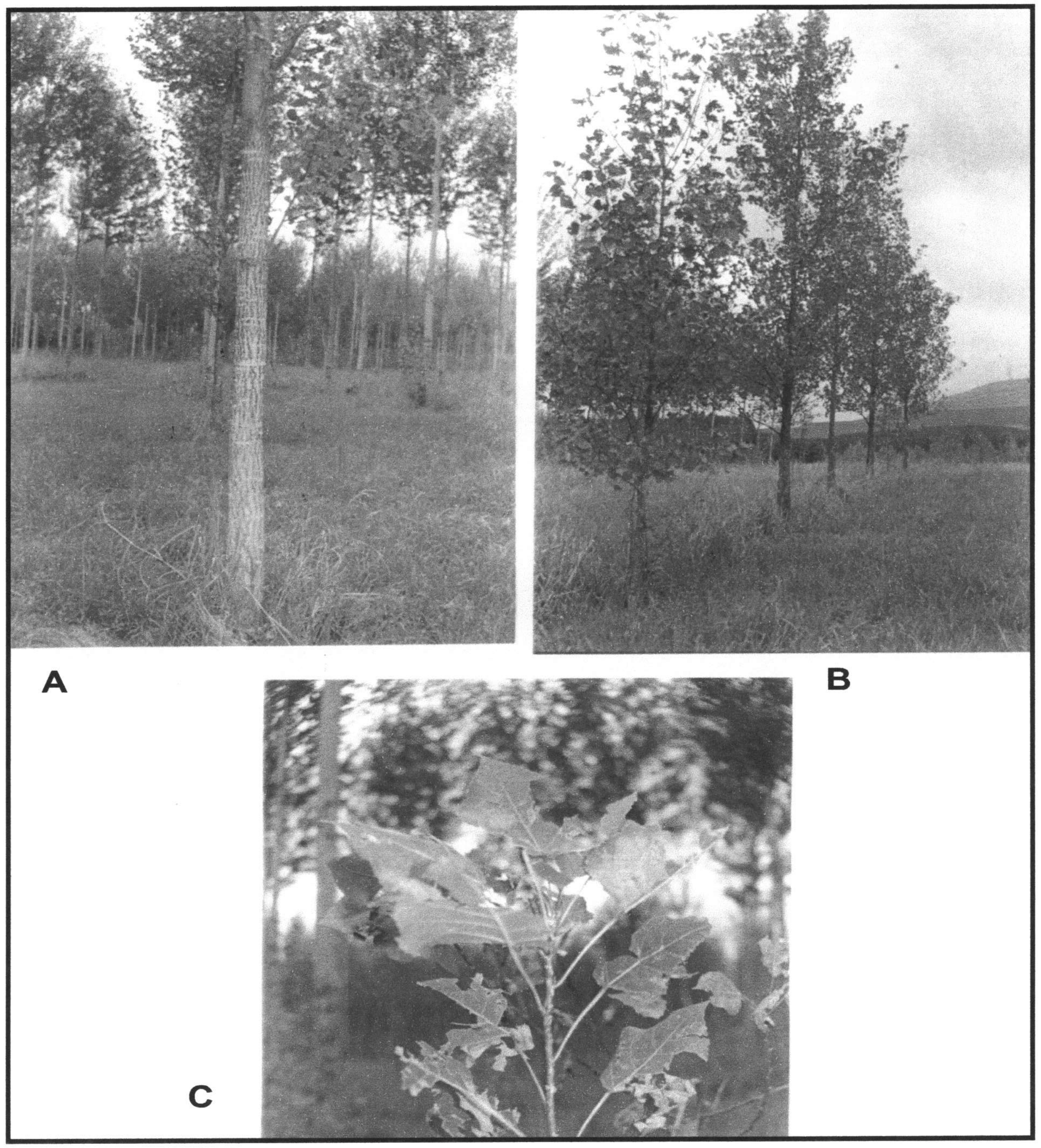


Fig. 1.10 Distinguishing features of the Clone 129 grown at the Lion Match Company Ltd, plantations, Seven Oaks, KwaZulu-Natal. A - bark, B - gross tree morphology, C - shoot tip

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Chapter 2 POPLAR RUST: *MELAMPSORA* SPP.

2.1 INTRODUCTION

Wherever poplar cultivation has been undertaken around the world, a number of fungal pathogens of *Populus* have also been introduced. Poplar leaf rust caused by the genus *Melampsora* Cast. is one of the most damaging leaf diseases of hybrid poplar plantations (Hubbes *et al.*, 1983). The most striking examples are *Melampsora larici-populina* Kleb. and *M. medusae* Thüm. f. sp. *deltoidae* Shain. Originally Eurasian and North American, respectively, these two species now occur in almost all parts of the world where hybrid poplars are grown (Newcombe and Chastagner, 1993a and b). *Melampsora larici-populina* had a significant, indirect effect on Argentina's fruit industry early last century. There, plantations of introduced poplar supplied wood for shipping containers used for fruit export, and for many years lumber production for these crates was markedly reduced by poplar rust until resistant clones of poplar were introduced (Littlefield, 1981). In native stands, however, poplar competes well with adversities, especially diseases, as a result of natural selection and a broad spectrum of genetic diversity (Ostry and McNabb, 1985).

The economical importance of rust infection is very variable, depending on the climatic conditions occurring throughout the year, the microclimate of the site, the proximity of the alternating host and the sensitivity of the primary host. The infection leads to an early defoliation which can result in a lack of lignification before winter which weakens the plants and makes them more susceptible to other adverse conditions (parasitic or edaphic). Reduction in growth through weak development of the root system also results and affects growth more so in the diameter, but also height. In severe infections these combined effects lead to the death of the plant (FAO, 1979).

Rust was first observed to be associated with abnormally severe damage on match poplar (*P. deltoides* var. *missouriensis*) in Greytown, KwaZulu-Natal, South Africa in December, 1985. This occurred in plantations belonging to the Lion Match Company. Similar symptoms were later observed in areas extending from south-eastern

Mpumalanga to southern KwaZulu-Natal, and in Zimbabwe (Trench *et al.*, 1988). Rust species known to occur in South Africa are *M. medusae, M. larici-populina* and *M. aecidioides* (D.C.) Shroet.. The presence of this species has not been confirmed by this author, however, Doidge (1927; 1950) and Gorter (1977) are among those that have collected samples of this species. Van Reenen (1995) describes *M. aecidiodes* as being *M. populnea* (Pers.) Karst. and the Commonwealth Mycological Institute (CMI) mention a synonym of *M. populnea* as being *M. pinitorqua* Rostrup (CMI, 1987). Chapter Three of this thesis discusses the discovery of the rust hybrid, *M. medusae-populina* (Hawke and Laing, 2001) in South Africa, a hybrid that has formed between *M. medusae* and *M. larici-populina.*

The incidence of rusts in poplar culture is such that the resistance criteria constitutes an important factor in selection. *Melampsora larici-populina, M. allii-populina* and *M. medusae* are poplar rusts whose damages are the greatest to the extent of compromising the cultivation of poplars in some countries (FAO, 1979).

2.2 TAXONOMY

The genus *Melampsora* is classified according to Agrios (1988) as follows:

DIVISION:	Eumycota
SUB-DIVISION:	Basidiomycotina (sexual basidiospores on basidia)
CLASS:	Hemibasiomycetes (basidia with cross-walls)
ORDER:	Uredinales (sperm cells fertilise receptive hyphae in spermagonia)
FAMILY:	Melampsoraceae (the teliospores are laterally united into crusts or columns)

The type species of the genus is *Melampsora euphorbiae* (Schub.) Cast.. Both autoecious species, e.g., *M. lini*, and heteroecious species, e.g., *M. medusae, M. larici-populina* and *M. occidentalis*, occur within the genus (Littlefield, 1981).

2.3 IDENTIFICATION

The Melampsoraceae family is recognised by the lateral union of the teliospores into subepidermal layers, crusts or columns (Alexopoulos and Mims, 1979). *Melampsora* and *Puccinia* species are identified by their respective telial stage morphologies. In the genus *Melampsora* there are five distinct fruiting structures with five different spore types (pycniospores, aeciospores, uredospores, teliospores and basidiospores). These have been called Stages 0, 1, 2, 3, and 4, respectively (Littlefield, 1981).

2.3.1 Pycnia Bearing Pycniospores and Receptive Hyphae (Stage 0)

Pycnia are subcuticular or subepidermal and produce receptive hyphae and haploid spermatia or pycniospores. These pycniospores are borne at the tips of sporophores (the spore-bearing cells), within the pycnia. They are small, haploid, uni-cellular spores that function as male gametes and are incapable of reinfecting the host. The flexuous hyphae act as the 'female' receptive structures with which the 'male' pycniospores fuse to initiate the dikaryotic N+N phase. Successful mating is only possible between + and - mating types i.e. between two separate pycnia. The pycnia may also contain sterile hairs called paraphyses which aid in the rupture of the overlying host epidermis (Littlefield, 1981).

2.3.2 Aecia Bearing Aaeciospores (Stage 1)

Aecia, usually on the abaxial leaf surface, bear chains of dikaryotic aeciospores on aeciosporophores or sporogenous cells which arise from large multi-nucleate cells in the aecium. An aeciospore (binucleate) initial is delimited from the mother cell by a septum. The initial then divides again to form a binucleate aeciospore and a small wedge-shaped, binucleate intercalary or disjunctor cell. This division process is repeated, resulting in a chain with the oldest spore at the top and the youngest at the bottom near the sporogenous cell, at the aecial base. A peridium usually arises around the spore chains (cup-like), and it may form a complete dome. When the aecium matures, the chains push through the peridium roof (Alexopoulos and Mims, 1979). The aecia of *Melampsora* are subepidermal in origin with the aeciospores catenulate and verrucose (Cummins, 1959). The aecia are diffuse instead of cup-shaped and the aeciospore chains are usually four to five spores in length. Such diffuse aecia are termed caeomata meaning rudimentary. The aeciospores have gently roughened walls and are orange-red in colour. Aeciospore infection occurs typically through host stomata and the mycelium which develops is dikaryotic (Cummins, 1959).

2.3.3 Uredia Bearing Uredospores (Stage 2)

Uredia of *Melampsora* are erumpent, with capitate paraphyses (Cummins, 1959). They develop subepidermally from dikaryotic mycelia originating from the germination of aeciospores or uredospores (Alexopoulos and Mims, 1979).

Spores are formed from buds in the sporogenous cells. The bud enlarges and is then divided by a septum into two cells. The upper cell enlarges into the spore, while the lower cell develops into a stalk-like pedicel. The spores press against the host epidermis from the inside and rupture it. The uredospores are dikaryotic with thick walls (Alexopoulos and Mims, 1979). They are echinulate or verrucose as well as being globose to oval in shape and yellow-orange in colour (Cummins, 1959).

2.3.4 Telia Bearing Teliospores (Stage 3)

Telia are groups of binucleate cells that give rise to special thick-walled cells called teliospores (synonym teleutospores). In many rusts, old uredia are actually converted to telia. Teliospores, which may be unicellular or composed of two or more cells, are formed from the tips of binucleate cells of the telium. Each cell of the spore is at first dikaryotic, but eventually karyogamy takes place, rendering each cell diploid and uninucleate. Most rusts overwinter in the teliospore stage, but in some species, the teliospore germinates soon after it is formed. In teliospores consisting of more than one cell, each cell is capable of germinating and giving rise to basidiospores (Alexopoulos and Mims, 1979).

The teliospores of *Melampsora* spp. are subepidermal in origin, or rarely subcuticular, not erumpent, and consist of crusts of laterally adherent spores, one spore layer in depth. The crusts form in a layer between the host mesophyll and the epidermis. Their teliospores are unicellular, sessile (some species have spore-like but presumably sterile cells below the teliospores), there is one germ pore and the wall is pigmented (Littlefield, 1981).

2.3.5 Basidia Bearing Basidiospores (Stage 4)

A teliospore is sometimes termed a probasidium as it is from this thick-walled structure that a metabasidium emerges. The diploid nucleus migrates into the metabasidium, undergoes meiosis and produces four haploid nuclei that distribute themselves at about equal distances in the metabasidium. Septa divide the metabasidium to form four uninucleate cells which later form four sterigmata and these, in turn, form four basidiospores (Alexopoulos and Mims, 1979). The basidium of *Melampsora* spp. is external (Cummins, 1959).

Basidiospores germinate, when a suitable host is found and conditions are suitable, to form a germ tube with a terminal appressorium. A penetration peg forms which penetrates directly through the host cuticle and epidermis and gives rise to an haploid mycelial thallus within the host. The *Melampsora* fungi obtain their nourishment from the host cells using haustoria (Littlefield, 1981). When the intercellular hyphae reach the host cell, the terminal portion is delimited by a septum. This portion is equivalent to the haustorial mother cell (HMC). From the HMC a penetration peg forms that penetrates the host cell wall and invaginates the plasma membrane (Cummins, 1959).

The CMI descriptions of pathogenic fungi and bacteria (Walker, 1979) describe *M. larici-populina* as follows:

> "Heteroecious-macrocyclic. *Pycnia* amphigenous, subepidermal, to 150μ high, pale yellow. *Aecia* on yellow spots, caeomoid, with a rudimentary cellular peridium, to 1mm diameter, orange. *Aeciospores* globoid to ovoid, 17-11 x 4-

19μ, wall colourless, finely verruculose, to 1.5μ thick (descriptions of pycnia and aecia adapted from Klebahn (1989) and Taris (1968) both in Walker, 1979). *Uredinia* subepidermal, erumpent, mainly hypophyllous, some epiphyllous especially in heavy infections, golden yellow to pale orange, often in groups of up to eight or more sori, 200-400μ in diameter, often surrounded by a zone of yellowed leaf tissue to 1mm wide, showing on upper leaf surface as angular yellow spots. *Urediniospores*", (Fig. 2.1A, D and E) "clavate to broadly ellipsoid, some oval to ovate, a few obovate to irregular, apex rounded, truncate base (25) 30-44 (50) x (12) 14-19 (22)μ, a few longer, contents golden yellow, wall colourless, 1.5-2μ thick above and below, thickened equatorially on two sides to 5μ. Wall surface echinulate, except for smooth patch 5-9μ wide on, or slightly to one side of, the apex; spines 1.5-2μ high, 0.7 (1)μ in diameter, 1.5-3μ apart, largest on lower half of spore and progressively smaller towards the smooth apical patch. Germ pores are not readily seen, germination from up to 4 scattered points on the spore surface. *Uredinial paraphyses*", (Fig. 2.1B, F) "abundant, scattered through the sori, capitate, 65-75μ long, stalk 4-6μ wide, swollen apex roughly spherical 14-21μ diameter to pyriform up to 25 x 17μ, wall colourless, or yellowish in old weathered sori, 1.5-2μ at sides, strongly thickened apically to 5-10 (12)μ, a few thicker, thick wall often showing laminations, the lumen of the capitate apex much reduced or eliminated by the wall thickening. *Telia*", (Fig. 2.1C) "develop on older infected leaves, abundant on fallen leaves, mainly epiphyllous, a few hypophyllous especially in heavy infections, at first pale amber darkening to deep reddish brown or almost black, colour due partly to discoloured host epidermal cells, slightly raised, subepidermal, roughly circular to angular or irregular in outline, 200-300μ in diameter, sometimes four or more fused into a larger composite sorus. *Teliospores* unicellular, roughly cylindrical in side view, angular in cross section, 35-50 x 7-12μ, often borne on one or two hyaline rectangular cells 10-12 x 7-9μ; teliospore wall hyaline to pale yellowish brown, 1μ thick, sometimes slightly thicker at the flattened apex.

M. larici-populina is distinguished from the several other *Melampsora* species on *Populus* by the combination of its strongly apically thickened uredinial paraphyses, its apically smooth, elongated uredospores with wall equatorially thickened on two sides, and its predominantly epiphyllous telia.

Pycnia and aecia on *Larix,* especially *L. decidua* and *L. leptoleptis*. *Uredinia* and *telia* on *Populus* spp., especially *P. nigra* L. and its varieties and hybrids, *P. balsamifera, P. yunnanensis* and others. Its exact host range on *Populus* spp. is not clear due to confusion with other *Melampsora* spp. and to uncertainty in the reported identity of some *Populus* spp. and clones."

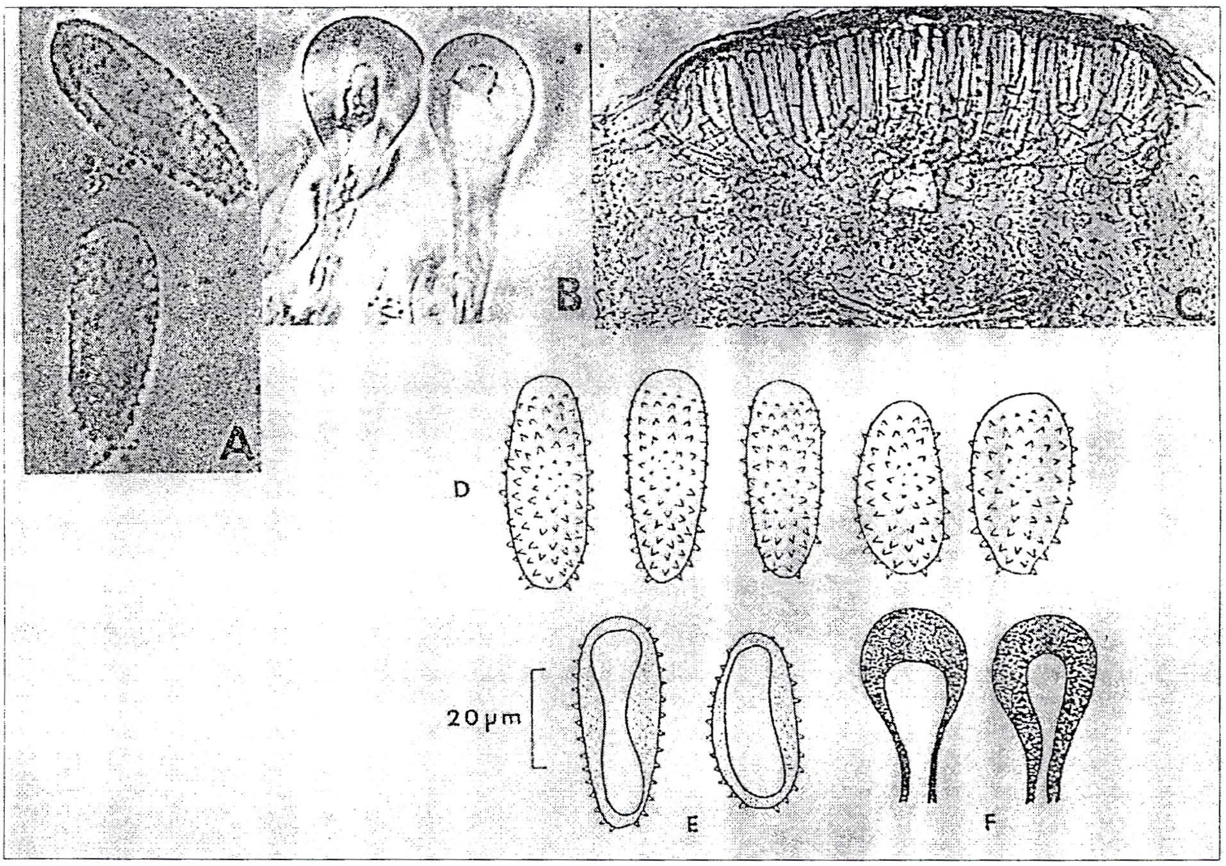

Fig. 2.1 Morphology of the various spore types and structures of *M. larici-populina* A, Uredospores x 800; B, uredinial paraphyses x 800; C, telium x 208; D-F, uredospores and paraphyses. (Photos: A. Searle from CMI descriptions of pathogenic fungi and bacteria, Walker, 1979)

The CMI descriptions of pathogenic fungi and bacteria (Walker, 1979) describe *M. medusae* as follows:

> "Heteroecious-macrocyclic. *Pycnia* mainly epiphyllous, subcuticular, scattered, pale yellowish, 40-55μ in diameter. *Aecia* mainly hypophyllus, caeomoid, numerous, pale yellowish, up to 0.5mm in diameter. *Aeciospores* catenulate, roughly spherical, 17-22μ in diameter, wall colourless 2.5-3μ thick, minutely roughened. *Uredinia* sub-epidermal, erumpent, mainly hypophyllous, a few smaller epiphyllous, golden yellow to orange, 150-250μ in diameter, showing as pale flecks on the upper leaf surface on some hosts. *Urediniospores*", (Fig. 2.2A, E and F) "obovate to oval, a few pyriform, apex rounded, truncate base, (23) 26-35 (37) x 15-19 (21)μ, golden yellow contents, wall colourless, 1-0.5μ thick above, to 2μ at base, and thickened on two sides to 3-5 (5.5)μ. Wall surface echinulate, except for a smooth equatorial patch 8-12μ wide usually extending more than half-way around the spore, rarely as a completely smooth equatorial band; spines 1-1.5 (2.5)μ high, 0.5-0.7μ in diameter, 1.5-3μ apart, smaller adjacent to the smooth patch. Germ pores not readily distinguished, germination seen from 2-4 scattered points on the spore. *Uredinial paraphyses*", (Fig. 2.2B, G) "abundant, scattered through the sori, capitate, to 70μ long, stalk 4-6μ wide, capitate apex roughly spherical, (12) 14-17 (19)μ in diameter, or oval to less commonly clavate 18-22 x 12-16μ, with wall uniformly 1.5-3μ thick or occasionally slightly thickened to 4μ at the apex. *Telia*", (Fig. 2.2C) "on older leaves mixed with uredinia, abundant on fallen leaves, mainly hypophyllous, a few epiphyllous especially in heavy infections, initially pale amber, darkening to deep reddish brown, almost black when dry, colour due partly to discoloured host epidermis, slightly raised, subepidermal, roughly circular to irregular in outline, 3-4 or more often fusing into a larger composite sorus, individual sori 150-350μ in diameter, occasionally smaller sori 90-100μ in diameter frequent on some leaves, some sori penetrate the leaf thickness and produce teliospores on both surfaces. *Teliospores* unicellular, roughly cylindrical in side view, circular to irregular in cross section, 30-45 x 11-14μ, wall pale yellowish brown uniformly 1-1.5μ thick, occasionally to 2-2.5μ at the apex, covered by the host epidermis. *Basidia*", (Fig. 2.2D) "as a golden layer on the surface of overwintered telia, arising from a small pore in the teliospore apex, borne on a short stalk 2-2.5μ wide, 2-3μ long; basidia cylindrical, rounded apex, straight or slightly curved 40-65 x 7-8μ, 4-celled, contents pale golden yellow, wall colourless. Each cell bears one single lateral sterigma, 2-2.5μ wide at the base, 10-12μ long and tapering to a fine point; sterigma of apical cell sometimes apical to subapical.

Basidiospores", (Fig. 2.2D) spherical, with a minute apiculus representing the point of attachment to the sterigma, 7.5-11μ in diameter, wall colourless, contents pale golden yellow, often showing secondary germination by a sterigma 10-12μ long bearing a slightly smaller secondary basidiospore.

M. medusae is distinguished from other *Melampsora* spp. that have been recorded on *Populus* spp. by having uredinia with uniformly thickened capitate paraphyses and large obovate to oval urediniospores with wall equatorially thickened on two sides and spiny except for a smooth equatorial patch usually extending ½ - ¾ around the spore."

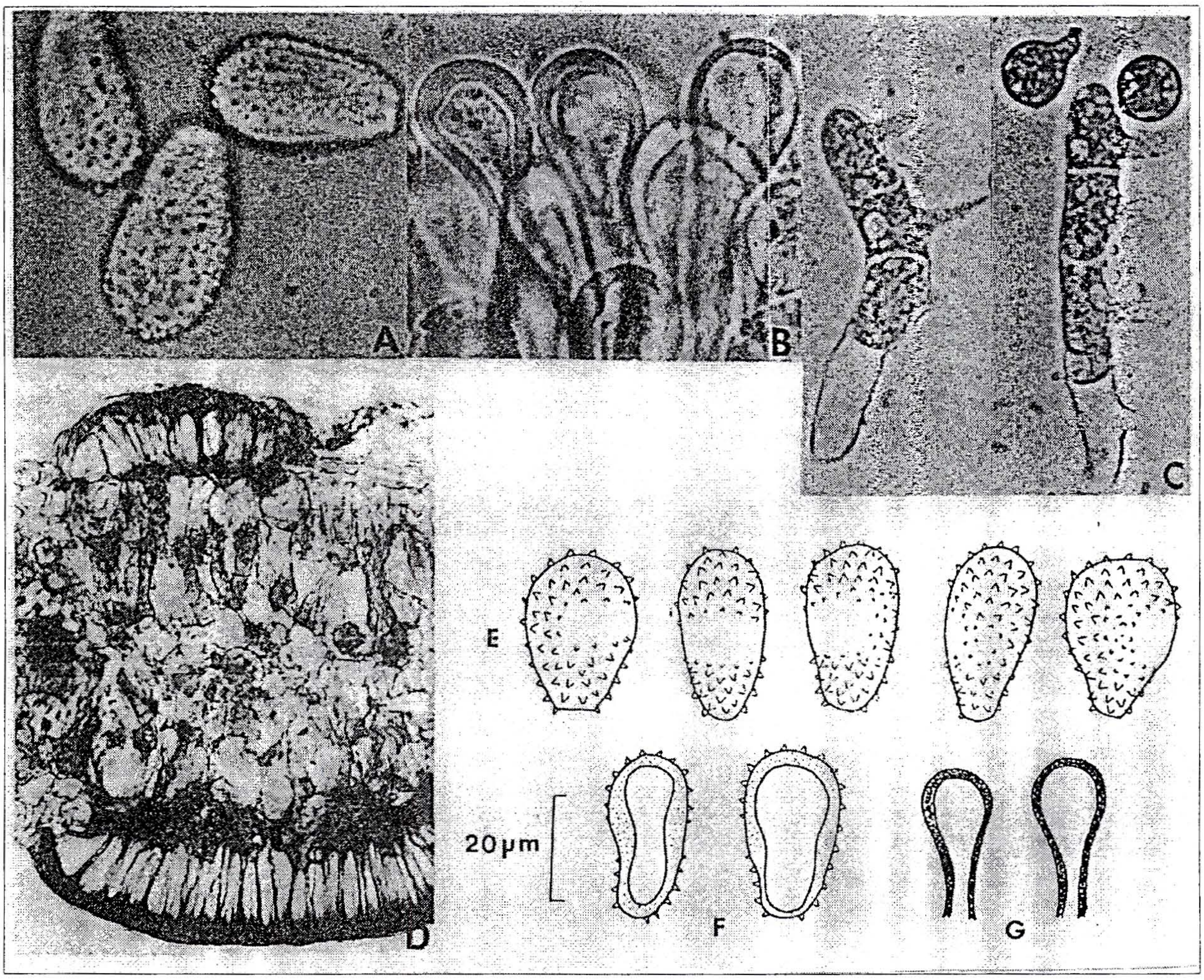

Fig. 2.2 Morphology of the various spore types and structures of *M. medusae* A, Uredospores x 800; B, uredinial paraphyses x 1 000; C, basidia and basidiospores x 800; D, telia x 208; E-G, urediniospores and paraphyses. (Photos: A. Searle from CMI descriptions of pathogenic fungi and bacteria, Walker, 1979).

Doidge (1927) gave the following description of *M. aecidioides*:

> "Uredo-sori hypophyllous, on small yellow leaf-spots, round, sub-pulverulent, 0.5-1mm. diam. Uredospores sub-globose, ovate or ellipsoid, 17-24 x 15-17μ. epispore 2.5-3μ thick, remotely verrucose-aculeate. Paraphyses usually capitate, 46-66μ long, 15-23μ broad at the tips, wall 3-6μ thick."

She also mentioned that this poplar rust was only known in the uredo-stage in South Africa, and that it was therefore not possible to assign it definitely to any one of the numerous species of *Melampsora* on *Populus* spp.. As mentioned earlier, Van Reenen (1995) described *M. aecidiodes* as being *M. populnea*.

Table 2.1 Comparisons of the different species of *Melampsora* occurring in South Africa (Doidge, 1927; Walker, 1979; Spiers and Hopcroft, 1994)

	M. larici-populina	*M. medusae*	*M. aecidioides*	*M. medusae-populina*
Uredia	hypophyllous, some epiphyllous	hypophyllous, a few, epiphyllous (smaller)	hypophyllous	mainly hypophyllous
Uredia diameter	200-400μ	150-250μ	500-1000μ	150-500μ
Uredospore length	(25)30-44(50)μ	(23)26-35(37)μ	17-24μ	(33.6)-34.6-(35.2)μ
Uredospore breadth	(12)14-19(22)μ	15-19(21)μ	15-17μ	(17.3)-17.9-(18.6)μ
Paraphyses length	65-75μ	70μ	46-66μ	
Paraphyses head diameter	14-21μ	(12)14-17(19)μ	15-23μ	(12.0)-15.9-(22.0)μ
Paraphyses wall thickness	1.5-2μ at sides	1.5-3μ	3-6μ	(2.0)-5.9-(12.0)μ
Paraphyses apical wall thickness	5-10(12)μ	occasionally 4μ		

() - indicate min. and/or max. ranges

2.4 DISTRIBUTION

Figures 2.3a and b show the distributions of *M. medusae*, *M. larici-populina*, *M. medusae-populina* and *M. populnea* which cause leaf rust of poplars (CMI, 1986 and 1991).

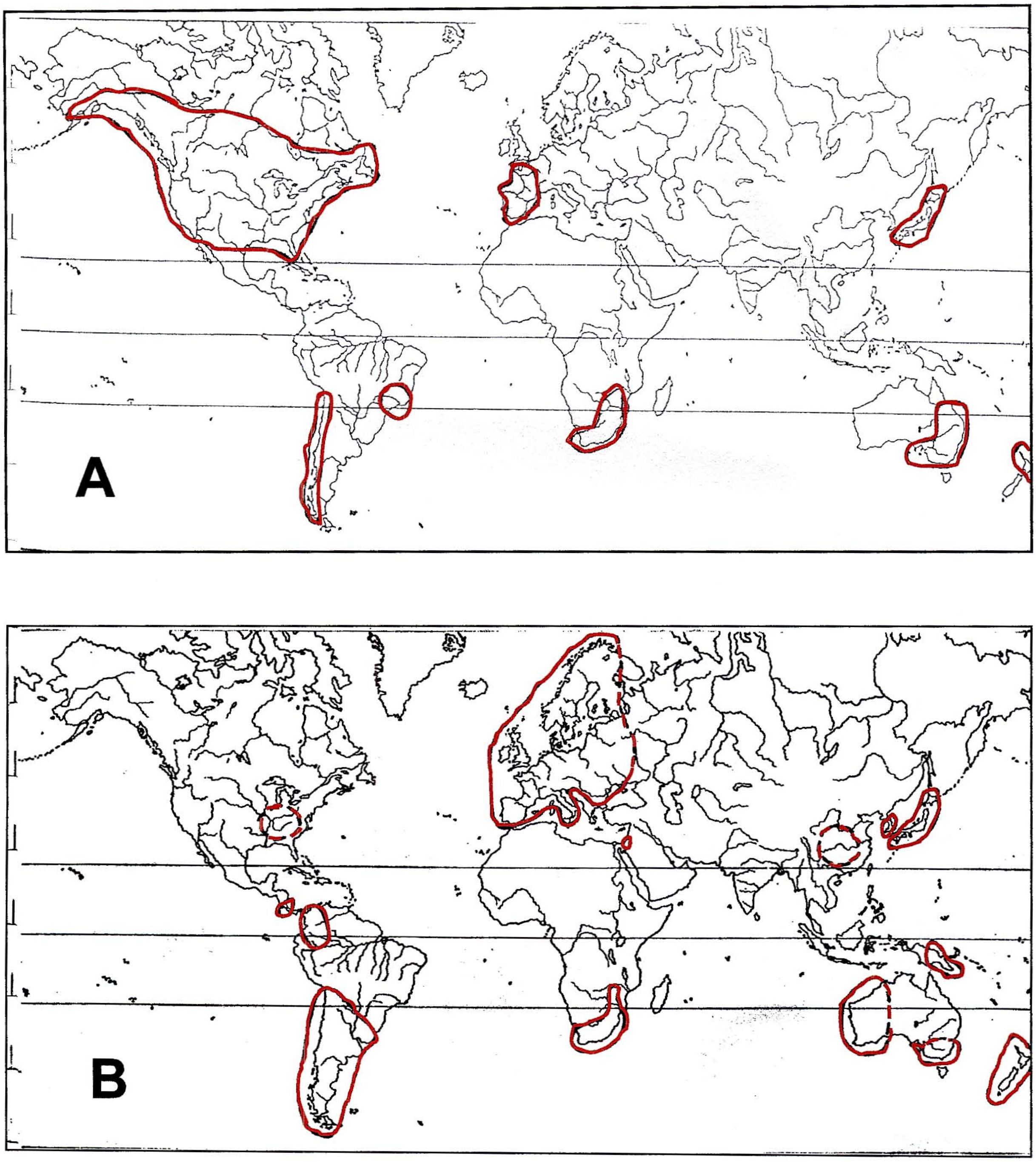

Fig. 2.3a CMI Distribution maps of *M. medusae* (A), *M. larici-populina* (B)

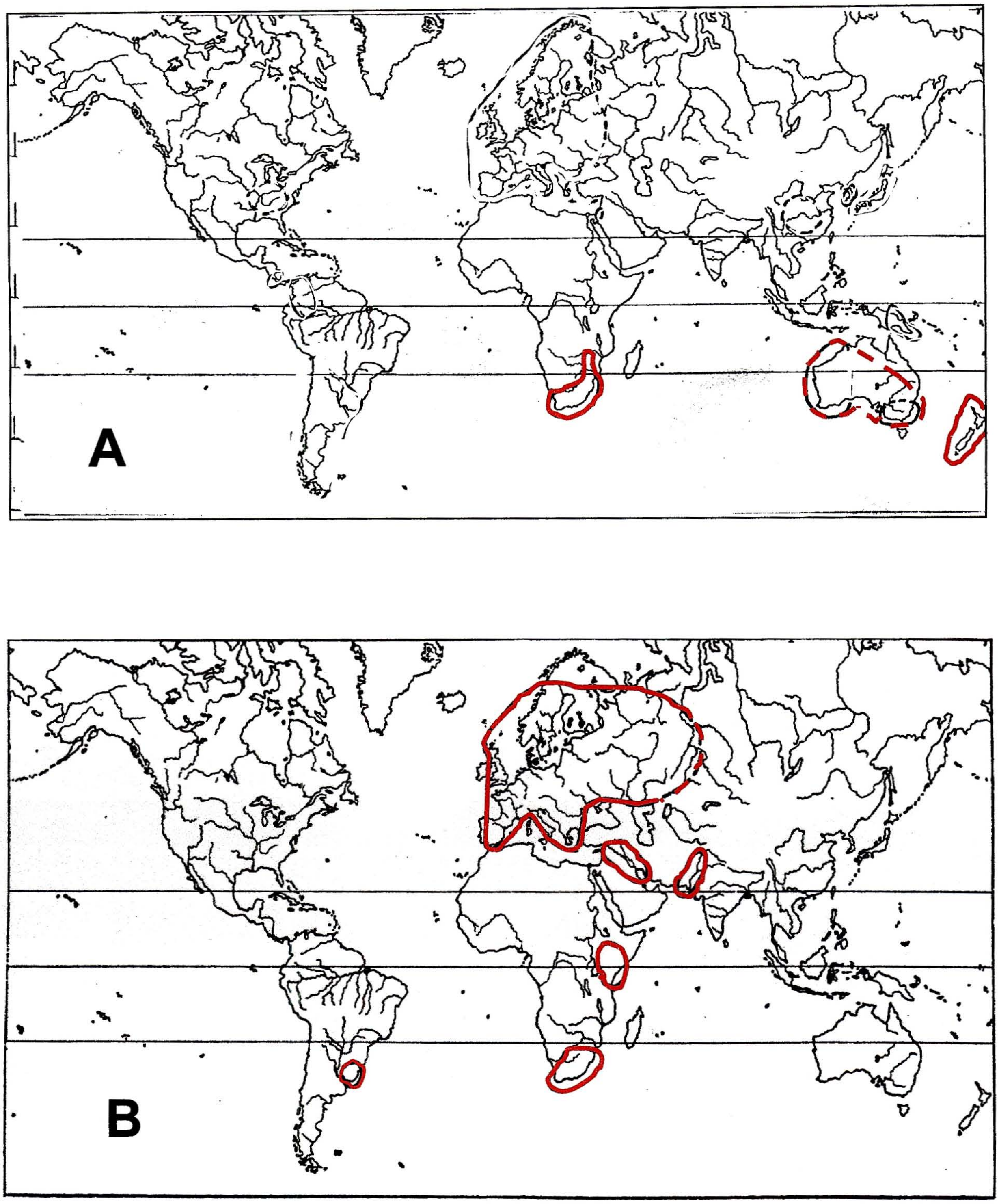

Fig. 2.3b CMI Distribution maps of *M. medusae-populina* (A), *M. populnea* (B)

2.5 THE LIFE CYCLE OF *MELAMPSORA* SPP.

A life cycle, compiled by Fleming (1996), is shown in Fig. 2.4. With the exception of teliospores, whose germination requires at least 12 hours exposure to free water, all spore types germinate in the presence of saturated humidity or free water within a few hours. Maximum infection of artificially inoculated detached leaves of eastern cottonwood with uredospores occurs after eight hours of continuous leaf wetness (Hamelin *et al.*, 1992). Thus infection is linked to the rainy seasons or conditions of mist and heavy, long lasting dew (FAO, 1979).

Temperature optima vary, depending on the spore stage and the species of *Melampsora*. Germination occurs most commonly between 5-30°C with 12 and 25°C being optimum. Peak infection using a leaf disc bioassay was reported to occur at 20°C, with no infection taking place below 2°C or above 30°C (Spiers, 1978). Laboratory studies have also indicated that exposure of *M. medusae* uredospores to 32°C for 1-5h reduces germination by 50%, while exposure to 35°C for over 3h or 36°C for 1h was lethal (Spiers, 1978). For a specific species, the temperature requirements are often lower for teliospores and aeciospores than for uredospores. When humidity decreases from saturation point, germination becomes possible only in a narrow temperature range with the optimum central. The germination tube enters a stoma and infection is effective within hours (FAO, 1979).

Survival of *Melampsora* species in temperate areas depends on their ability to overwinter. This is thought to be in the uredial stage on persisting green leaves of semi-evergreen poplar varieties, fallen leaves (as uredospores lodged under bud scales) or as mycelium in buds (Walker, 1979; Newcombe and Chastagner, 1993b). In New Zealand, *M. larici-populina* has more impact than did *M. medusae* because, unlike the latter species, it is able to overwinter on the semi-evergreen Lombardy poplar (Trench *et al.*, 1988). In Japan, uredospores of *M. larici-populina* are produced in autumn and can remain viable and pathogenic until the following spring (Walker, 1979).

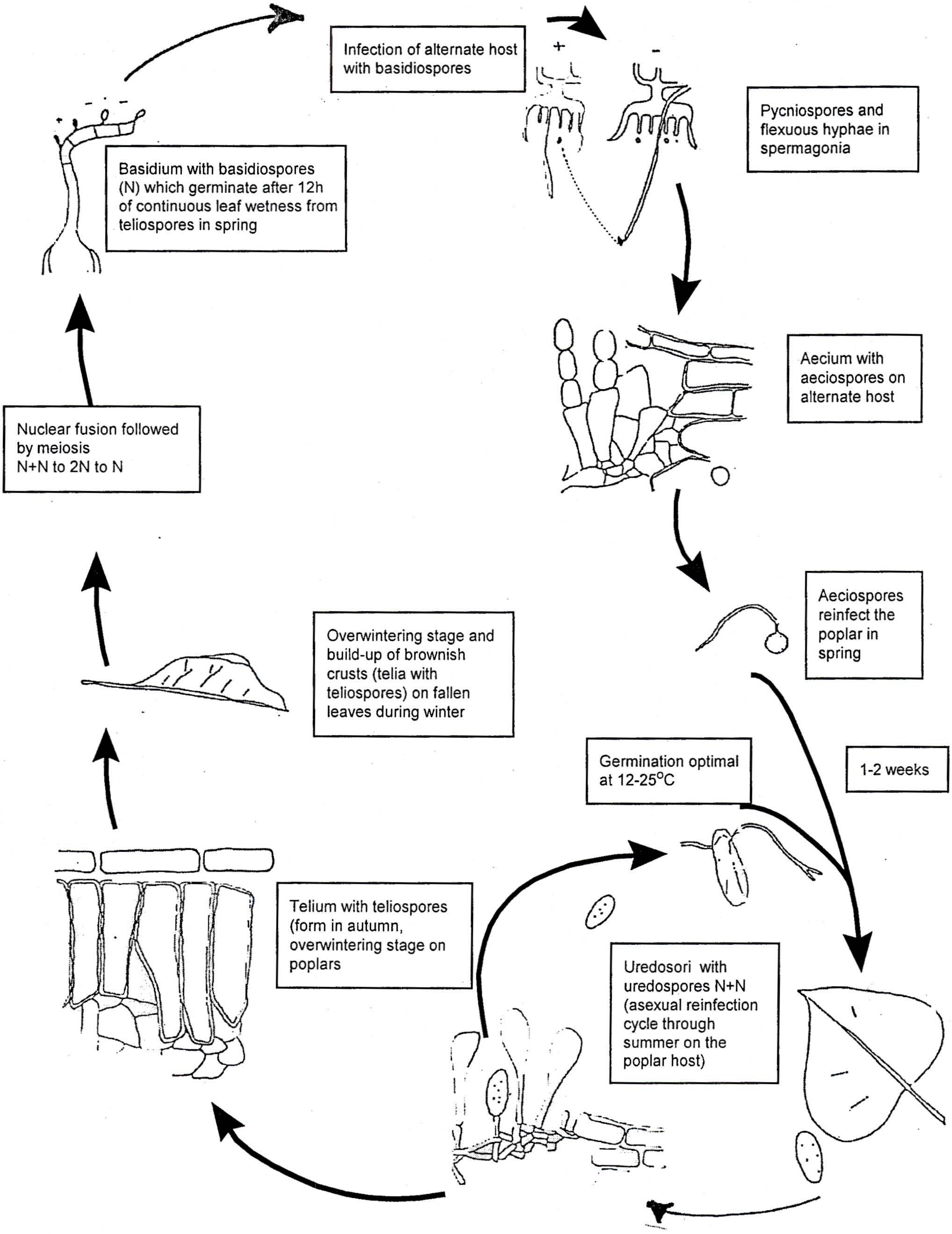

Fig. 2.4 The life cycle of a typical rust species infecting poplar (Fleming, 1996)

2.6 HOSTS OF *MELAMPSORA* SPP.

Melampsora medusae, M. occidentalis and *M. larici-populina* produce telia and uredia on *Populus* and *Salix* species and aecia and pycnia on various plants including *Larix, Abies, Ribes, Pinus* and *Allium* spp.. In the Eastern United States, *M. medusae* has the uredial and telial stages on eastern cottonwood (*P. deltoides* Bartr. Ex Marshall) and trembling aspen (*P. tremuloides* Michx.). *Larix laricina* (Du Roi) K.Koch (Tamarack) is the favoured aecial host in its native habitat, sharing an extensive common range in North America with the poplar. *Larix decidua* Mill. (European larch), *L. leptolepis* (Siebold & Zucc.) Gordon (Japanese larch), and *Pinus radiata* D. Don (Monterey pine) have been reported as aecial hosts to the heteroecious rusts in Europe, Japan and Australia, respectively (Newcombe and Chastagner, 1993b). Shain (1988) reports the existence of *M. medusae* f. sp. *deltoidis* which is primarily pathogenic to *Populus deltoides* and *M. medusae* f. sp. *tremuloidis*, which is primarily pathogenic to *P. tremuloides*. Newcombe and Chastagner (1994) later reported additional coniferous aecial hosts of *M. medusae* and *M. larici-populina*. *M. larici-populina* was able to form aecia on *Pinus ponderosa* Douglas ex P. Laws. & C. Laws. (Ponderosa pine), while aecia of *M. medusae* f. sp. *deltoidae* were found on five previously unreported hosts, *L. occidentalis*, *Pseudotsuga menziesii* (Mirb.) Franco (Douglas fir), *P. contorta*, *P. ponderosa* and *P. radiata*.

Melampsora lini is an autoecious rust which is common on *Linum usitatissimum*. Other *Melampsora* species are parasitic on *Salix* species. *Melampsora populnea* has uredospores and teliospores on *Populus* species and pycnia and aecia on *Mercuriales perennis*.

2.7 DISSEMINATION

Natural barriers such as oceans and mountain ranges can drastically retard movement of rust between geographic areas. Once the barrier has been crossed either by wind dissemination or inadvertent human introduction, winds may rapidly carry spores from the new focus over large areas.

Release of uredospores of *M. larici-populina* is effective from 10°C and reaches a max. at about 20°C. Optimal temperature ranges for uredospore dissemination were 18-22°C in France (Taris, 1968) and 15-20°C in North America (Widin and Schipper, 1980). An atmospheric humidity greater than 80% and a wind speed of >1ms^{-1} are needed (FAO, 1979).

Melampsora medusae and *M. larici-populina*, long established pathogens of poplars and cottonwoods in many regions of the world, did not appear on the Australian continent until early 1972. Within 14 weeks, these newly arrived pathogens spread inland up to 400km, extending from the north end of New South Wales southward to near Melbourne, Victoria (Fig. 2.5). The wet cyclonic weather that occurred during January and February coupled with the prevailing easterly winds was particularly favourable for the development and spread of these rusts. Within approximately one year of the discovery of these rusts in Australia, they were also found for the first time in New Zealand, more than 1 600km across the Tasman Sea. This spread appears to have been by wind-borne uredospores (Littlefield, 1981).

Rain was found to play an important mechanical role in the dissemination of uredospores in France (Taris, 1968). In north central North America the number of uredospores caught on traps increased one week after rainfalls of 15mm or more (Widin and Schipper, 1980). These authors also reported that fewer uredospores were trapped in 1976, a year with lower early season rainfall, as compared with 1975. Uredospores of *M. medusae* were not trapped >50m above ground, after leaf fall or in the spring in a survey conducted over a poplar plantation near Filter, Mississippi, USA (McCracken, 1989). However, Sheridan *et al.* (1975) recovered uredospores when conditions were calm at 500m altitude.

Other means of dispersal include plant material being transported to new areas, spores adhering to human clothing, sheep grazing under poplars, or birds nesting or feeding among plantations (Sheridan *et al.*, 1975).

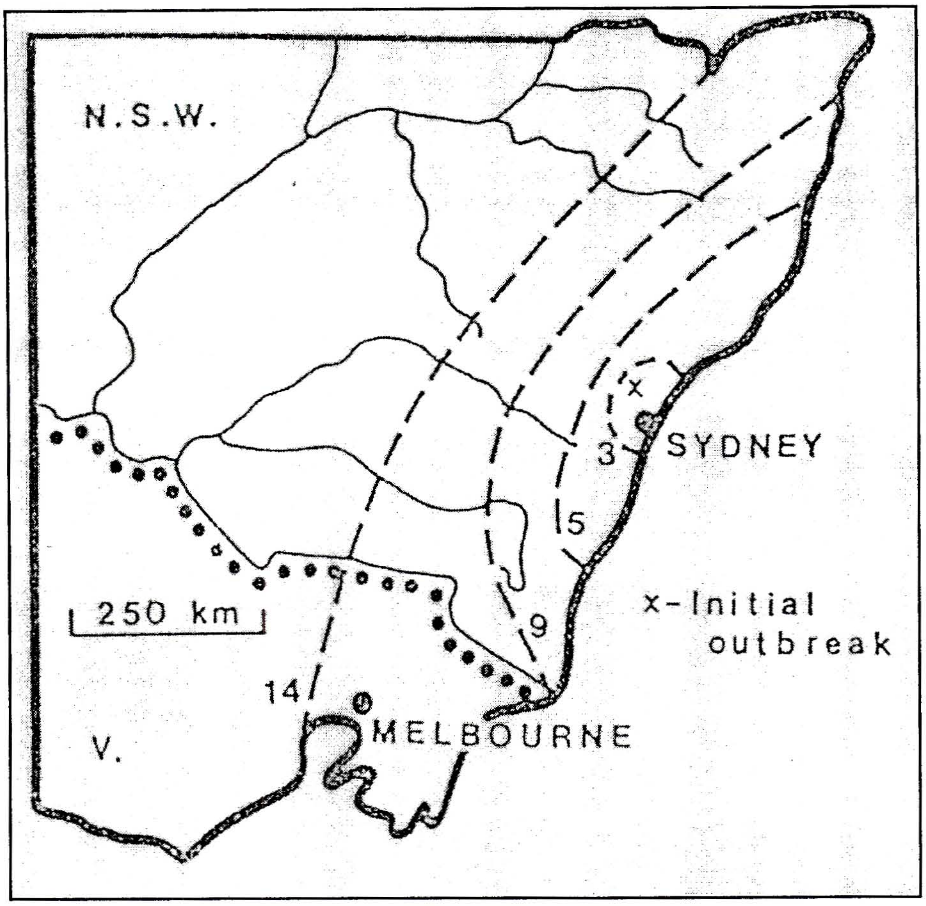

Fig. 2.5 Spread of *Melampsora medusae* into south eastern Australia in 1972 after 3, 5, 9 and 14 weeks. (N.S.W.-New South Wales, V.-Victoria) (Adapted from Walker *et al.*, 1974)

2.8 SYMPTOMS

The first symptoms are minute chlorotic spots on the leaves. These appear in spring and enlarge, developing into yellow/orange powdery pustules (2-3mm in diameter). The pustules which rupture to release large numbers of powdery orange-yellow spores (uredia) are often surrounded by a chlorotic region that is bright yellow (Fig. 2.6A). The areas surrounding and including the pustule eventually necrose. Defoliations will occur when 50% or more of the leaf tissue is covered with rust (Peterson and Smith, 1975) (Fig. 2.6B). At the end of the vegetative season, and chiefly on the upper surfaces of the leaves, blackish telia appear, formed by groupings of teliospores in dense rows under the epidermis. The teliospore membrane is thick and strongly coloured which makes the spores appear dark and heightens the crusty look of the close-knit teliospores (FAO, 1979; Shain and Filer, 1989).

Pustules on the underside of a poplar leaf

Defoliation caused by *Melampsora* rust

Fig. 2.6 Typical visual symptoms of rust infection on poplar

Infected plants have an abnormally high respiratory rate and a slow photosynthetic rate. These two processes combine, reducing the food reserves of infected plants. The following season, budding is delayed, less complete, and flowering is sometimes jeopardised. In the parenchyma layer, the mycelium follows an intercellular pathway, enters the host cells and produces haustoria. The chromoplasts are reduced and altered in their fine structure (FAO, 1979). After inoculation, the total content of sugar and sugar alcohols in diseased leaves increases rapidly and this occurs before visual symptoms appear (Suzuki, 1975). Some phytotoxins, in addition to their cytotoxic effects, could directly suppress specific defence responses in plant cells during pathogenesis (Vurro and Ellis, 1997). Premature defoliation and growth suppression of the poplar are also a result of rust infection (FAO, 1979). Defoliation can occur two or three times in a season, each defoliation being followed by a new growth flush (Walker, 1979). It has been found that the stem diameter increment, especially, is affected by rust attacks in previous years (Pichot and Du Cros, 1993). Rust also predisposes the poplar tree to cold injury, i.e., frost, and to secondary pathogens such as *Cytospora* and *Dothichiza* (FAO, 1979; Hubbes *et al.*, 1983; Chang *et al.*, 1989). *Melampsora medusae* has been known to cause death of trees, especially nursery stock and trees of one to two years old (Walker *et al.*, 1974). Repeated infections over successive years can result in the loss of an entire plantation (Laurans and Pilate, 1998).

2.9 CONTROL

2.9.1 Chemical

During years of early and bad infections, chemicals which effectively control poplar rusts should be used to treat trees (FAO, 1979). Chemical spraying can also be used to provide protection against loss of resistance by emergence of new physiological races. Sheridan *et al.* (1975) found benodanil (BAS 317 F) with oil (0.25%) or water to be the most effective treatment in control of poplar rust. Plantvax 75W in oil and copper oxychloride also reduced disease levels significantly. Benomyl gave good control early in the season, but failed to do so later as disease pressure increased. Foliar application of Bayleton (triadimefon at 0.08%) is effective in controlling poplar rust (Pandey *et al.*, 1996). Findings in this thesis have been that cyproconazole (Alto at 0.3ml/L) was the most effective fungicide, of the fungicides tested, for controlling rust infection (Hawke and Laing, 1998). This fungicide is registered in Europe for the control of poplar rust (Pinon, 2000).

2.9.2 Mixed Plantings

Planting of a few resistant clones in extensive plantations (i.e., a monoclonal situation) is dangerous. Stands such as these favour the build-up of pathogenic races, caused by selection pressure on the fungus. A patchwork planting design of clones with different resistant genes has been proposed to combat this problem. In this way it is hoped that a clone that is susceptible to a physiologic race will then be surrounded by clones that are resistant to that particular physiologic race (Shain and Filer, 1989). Clones of superior resistance may also be used with clones of reduced resistance in mixed or mosaic plantations, in order to provide genetic and physical barriers to pathogen spread (Schoeneman, 1986).

2.9.3 Aspects of Resistance Breeding

Resistance of poplars to rust has been reported to be under genetic control. The control of rust on poplar plantations therefore relies widely on the planting of clones which are resistant to *Melampsora* spp. (Chang *et al.*, 1989). On resistant clones, fewer sori develop and defoliation is not as severe as it is on the susceptible clones (Chang *et al.*, 1989). Breeding programmes have been instituted with the primary aim of breeding resistant lines. A priority is to screen new clones for disease resistance to maintain a broad genetic base that will provide diversity in plantings. This is vitally important as there are many races of *Melampsora* which are host specific. Breeding programmes are possible because of the existing genetic diversity in the *Populus* genus (Ostry and McNabb, 1985).

Flor (1954, In Littlefield, 1981), constructed a set of differential varieties of flax to identify races of *M. lini*, the flax rust fungus. He developed 25 lines of flax, each containing a single, mutually exclusive gene for rust resistance with all lines having the common genetic background of the cultivar, Bison. Thus, based on which resistance genes in the set of differentials a rust species would overcome, it became possible to identify races of *M. lini* by their specific pathogenetic gene(s). If the host and pathogen genotypes were not compatible, a hypersensitive type reaction resulted (Littlefield, 1981). This principle of gene-for gene susceptibility is widely used in resistance breeding today.

Resistance to pathogens depends partly on the poplar species. Fair resistance is common to the oozing canker in *P. nigra*, to *Marssonina brunnea* in *P. deltoides, P. nigra* and *P. trichocarpa*, to rusts in *P. deltoides* and to *Discosporium populeum* in *P. trichocarpa*. A better appraisal of damage is necessary for foliar diseases (Pinon and Valadon, 1997). Necrotic flecking in the field and growth-room experiments was found to be governed by a single, dominant gene (Mmdl) inherited from the *P. trichocarpa* parent. This gene played a major role in resistance to *M. medusae* f. sp. *deltoidae* in the Pacific Northwest (Newcombe *et al.*, 1996). Goue Mourier *et al.* (1996) found all *P. trichocarpa* parents to be susceptible and most of the *P. deltoides* parents resistant with both quantitative and qualitative resistance. The hybrids had qualitative resistance controlled by a few genes. One genomic area was revealed which was involved in qualitative resistance, although it was not sufficient to assure durability. Other genes involved in quantitative resistance were masked (Goue Mourier *et al.*, 1996). The segregation ratio of resistant to susceptible plants suggested that a single, dominant locus (Mer) defined the resistance to *Melampsora* (Cervera *et al.*, 1996). In a study conducted by Schoeneman (1986) resistance was greatest in *P. maximowiczii* seedlings. *Populus deltoides* imparted rust resistance to its progeny, while *P. trichocarpa* and *P. nigra* imparted susceptibility. Male clones of *P. trichocarpa* and female clones of *P. nigra* imparted race specific resistance to progeny. *Populus trichocarpa* females and *P. nigra* males seemed to carry genes for non-specific resistance. Resistance in *P. deltoides* was polygenic. Progeny of *P. maximowiczii* inherited resistance to a high degree and this species may be useful for breeding

(Krzan, 1981). Studies on interspecific hybridization between *P. lasiocarpa* (resistant to the poplar rust *M. larici-populina*) and *P. nigra* (susceptible to it) showed several valuable traits including resistance to leaf diseases, and may also be incorporated into breeding programmes (Werner and Siwecki, 1994).

Pinon and Du Cros (1976) made a study of resistance to *Melampsora* spp. on nursery stock of many clones of *P. nigra, P. deltoides, P.* X *euramericana* (*P.* X *canadensis*) and *P. trichocarpa*. Most clones of *P. deltoides* were found to be resistant, with some variation related to the country of origin. The other three species were more or less susceptible, but it is suggested that selective breeding for resistance should be possible. *Populus nigra* and *P. trichocarpa* were particularly susceptible to attack by *M. larici-populina*. Some Belgian clones resistant to *M. larici-populina* were susceptible to *M. allii-populina*. Seedlings resulting from free pollination of *P. nigra* showed a strong correlation between the resistance of the female parent and that of the offspring (Pinon and Du Cros, 1976).

Breeders may choose to develop clones with vertical or qualitative resistance or alternatively with horizontal or quantitative resistance. Qualitative resistance can be defined as the absolute absence of any sporulation on the leaf tissue, while quantitative resistance considers the different levels of infection observed. These definitions do not directly refer to the number of genes involved in resistance. Quantitative resistance is a combination of complementary traits of the host that decrease the pathogen development and multiplication, slowing the epidemic and thus limiting the total damage on hosts (Pichot and Du Cros, 1993). A new male clone, *Populus* x 'Assiniboine', developed in Canada has superior growth and resistance to *Melampsora* leaf rust (*M. medusae*) (Spiers and Hopcroft, 1990).

The discovery in N. France of a new strain of poplar leaf rust (also identified in Belgium), *M. larici-populina* E4, on poplar clones previously considered resistant to the disease (Ministere de l'Agriculture et de la Peche, 1995). It is concluded that race E1 predominates in locations sampled in both California and Washington, while three other, unnamed races occur at a low frequency (Pinon *et al.*, 1994).

There are shortcomings in breeding resistant varieties. It is possible to select for trees with high partial resistance combined with a hypersensitive-type resistance against specific races of the pathogen (Prakash and Heather, 1989). However, it is not always possible to combine resistance with other factors required such as fast growth and desirable wood quality. The existence of several races of *M. medusae* have already been found to impede the management of the rust pathogen by employing resistant cultivars (Prakash and Thielges, 1987).

Thielges *et al.* (1988), after conducting periodic quantitative observations for incidence and severity of leaf rust, emphasized the following:

(i) the importance of obtaining local sources of resistance to incorporate into breeding populations,
(i) the need to conduct nursery and field screening for disease resistance at or near the site of production plantation, and
(i) the use of laboratory screening against a diverse collection of pathogen isolates to detect a wide spectrum of resistance.

2.9.4 Cultural Practices

Cultural methods of control should also be taken into consideration. Before establishing a poplar stand, the choice of site should not be in valleys containing lasting mists. If the stand is already established, then one should make sure that there is good crown aeration and adequate fertilisation (i.e. sufficient potassium but not an excess of nitrogen) (FAO, 1979).

Prior to a predicted epidemic, susceptible plant material can be inoculated with a non-disease causing agent that stimulates resistance in the plant material. However, prior inoculation of poplar leaves with *Ceratocystis fimbriata*, to stimulate a resistance response, failed to immunize poplar shoots against pathogenic fungi (Kozlowska and Krzyanski, 1992).

Semi-evergreen Lombardy poplars were the initial source of disease outbreaks each year in New Zealand (Sheridan *et al.*, 1975). These should be destroyed by injection or by cutting down and painting the stumps with a killing agent. Removal of susceptible semi-evergreens, to reduce the inoculum available in spring may eventually eliminate disease (Sheridan *et al.*, 1975).

2.9.5 Biological Control

Biological control is discussed in Chapter Seven.

2.10 ARTIFICIAL TECHNIQUES

Until the 1950's rust could not be cultured artificially. Some rusts can now be axenically cultured in spite of the fact that they are obligate parasites (Rijkenberg, 1969). Axenic culture is the growth of an organism of a single species in the absence of living organisms or cells of any other species (Maclean, 1982). Rust fungi are biotrophs when they extract nutrients from the living cells of their host, and saprotrophs when they extract nutrients from laboratory media.

Axenic culture of rust from spores works best if uredospores are used. These structures are numerous and the most common spore type on plants during summer. The rust fungus has an overall need for a carbohydrate as the bulk carbon source, a sulfur-containing amino acid as the sulfur source, and another amino acid as the bulk nitrogen source (Rijkenberg, 1969).

The key component is the organic nitrogen source. Difco yeast extract or Evans peptone have been used successfully in the past. The carbohydrate source can be sucrose, glucose, D-fructose, D-mannose or D-mannitol, but not D-galactose. The following were found not to support growth from uredospores: potato dextrose agar, potato carrot agar, oatmeal agar, beef extract agar and malt agar. The mineral nutrition and pH of the media are also important. Czapek's minerals or inclusion of 2-6g/l of $Ca(NO_3)_2.4H_2O$ should be used in the medium. A pH of 6 to 6.4 is optimal. The addition of citrate to the medium prevents the precipitation of phosphates during storage (Maclean, 1982). Lane and Shaw (1974) successfully used the mixture shown in Table 2.2 for the axenic culture of *M. occidentalis* Pers..

Single rust pustules, including a surrounding zone of uninfected tissue 1mm wide were excised and placed, pustule side up on the above agar medium. The pustules as well as the medium were sterilised. After three weeks of incubation at 17°C in darkness, mycelium emerged from some pustules, and after four months mycelium had grown onto the agar medium from 30% of the pustules.

Some uredospores retained their viability and infectivity to eastern cottonwood (*P. deltoides*) up to five years after being freeze-dried. A cyclone separator (preferably with a suction <600mm Hg) was used to collect uredospores from infected leaves. Air-dried uredospores were quick-frozen and placed on a vacuum manifold (5-10μm Hg) for 5 - 6h before their containers were sealed under vacuum. Collections were then stored for use as needed in a refrigerator at 1-2°C. Neither heat shock nor rehydration seemed to affect the germination of these freeze-dried spores. There was no indication that the pathogenicity of uredospores to selected poplar clones changed after freeze-drying and storage (Shain, 1979).

Shain (1974) describes a technique for evaluating host response to obligate and non-obligate parasites. The lower surface of leaf discs of *P. deltoides* are inoculated with 10μl of standardized spore suspensions of the obligate parasite *Melampsora* spp. ($2X10^4$spores/ml) or the non-obligate *Septoria musiva* ($3X10^5$spores/ml). Inoculations were more successful when inoculum droplets dried within 20h. Incubation temperatures for discs inoculated with *Melampsora* sp. and *S. musiva* are 20 and 25°C, respectively. Uredial sori and/or necrotic lesions will develop in about six days (Shain, 1974).

Table 2.2 Nutrient mixture successfully used by Lane and Shaw (1974) for the axenic culture of *M. occidentalis*

Substance	Quantity in g/L (unless otherwise stipulated)
KNO_3	0.25
$MgSO_4.7H_2O$	0.25
KH_2PO_4	0.25
K_2HPO_4	0.75
NH_4NO_3	0.02
$Ca(NO_3)_2.4H_2O$	2
Sucrose	50
Aspartic acid	5.99
Cysteine	0.558
Micronutrient stock solution (Table 2.3)	0.8 ml/L

Table 2.3 Contents of the micronutrient stock solution (mg/200 ml) used in Lane and Shaw's (1974) nutrient mixture

Substance	Quantity in mg/200 ml unless otherwise stipulated
13% NaFe (Geigy)	10 000
$MnSO_4.H_2O$	447
KI	10
$NiCl_2.6H_2O$	18
$CoCl_2.6H_2O$	18
$Ti(SO_4)_2.9H_2O$	42
$ZnSO_4.7H_2O$	35
$CuSO_4.5H_2O$	15
$BeSO_4$	20
85% H_3PO_4	10
H_2SO_4 (concentrated)	0.2 ml

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Chapter 3 DISTRIBUTION OF *MELAMPSORA* SPP. ON DIFFERENT POPLAR CLONES GROWN AT THE LION MATCH COMPANY REDCLYFF NURSERY, KWAZULU-NATAL, SOUTH AFRICA

ABSTRACT

An assessment of infection on poplar clones grown at the Lion Match Company Redclyff Nursery in Seven Oaks, KwaZulu-Natal was made to ascertain the nature of infection of the three common species of *Melampsora* infecting poplars in South Africa. These three species are *M. larici-populina, M. medusae* and the hybrid, *M. medusae-populina.* Their contrasting wall echinulations were used to differentiate these species using a scanning electron microscope. A visual rating scale measuring percentage leaf area infected (LAI) was used to determine disease severity. Rust development was slow in new material obtained from New Zealand, indicating rust resistance. This new material was not infected with *M. larici-populina.* Plant material from Europe showed severe susceptibility to *M. larici-populina. Melampsora medusae-populina* was the most prevalent species found at Lion Match Company plantations, Seven Oaks, KwaZulu-Natal, South Africa in the survey conducted from January to April, 1998.

3.1 INTRODUCTION

Poplar leaf rust caused by species of the genus *Melampsora* Cast. is one of the most damaging leaf diseases of hybrid poplar plantations. Damage varies according to climatic fluctuations, microclimate of the plantations, proximity of alternate hosts, metabolic state of poplar hosts, and susceptibility of the poplar clones planted. Heavy rust infections, particularly those early in the growing season, weaken the poplar host and predispose it to other biotic or abiotic diseases. In addition, shoot growth and development of the root system are reduced (Taris, 1979, In Hubbes *et al.*, 1983).

In his study on poplar rust in France, Pinon (1973) described eight species of *Melampsora* based on the characteristic features of uredospores and associated paraphyses. Trench and Churchill (1987) reported that *M. medusae* Thüm, *M. larici-populina* Kleb. and *M. aecidioides* (DC.) Schroet. occur on poplars in South Africa. Two of these, *M. medusae* and *M. larici-populina* are the more common fungi to attack commercially grown clones of poplars, globally.

Many physiological races, being distinguished by their virulence, have been identified within *M. larici-populina* (Netherlands and Australia), *M. medusae* (Australia) and *M. allii-populina* (Italy) (FAO, 1979).

The benefit of conducting a disease survey of this nature, is to identify susceptible or resistant clones and determine the nature of the *Melampsora* spp. infecting different poplar clones in South Africa. Disease surveys of this kind should be continuous so that susceptible clones are not propagated in the country. Because specific physiological races infect specific clones, race identification was conducted to identify the different races that occur in South Africa.

3.2 MATERIALS AND METHODS

3.2.1 Site Description

Seven Oaks lies at an altitude of 1040m above sea level, 29°12'45" South and 30°29'30" East about 120km North West of Durban in KwaZulu-Natal, South Africa. This is a summer rainfall area (800mm/annum) with max. daily temperature of 36°C, average 28°C and mean monthly temperature of 20°C. Winters are cool with early morning frost, a min. temperature of -8°C and a mean monthly temperature of 12°C. The soil type is of the Kranskop form, and the Fourdoun type (MacVicar, 1991). The soil depth is 800-1200mm. It has been described as being a dark brown to greyish brown, apedal, fine sandy clay loam topsoil (150-300mm thick) on dark brown to strong brown, apedal, fine sandy clay (500-700mm thick) on red to dark reddish brown, apedal, fine clay subsoil (800-1200mm deep) on saprolite.

3.2.2 Data Collection

Samples of diseased leaves from different clones were selected from the beginning of January, 1998 through to the end of April of the same year. This would have covered the peak rust epidemic period. In all, 18 clones were sampled and of these, four of the clones (65/31, 65/29, K12-2 and I 488) had been in the country more than six years. Eleven were clones originally obtained from New Zealand but grown in Lesotho and three were new clones from Europe that had recently come out of quarantine (Table 3.1). Clones were assessed as resistant (no pustules present), slightly susceptible (few pustules present) and susceptible (many pustules). Single pustules were removed from the leaves using a single edged blade, placed on a stub covered with glue and treated with "Sputter Cryo Low Temperature System" fixation. This involved plunge-freezing in liquid nitrogen and being held under a vacuum whilst being carbon coated, all steps being conducted at ultra-low temperatures (-270°C). They were then viewed under Cryo SEM at ultra-low temperatures. The pustules were identified as *Melampsora medusae, M. larici-populina* or *M. medusae-populina* by their wall echinulations (Fig. 3.1).

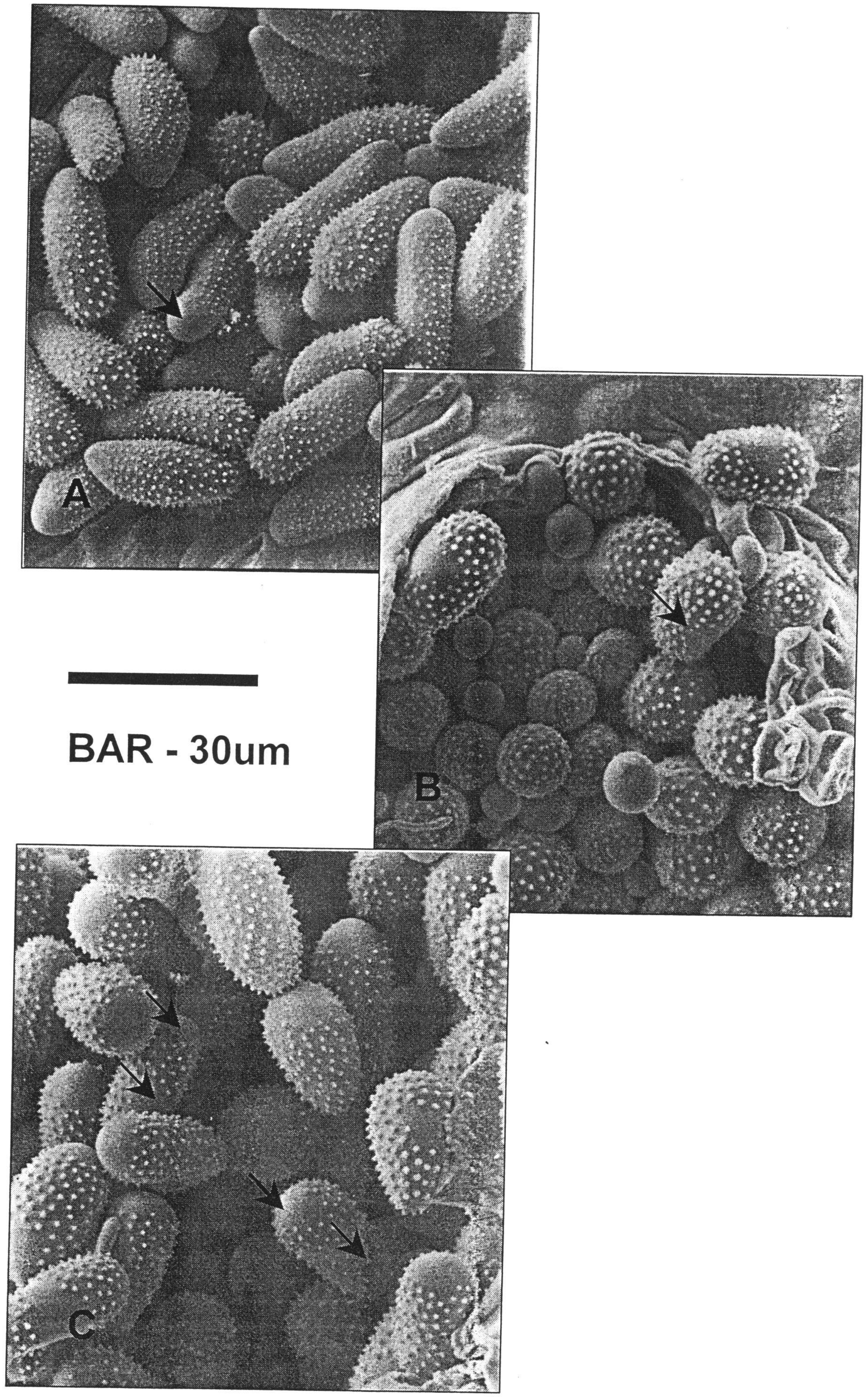


Fig. 3.1 Scanning electron micrographs of the different wall echinulations of *M. larici-populina* (A), *M. medusae* (B) and *M. medusae-populina* (C).

3.3 RESULTS

Most clones showed increased severity of disease towards the end of the season (Table 3.1and 3.2). Clones 65/29, K12-2, 87004-46, 5006, 80015-31, 87002-05 and 5007 were among the clones with the least disease. Clones 87002-05 and 87004-46 showed typical symptoms of rust infection, i.e., chlorotic spots. However, on close examination the pustules had not burst and remained erumpent but enclosed by the cuticle. For electron microscopy analysis they were pried open so that the species of rust could be determined. Clones 65/31, I488, 50-197, 15-079 and 184-411 were among the clones with the most disease.

Table 3.1 Severity of rust infection on different poplar clones over the season of assessment (January-April, 1998) at Lion Match Company plantations, Seven Oaks, KwaZulu-Natal

	Origin	Parentage	05/01	21/01	06/02	17/02	24/02	13/03	01/04	23/04
65/31	At site >5yrs	P. X nigra cv Chile	ss		s			s		s
65/29	At site >5yrs	P. X nigra cv Chile	r		ss			ss		s
K12-2	At site >5yrs	unknown	ss		ss			ss		ss
I 488	At site >5yrs	P. euramericana	ss		s			s		s
87004-46	NZ-Lesotho	unknown		r		r			ss	
5006	NZ-Lesotho	P. X yunanensis		r			ss	r		ss
87002-21	NZ-Lesotho	P. X szechuanica		r			ss	s		
80015-31	NZ-Lesotho	P. X nigra		r			ss			
87002-10	NZ-Lesotho	P. X szechuanica				r			s	
87005-06	NZ-Lesotho	P. X szechuanica		r		r			s	
87002-05	NZ-Lesotho	P. X szechuanica		r			r			
87004-12	NZ-Lesotho	unknown		r		ss			s	
5007	NZ-Lesotho	P. X yunanensis		ss			ss		ss	
87004-32	NZ-Lesotho	unknown		ss		ss			s	
87004-11	NZ-Lesotho	unknown		r			ss		s	
50-197	Europe	P. X trichocarpa							s	
15-029	Europe	unknown							s	
184-411	Europe	P. X trichocarpa							s	

NZ-Lesotho =	Clones originally from New Zealand were imported to Lesotho in June, 1991
s =	susceptible (many pustules)
ss =	slight susceptibility (one or two pustules)
r =	resistant (no pustules)
gaps =	not sampled

Table 3.2 Relative incidences of *Melampsora* spp. causing infection on different poplar clones at Lion Match Company plantations, Seven Oaks, KwaZulu-Natal (1998)

Clones	Species	05/01	21/01	06/02	17/02	24/02	13/03	01/04	23/04	Total
65/31	*M. medusae*	0		0			0		0	0
	M. medusae-populina	40		37.5			35.3		7.7	30.1
	M. larici-populina	60		62.5			64.7		92.3	69.9
65/29	*M. medusae*	0		6.7			0		0	1.7
	M. medusae-populina	100		26.7			100		6.3	58.2
	M. larici-populina	0		67.7			0		93.7	40.4
K12-2	*M. medusae*	0		0			0		0	0
	M. medusae-populina	100		44.4			7.1		100	62.9
	M. larici-populina	0		55.6			92.9		0	37.1
I 488	*M. medusae*	0		0			0		8.3	2.1
	M. medusae-populina	10		0			100		25	33.8
	M. larici-populina	90		100			0		66.7	64.2
87004-46	*M. medusae*		100		100			0		66.7
	M. medusae-populina		0		0			100		33.3
	M. larici-populina		0		0			0		0
5006	*M. medusae*		100			92.9	100		71.4	91.1
	M. medusae-populina		0			7.1	0		28.6	8.9
	M. larici-populina		0			0	0		0	0
87002-21	*M. medusae*		12.5			100	100			70.8
	M. medusae-populina		87.5			0	0			29.2
	M. larici-populina		0			0	0			0
80015-31	*M. medusae*		100			5.6				52.8
	M. medusae-populina		0			94.4				47.2
	M. larici-populina		0			0				0
87002-10	*M. medusae*				100			95.2		97.6
	M. medusae-populina				0			4.8		2.4
	M. larici-populina				0			0		0
87005-06	*M. medusae*		20		62.5			0		27.5
	M. medusae-populina		80		37.5			100		72.5
	M. larici-populina		0		0			0		0
87002-05	*M. medusae*		100			0				100
	M. medusae-populina		0			0				0
	M. larici-populina		0			0				0
87004-12	*M. medusae*		100		53.8			5.3		53
	M. medusae-populina		0		46.2			94.7		47
	M. larici-populina		0		0			0		0
5007	*M. medusae*		0			0		0	0	0
	M. medusae-populina		100			100		100	100	100
	M. larici-populina		0			0		0	0	0
87004-32	*M. medusae*		0		33.3			26.7		20
	M. medusae-populina		100		66.7			73.3		80
	M. larici-populina		0		0			0		0
87004-11	*M. medusae*		60			94.7		63.6		72.8
	M. medusae-populina		40			5.3		36.4		27.2
	M. larici-populina		0			0		0		0
50-197	*M. medusae*							0		0
	M. medusae-populina							0		0
	M. larici-populina							100		100
15-079	*M. medusae*							0		0
	M. medusae-populina							21.1		21.1
	M. larici-populina							78.9		78.9
184-411	*M. medusae*							0		0
	M. medusae-populina							0		0
	M. larici-populina							100		100
	M. medusae	0	45.8	1.5	59.6	56.4	31.4	19.1	13.4	36.5
	M. medusae-populina	52.6	54.2	26.2	40.4	43.6	40.7	49.4	43.9	36.3
	M. larici-populina	47.4	0	72.3	0	0	27.9	31.5	42.7	27.2

gap - not sampled

Proportions of the different species have fluctuated over the duration of the survey (Table 3.3). *M. larici-populina* became more prolific towards the end of the season whilst *M. medusae* populations decreased on the plant material examined. Populations of *M. medusae-populina* remained steady throughout the survey.

Clones 65/31, K12-2, 5007, 50-197, 15-079 and 184-411 were not infected by *M. medusae* (Fig. 3.2, 3.3, 3.5 and Table 3.2). Clones 65/29 and I488 had very little *M. medusae* infection (1.7% for 65/29 and 2.1% for I488) with *M. medusae* only recorded infecting Clone I488 at the end of the season (Fig. 3.2, 3.3 and Table 3.2). Clones 5006, 87002-10 and 87002-05 were infected primarily by *M. medusae* (Fig. 3.3 and 3.4). Clones 50-197, 15-079 and 184-411 were infected primarily by *M. larici-populina*. Eleven clones (all clones from Lesotho) were not infected with *M. larici-populina* (Fig. 3.2 and Table 3.4). Only two clones (European sources) were exclusively infected with this species. No *M. medusae/M. larici-populina* interactions were observed except where a clone was infected by all three species (i.e., Clones 65/29 and I488) but the *M. medusae* contribution was relatively small. Over half of the clones surveyed were susceptible to both *M. medusae* and *M. medusae-populina*. Figure 3.7 shows the close proximity of pustules from different species that was occasionally seen.

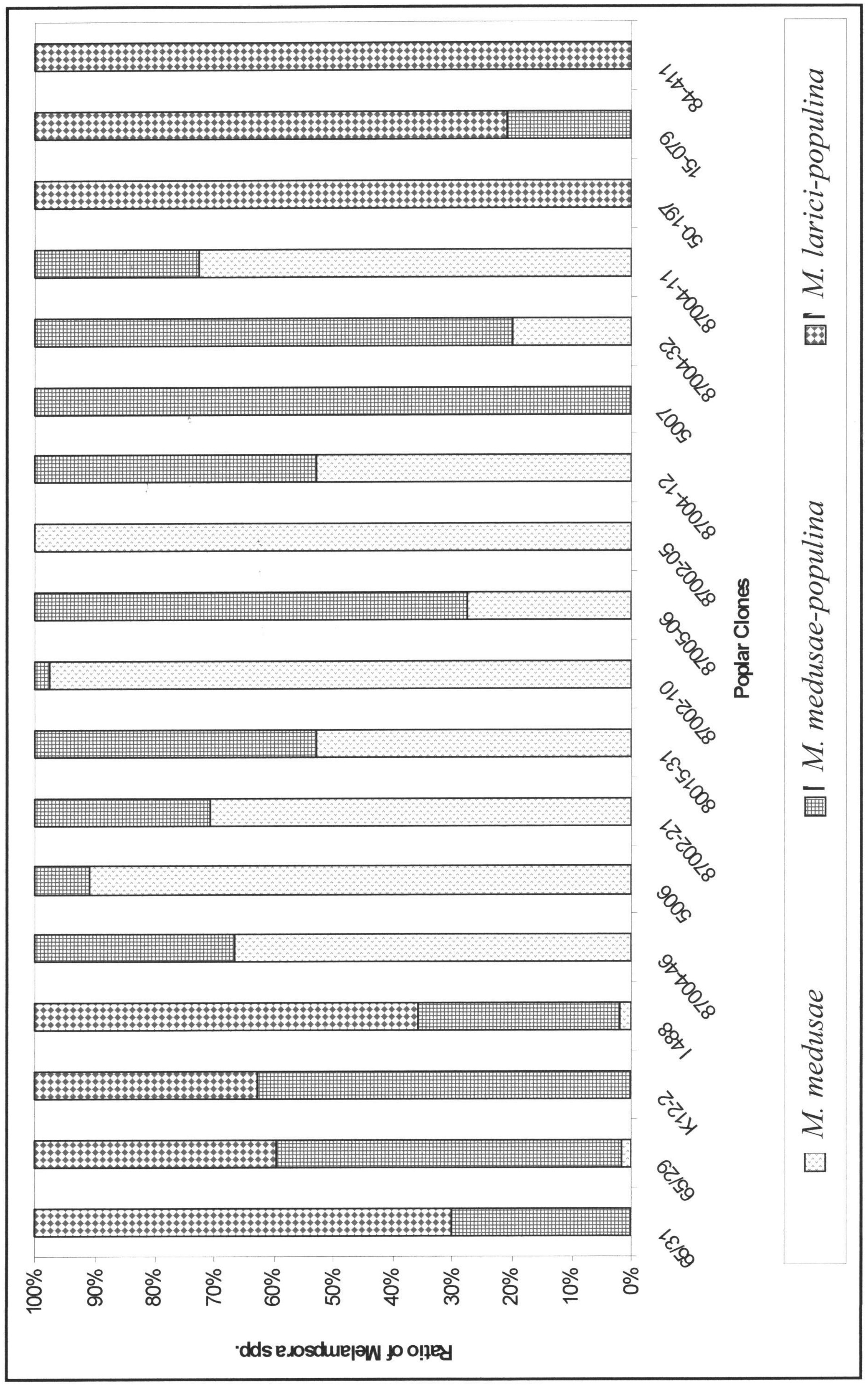
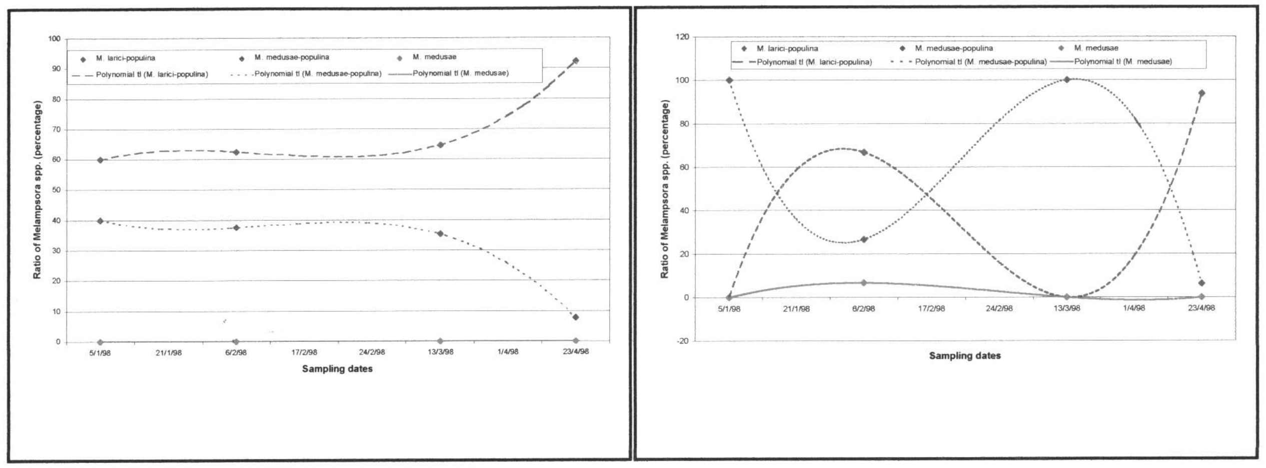
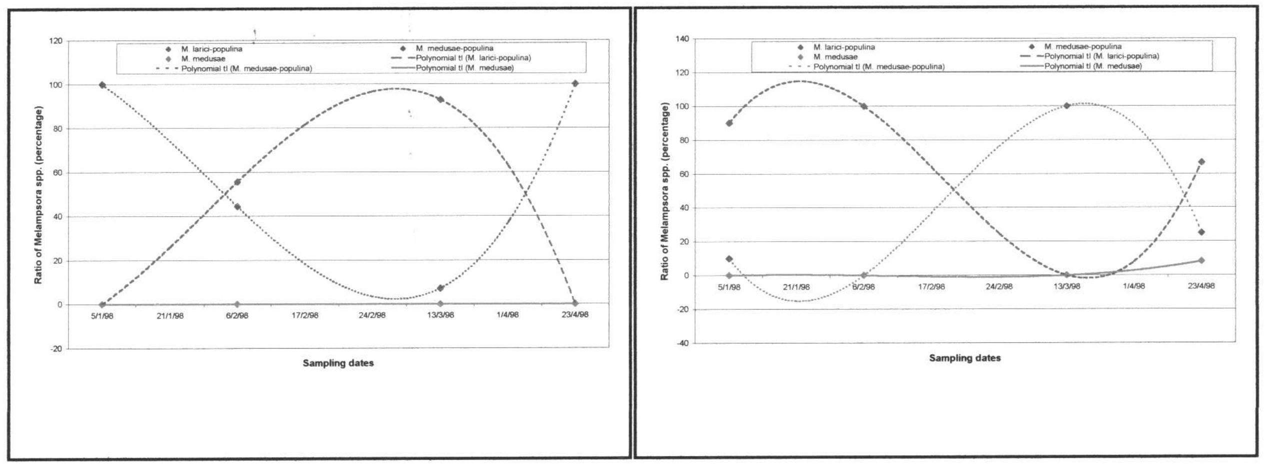


Fig. 3.2 Relative incidence of *Melampsora* spp. infecting commercial and experimental poplar clones from a survey conducted from January to April, 1998 at Lion Match Company plantations, Seven Oaks, KwaZulu-Natal, South Africa.



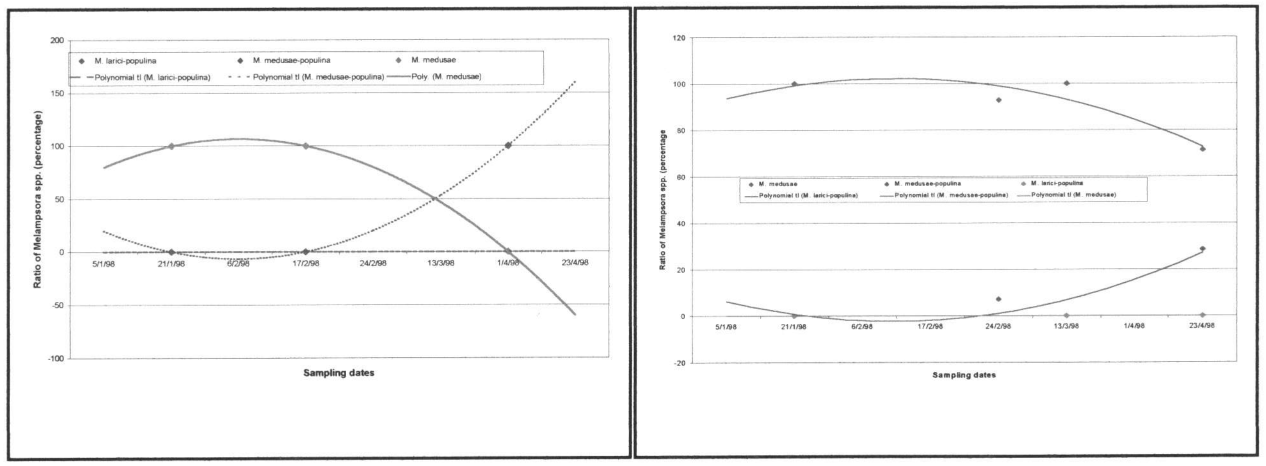
Clone 65/31

Clone 65/29



Clone K12-2

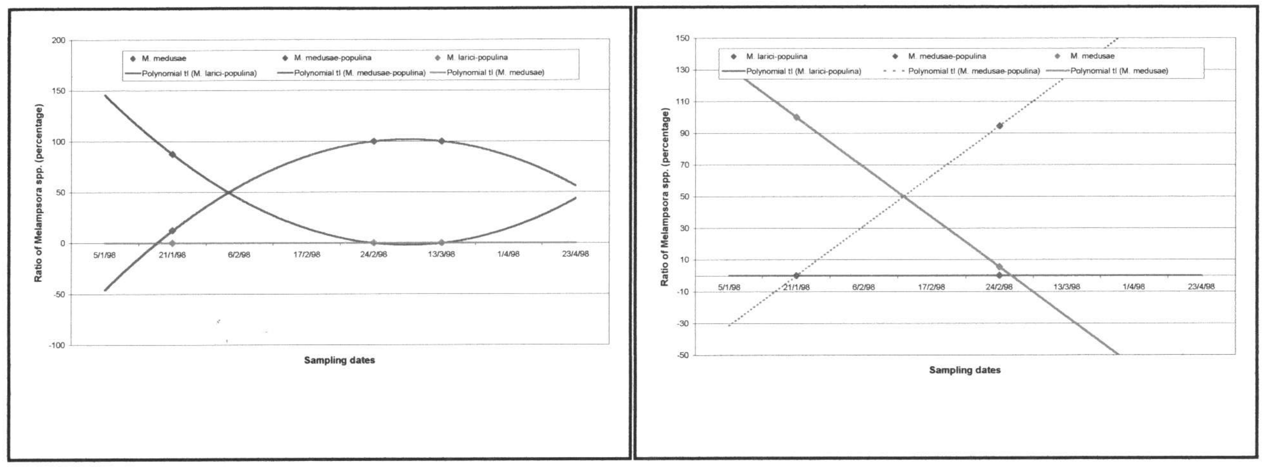
Clone I488



Clone 87004-46

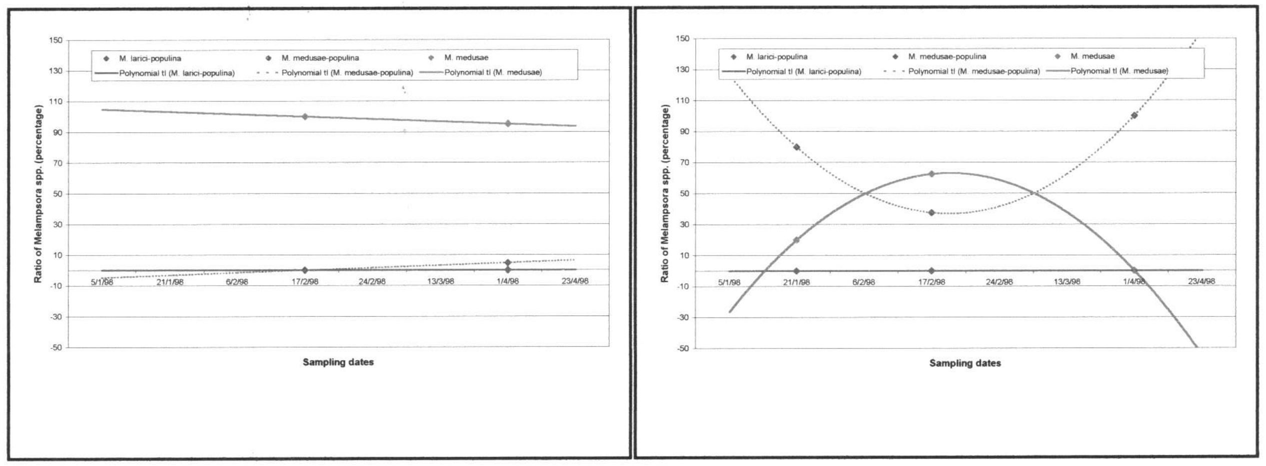
Clone 5006

Fig. 3.3 Relative incidence of rust species infecting commercial and experimental poplar clones (Clone 65/31, Clone 65/29, Clone K12-2, Clone I488, Clone 87004-46, Clone 5006) at each sampling date from a survey conducted from January to April, 1998 at Lion Match Company plantations, Seven Oaks, KwaZulu-Natal, South Africa.



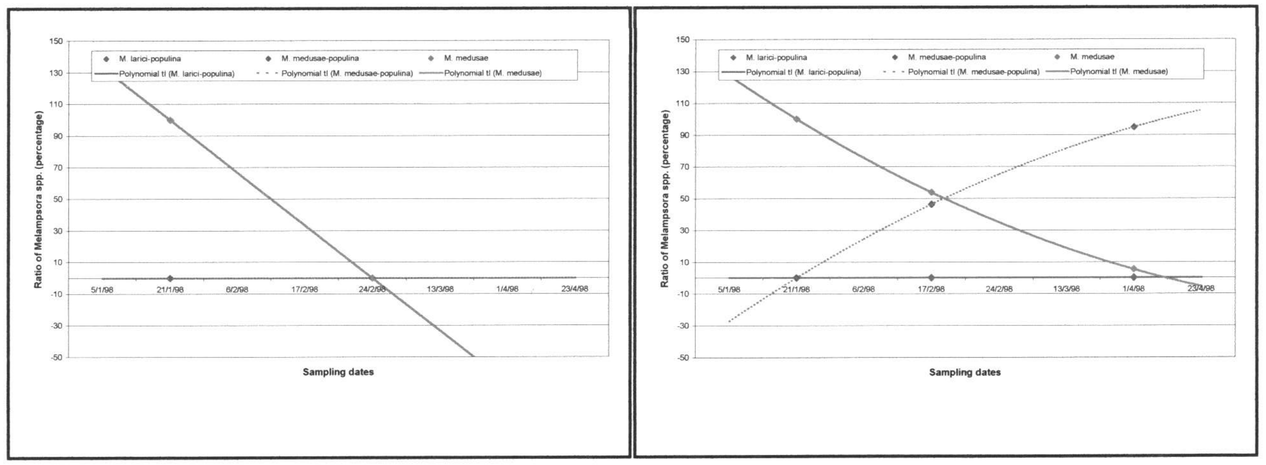
Clone 87002-21

Clone 80015-31



Clone 87002-10

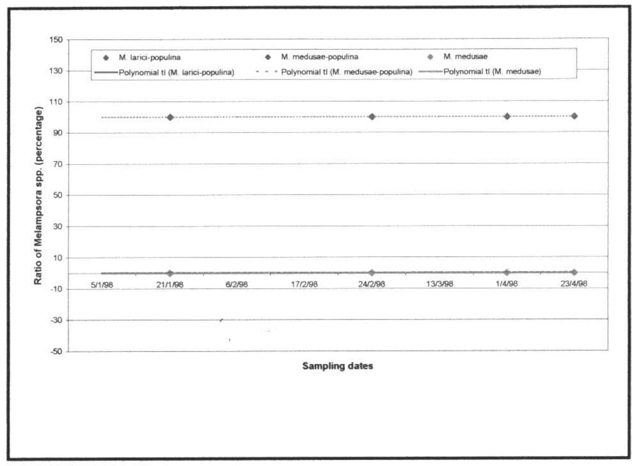
Clone 87005-06



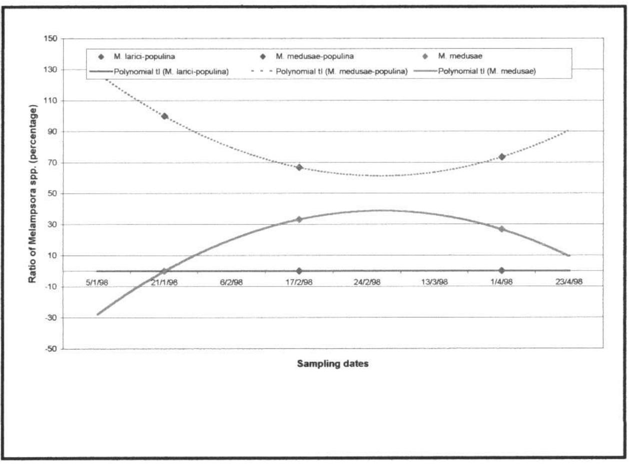
Clone 87002-05

Clone 87004-12

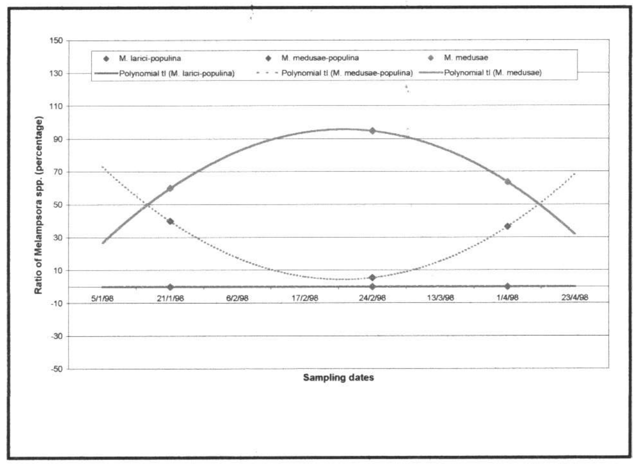
Fig. 3.4 Relative incidence of rust species infecting commercial and experimental poplar clones (Clone 87002-21, Clone 80015-31, Clone 87002-10, Clone 87005-06, Clone 87002-05, Clone 87004-12) at each sampling date from a survey conducted from January to April, 1998 at Lion Match Company plantations, Seven Oaks, KwaZulu-Natal, South Africa.



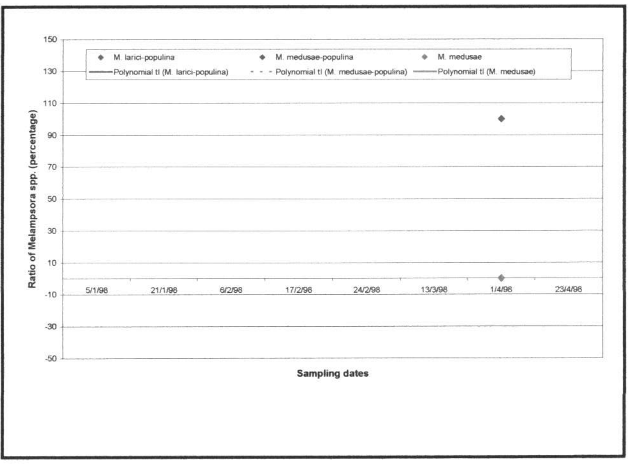
Clone 5007



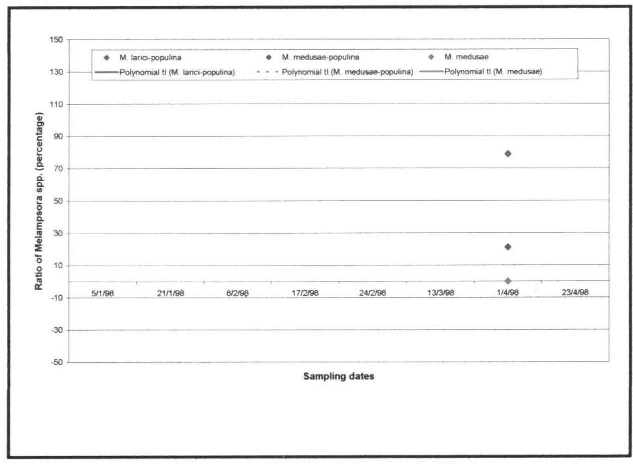
Clone 87004-32



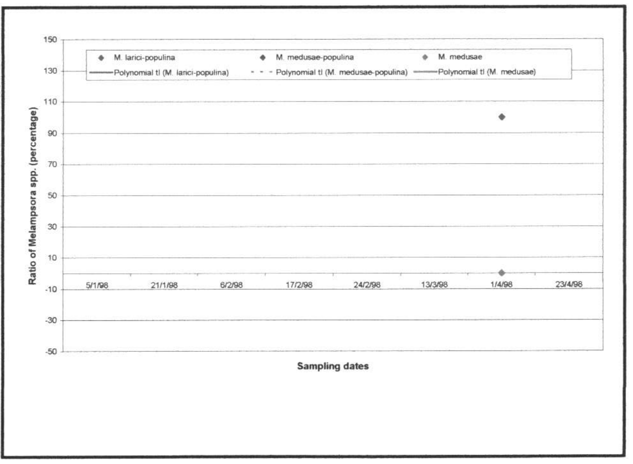
Clone 87004-11



Clone 50-197



Clone 15-079



Clone 84-411

Fig. 3.5 Relative incidence of rust species infecting commercial and experimental poplar clones (Clone 5007, Clone 87004-32, Clone 87004-11, Clone 50-197, Clone 15-079, Clone 184-411) at each sampling date from a survey conducted from January to April, 1998 at Lion Match Company plantations, Seven Oaks, KwaZulu-Natal, South Africa.

Table 3.3 Fluctuations of the relative incidence of *Melampsora* spp. infecting commercial and experimental poplar clones from a survey conducted in 1998 at Lion Match Company plantations, Seven Oaks, KwaZulu-Natal, South Africa

Month	***M. medusae***	***M. medusae-populina***	***M. larici-populina***
January	25.6	53.5	20.9
February	39.0	36.9	24.1
March	23.2	46.5	30.3
April	13.4	43.9	42.7
Overall	27.2	44.1	28.7

Table 3.4 Summary of Table 3.2 showing differential susceptibility of the different clones to the three *Melampsora* spp. found infecting commercial and experimental clones at Lion Match Company plantations in Seven Oaks, KwaZulu-Natal, South Africa. A clone appearing twice in a row indicates the pathogen species by host clone interaction as well as the dominant species occurring in the interaction (only when the single species contribution >70%)

***M. medusae* (only)**	***M. medusae-populina* (only)**	***M. larici-populina* (only)**	***M. medusae/ M. medusae-populina***	***M. medusae/ M. larici-populina***	***M. medusae-populina/ M. larici-populina***	***M. medusae/ M. medusae-populina/ M. larici-populina***
		65/31			65/31	
					65/29	65/29
					K12-2	
					I488	I488
			87004-46			
5006			5006			
87002-21			87002-21			
			80015-31			
87002-10			87002-10			
	87005-06		87005-06			
			87004-12			
87002-05						
	5007					
	87004-32		87004-32			
87004-11			87004-11			
		50-197				
	15-079					
		184-411				

Fig. 3.6 Adjacent pustules of two different species of rust. A. *M. medusae-populina*: large pustule at the top (MP) and *M. medusae*: smaller pustule at bottom (M) B. small pustule of *M. larici-populina* (LP) with larger pustule of *M. medusae* (M)

3.4 DISCUSSION

In clones that have been in circulation in South Africa for more than five years (Clone 65/31, Clone 65/29, Clone K12-2, Clone I488), rust epidemics would have stabilised. Mid-January in South Africa falls in the middle of summer. Rust epidemics are greatest at this time due to the warm temperatures and high humidity. However, as the survey progressed, more disease became apparent. This could be due to increased plant susceptibility towards the end of the growing period, or that conditions towards the end of summer are more favourable for disease development. The two known susceptible clones, Clone 65/31 and Clone I488, became susceptible earliest out of all the sampled material. Clone K12-2 maintained low infection levels throughout the survey, whereas, the resistant Clone 65/29 became susceptible only towards the end of April.

On the first sampling date, the newer clones from New Zealand (Clone 87004-46, Clone 5006, Clone 87002-21, Clone 80015-31, Clone 87002-10, Clone 87005-06, Clone 87002-05, Clone 87004-12, Clone 5007, Clone 87004-32 and Clone 87004-11) appeared to be resistant to all species of *Melampsora* rust. Chlorosis did occur on some leaves on which pustules were present that had not burst. In samples taken from these same clones towards the end of the survey burst, pustules became apparent. Susceptibility increased towards the end of the survey so that all clones that were resistant became either slightly susceptible or susceptible, with the exception of Clone 87002-05 which was last sampled toward the end of February. Therefore, it may well be that it, too, became more susceptible.

Material from Europe had just come out of quarantine and therefore could not be sampled earlier. However, this material was very susceptible, with large and prolific pustules. This new material was mostly infected with *M. larici-populina*. This is surprising as the race of *M. larici-populina* found to occur in South Africa is an old race and it would be expected that this race would be incorporated into European breeding programs in screening for resistance. Clone 15-079 was also infected with *M. medusae-populina*. Unfortunately, the parentage of this clone was unknown at the time of writing, but it may be assumed that it is different to Clone 50-197 and 184-411. Race identification conducted in France (Hawke, 1997) showed that *M. medusae-populina* was found to have all the virulences known to occur in *M. larici-populina* as well as one virulence effective on two clones which were still completely resistant to *M. larici-populina* in France (Pinon, 1997). From this it is not surprising that this new material was infected by *M. medusae-populina.*

Figure 3.2 summarises the survey, and patterns in clone types appear clear. Most of the clones from Lesotho (originally New Zealand) appear to have the same overall resistance make-up. This does not appear to be genetic since their clonal parentage is very different, ranging from *P. yunanensis, P. szechuanica* and *P. nigra* crosses with *P. deltoides*. Clone 80015-31, Clone 65/31 and Clone 65/29 have similar parentage but appear to have no similarity in genetically inherited resistance. Because all the Lesotho material was infected in the same way, it is possible that the environment was responsible for the composition of infection and not genetics. These clones were planted away from the other clones in a relatively new nursery site with established poplar stands approximately 500m away.

The composition of species of *Melampsora* fluctuated on all clones during the course of the survey (Fig. 3.3-3.5). The patterns of composition appear to be random. This could be due to the fact that most of the clones were newly introduced and populations of *Melampsora* species were still adjusting. Of the established clones; Clone 65/31, the dominant species of rust (*M. larici-populina*) increases towards the end of the survey; Clone 65/29, a random fluctuation of populations exists; Clone K12-2, *M.medusae* populations were dominant and replaced by *M. medusae-populina* populations; Clone I488, *M. larici-populina* is dominant with *M. medusae-populina* causing low infection towards the end of the survey.

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Chapter 4 DISTRIBUTION OF *MELAMPSORA* SPP. ON POPLARS IN SOUTH AFRICA: A PRELIMINARY SURVEY

ABSTRACT

A once-off survey of rust infected poplar leaves from ten different locations (over 1 500km apart) was conducted to ascertain the effect of geographical and meteorological conditions on the presence and severity of rust on poplars. The most popularly grown clones in South Africa are Clone 65/29, Clone 65/31, Clone I488, Clone 129 and the old clone *Populus deltoides* var. *missouriensis*. Clone 65/31 had the greatest severity of disease (10.4%) closely followed by Clone I488 (9.5%). Clone 129 had the least amount of disease (1.8%). The most common rust species occurring in South Africa was M. larici-populina. The hybrid, *M. medusae-populina*, was the least prevalent, although race studies found this species to be the most virulent.

4.1 INTRODUCTION

Poplar leaf rust caused by species of the genus *Melampsora* Cast. is one of the most damaging leaf diseases of hybrid poplar plantations. Damage varies according to climatic fluctuations, microclimate of the plantations, proximity of alternate hosts, metabolic state of poplar hosts, and susceptibility of the poplar clones planted. Heavy rust infections, particularly those early in the growing season, weaken the poplar host and predispose it to other biotic or abiotic diseases. In addition, shoot growth and development of the root system are reduced (Taris, 1979, In Hubbes *et al.*, 1983).

In his study on poplar rust in France, Pinon (1973) described eight species of *Melampsora* based on the characteristic features of uredospores and associated paraphyses. Trench and Churchill (1987) reported that *M. medusae* Thüm, *M. larici-populina* Kleb. and *M. aecidioides* (DC.) Schroet. occur on poplars in South Africa. Two of these, *M. medusae* and *M. larici-populina* are the more common fungi to attack commercially grown clones of poplars, globally.

The benefit of doing a disease survey is to determine the extent of the spread of poplar rust and to identify susceptible or resistant clones. The aim of this survey was to determine if the seasons and time influenced populations of the different rust species and to determine whether a relationship exists between clones and the species that infect them.

4.2 MATERIALS AND METHODS

4.2.1 Collection of Data

A list of poplar growers was obtained from the Lion Match Company and growers were contacted. Various growers were selected as sites to be represented in the survey resulting in 11 areas in total. Twenty-five samples of 50 leaves (over the month of May, 1999) on approximately the same position on a branch were picked, bagged and sealed. These were preserved in a portable refrigerator for the duration of the trip. Each leaf was assessed for percentage leaf area infected (LAI) and percentage leaf necrosis (LN) using a visual rating scale (Fig. 4.1). Single pustules were removed from the leaves using a single edged razor blade to make up 100 pustules from each clone from each site. The pustules were then placed on a stub (Fig. 4.2), fixed with osmium tetroxide, coated with gold: palladium and viewed under an Hitachi Scanning Electron Microscope. The pustules were identified as *Melampsora medusae, M. larici-populina* or *M. medusae-populina* by their wall echinulations (Fig. 3.1). Pustules of the same species were converted to a percentage of the LAI and this conversion factor was used to calculate the percentage of each species making up the LN. Temperature and rainfall data was obtained from the Computing Center for Water Research (CCWR), Pannar Research Services (Pty) Ltd and SASEX, Umfolozi, KwaZulu-Natal. A computer program, Arcview, was used to generate grid maps of temperature and rainfall in the sampling areas using 50 year means.

4.2.2 Site Descriptions

Table 4.1 lists questions asked of each farmer participating in the survey. Various farms were selected as sites to be represented in the survey. The sites are listed in Table 4.2.

Table 4.1 Questionnaire used for conducting the survey of poplar growers in South Africa

QUESTIONNAIRE

How many hectares of poplars do you have?

How long do you expect from a rotation?

What other growers do you know?

How old are your plantations?

Where are you? Directions:

What clones do you grow?

Soils: Have any analyses been done? What types of soils do you grow your poplars on?

What is your mean annual rainfall?

What fertilizer applications have been made?

What other cultural practices have been employed?

What was your propagation type and where did you get it from?

What problems have you experienced (e.g. rust, caterpillars, site, etc...)?

Did you notice a defoliation date? When?

Do you have any yield data?

Table 4.2 Site descriptions of farms represented in a survey evaluating rusts of poplars in South Africa. Individual farming practices have been listed

Town	Latitude/ Longitude	Soils	Fertilizer applications	Rainfall (mm/yr)	Other
Bergville	28°45'S 29°25'E			800	
Elandslaagte	28°20'S 30°00'E	Deep, alluvial	One handful of 2:3:2 at planting	727	Rust and frost are main problems that have been experienced
Ladysmith	28°35'S 29°35'E		None	800	Problems have been experienced with field mice ring-barking the trees; Rust is particularly bad on older clones
Loui Trichardt	23°05'S 29°55'E	Vleis with poor drainage	None	540	
Mtubatuba	28°25'S 32°20'E	Alluvial dark and heavy, often waterlogged	150g superphosphate with a small handful of urea at planting	1400-1500	Squatters have been stealing trees
Paulpietersburg	27°25'S 30°50'E	Pongola river food plain (Katspruit)	100g 2:3:2 at planting and 400kg LAN at the beginning of each season	822	Intercrop plantations with grazing and hay; defoliation has previously been noticed to occur in November when there is heavy rainfall and high temperatures
Seven Oaks	29°13'45"S 30°29'30"E	Kranskop form of the Fourdoun type	Handful of 7:1:7 annually	800	Drip irrigated experimental stand
Sheepmoor	26°45'S 30°20'E		Handful of 2:3:2 at planting	773	
Winterton	28°50'S 29°35'E	Hutton	Handful of 2:3:2 at planting	740	Stand received drainage from a pivot irrigating maize and wheat

4.2.3 Statistical Analysis

Data was analysed using the correlation function in Quattro Pro (Corel Corp.). No rain fell on any of the sites on Day0, Day1 and Day2 prior to sampling and these days have been excluded from correlation and regression analysis. Linear regression analysis was also conducted on data sets using the "least squares" method to fit a line through a set of observations. Correlation and linear regression values were converted to percentages by multiplying by 100. Because the objective of this survey was to determine whether a sensitivity to temperature and rainfall of the rust species existed, R^2 was only calculated for LAI of the different *Melampsora* spp.

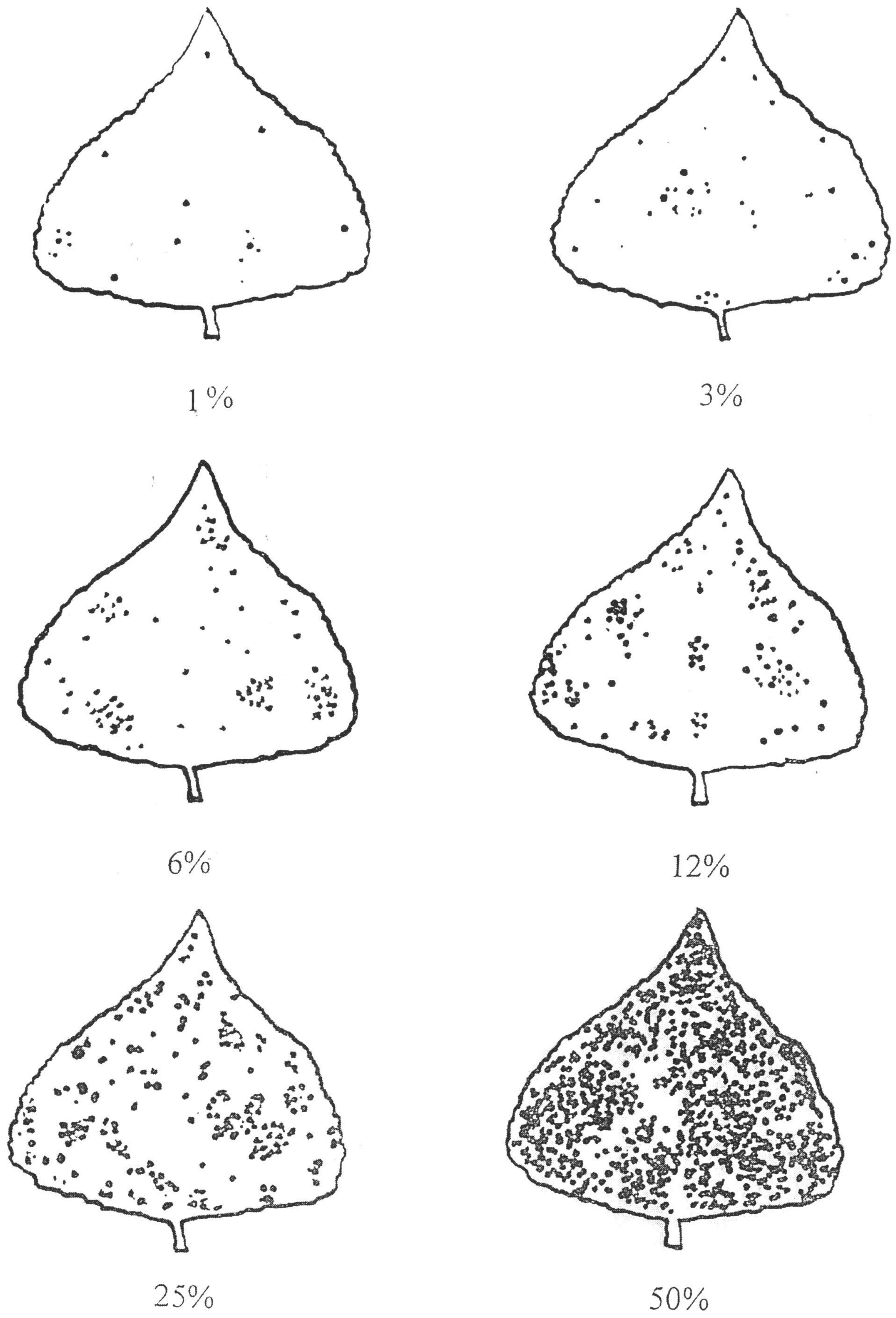

Fig. 4.1 Visual rating scale used to aid in rating diseased leaves for percentage leaf area infected and percentage leaf necrosis.

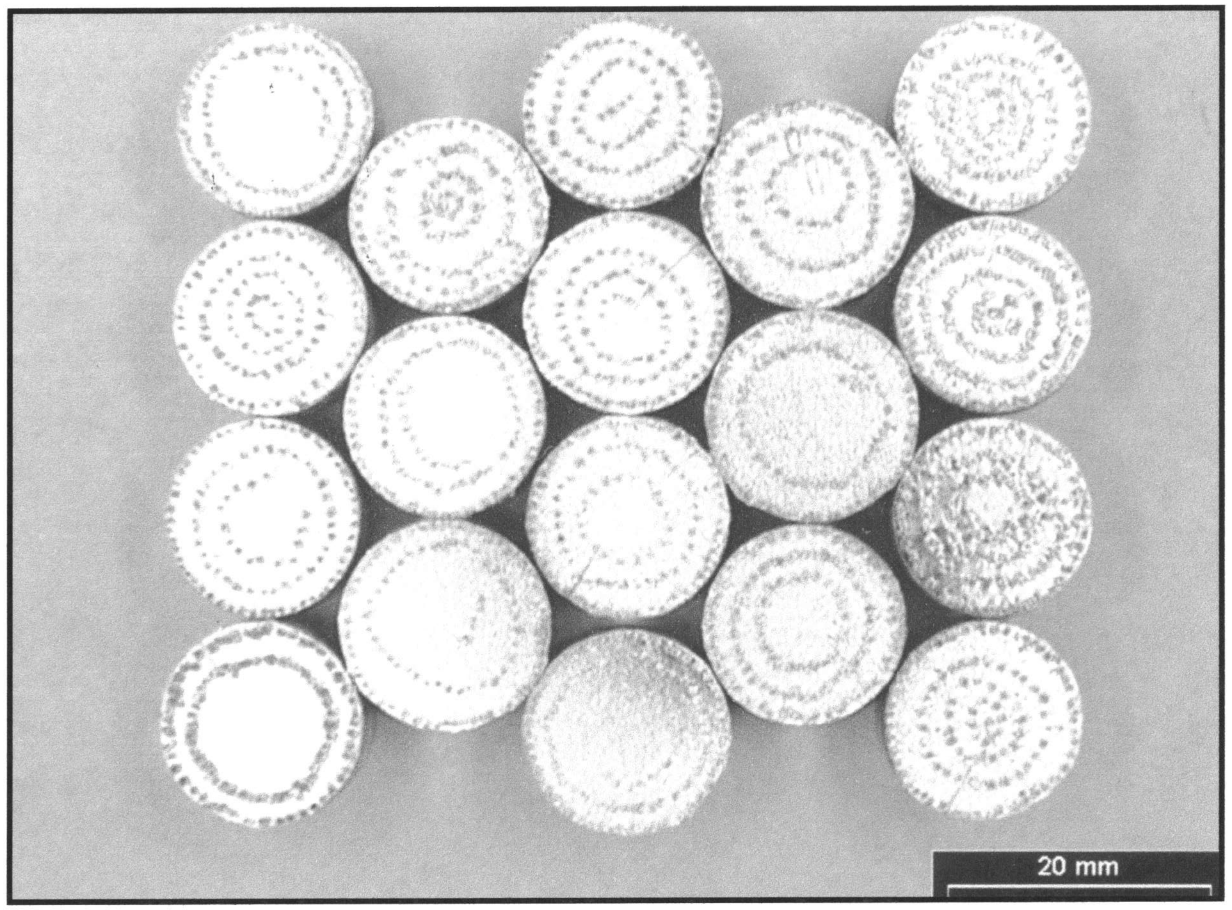

Fig. 4.2 Leaf sections on a stub that have been fixed with osmium tetroxide and coated with gold: palladium, in preparation for viewing under an Hitachi S570 Scanning Electron Microscope.

4.3 RESULTS

Highest disease level (19% leaf area infected, LAI) was found at Elandslaagte on Clone 65/31 (low rainfall of 5-10mm in May), 4-24°C (min. and max. for May). Trees had been planted next to a perennial water source. Aside from Clone 65/29 in Matubatuba which had no disease, the least disease level was found in Bergville on the Clone 65/31 (3.2% LAI) (low rainfall of 5-10mm in May), 4-24°C (min. and max. for May). Trees had not been planted adjacent to a watercourse.

Most prevalent species of *Melampsora* was *M. larici-populina* (65%) and the most susceptible clone was Clone 65/31 with a mean LAI of 10.6%. Least prevalent species of *Melampsora* was *M. medusae-populina* and the least susceptible clone was Clone 129 with 1.5% LAI mean. Clone *Populus deltoides* var *missouriensis* and Clone 129 were only infected by *M. medusae*. Clone I488, 65/31and 65/29 were infected by all three species of *Melampsora* (Fig. 4.3, Fig. 4.4 and Fig. 4.7). Clone *P. deltoides* var. *missouriensis* had the greatest mean LN (21.9%) whilst Clone 65/29 had the least (5.8%).

Table 4.3 Correlation percentages (R and R^2) of comparisons between the different species of *Melampsora* and Clone, Site, LAI (leaf area infected) and LN (leaf necrosis). I-p = *Melampsora larici-populina*, m = *M. medusae* and m-p = *M. medusae-populina*

FACTOR	LAI (l-p)		LAI (m)		LAI (m-p)	
	R	R^2	R	R^2	R	R^2
Clone	-24.92	6.21	24.91	6.21	-12.24	1.5
Site	-29.25	8.56	21.52	4.63	9.77	0.96
LAI	79.55	63.28	0.24	0	27.66	7.65
LAI (l-p)	100	100	-43.28	18.73	-16.21	2.63
LAI (m)	-43.28	18.73	100	100	-5.2	0.27
LAI (m-p)	-16.21	2.63	-5.2	0.27	100	100
LN	1.21	0.01	4.01	0.16	-6.31	0.4
LN (l-p)	73.73	54.46	-53.26	28.37	-17.72	3.14
LN (m)	-44.55	19.85	43.53	18.95	-14.61	2.13
LN (m-p)	-19.71	3.89	-15.89	2.53	93.06	8.66

Table 4.4 R and R^2 percentages between LAI (leaf area infected) of each *Melampsora* spp. and Rain (rainfall), Temp (mean temperature), whether sampling stand was near a water source, TMax (max. temperature for the day), TMin (min. temperature for the day) up to two weeks prior to sampling l-p = *Melampsora larici-populina*, m = *M. medusae* and m-p = *M. medusae-populina*

FACTOR	LAI (l-p)		LAI (m)		LAI (m-p)	
	R	R^2	R	R^2	R	R^2
Rain 3	-22.01	4.64	-20.06	4.02	64.04	41.01
Rain 4	-22.01	4.64	-20.06	4.02	64.04	41.01
Rain 5	-22.01	4.64	-20.06	4.02	64.04	41.01
Rain 6	-22.01	4.64	-20.06	4.02	64.04	41.01
Rain 7	-22.01	4.64	-20.06	4.02	64.04	41.01
Rain 8	-22.01	4.64	-20.06	4.02	64.04	41.01
Rain 9	-14.2	2.02	26.75	7.16	11.26	1.27
Rain 10	-14.6	2.13	-19.83	3.93	55.28	30.56
Rain 11	6.968	0.49	-27.03	7.31	26.92	7.25
Rain 12	-22.01	4.84	-20.06	4.02	64.04	41.01
Rain 13	-15.57	2.42	-11.28	1.27	16.27	2.65
Temp 0	7.12	0.51	-34.07	11.61	33.1	10.95
Temp 1	12	1.44	-34.78	12.1	14.25	20.3
Temp 2	19.08	3.64	-31.11	9.68	-3.43	0.12
Temp 3	16.01	2.56	-32.89	10.82	-0.16	0
Temp 4	10.57	1.12	-32.78	10.74	12.59	1.59
Temp 5	10.42	1.09	-31.82	10.12	6.78	0.46
Temp 6	18.77	3.52	-33.41	11.16	-2.86	0.08
Temp 7	7.267	0.53	-31.4	9.86	10.98	1.21
Temp 8	4.602	0.21	-34.09	11.62	21.54	4.64
Temp 9	3.037	0.09	-29.12	8.48	19.33	3.74
Temp 10	10.52	1.11	-38.27	14.65	16.5	2.72
Temp 11	10.84	1.17	-31.83	10.13	7.97	0.64
Temp 12	14.88	2.22	-34.46	11.82	6.36	0.4
Temp 13	14.11	1.99	-27.88	7.77	0.86	0.01
Water Source	19.63	3.85	12.48	1.56	3.13	0.1
Tmax 0	-12.14	1.47	-23.62	5.58	48.87	23.88
Tmax 1	-6.2	0.38	-25.13	6.37	40.61	16.49
Tmax 2	4.08	0.17	-27.38	7.5	3.59	0.13
Tmax 3	14.67	2.15	-29.52	8.72	-8.84	0.78
Tmax 4	10.31	1.06	-30.53	9.32	-3.13	0.1
Tmax 5	1.646	0.03	-28.01	7.84	5.51	0.73
Tmax 6	8.57	0.73	-21.92	4.81	-18.71	3.5
Tmax 7	4.1	0.17	-29.1	8.47	6.59	0.43
Tmax 8	-4.31	0.19	-33.45	9.06	30.1	9.06
Tmax 9	-2.57	0.07	-26.53	7.04	44.42	19.73
Tmax 10	-26.71	7.13	-16.27	2.65	43.99	19.35
Tmax 11	2.04	0.04	-33.26	11.07	23.09	5.33
Tmax 12	-1.75	0.03	-29.29	8.58	27.02	7.3
Tmax 13	18.19	3.31	-28.77	8.28	-5.85	0.34
Tmin 0	20.95	4.39	-34.98	12.24	14.64	2.14
Tmin 1	16.68	2.78	-28.34	8.03	-5.09	0.26
Tmin 2	26.82	7.19	-25.02	6.26	-8.58	0.74
Tmin 3	12.92	1.67	-30.67	9.41	10.94	1.2
Tmin 4	11.2	1.25	-32.94	10.85	20.85	4.35
Tmin 5	13.23	1.75	-31.99	10.23	7.25	0.53
Tmin 6	16.45	2.71	-32.51	10.57	8.36	7
Tmin 7	15.67	2.46	-36.61	13.4	10.54	1.11
Tmin 8	6.02	0.36	-27.24	7.42	13.92	1.94
Tmin 9	13.52	1.83	-29.55	8.73	4.09	0.17
Tmin 10	20.18	4.07	-31.12	9.69	-3.02	0.09
Tmin 11	20.08	4.03	-31.16	9.7	-5.37	0.29
Tmin 12	16.85	2.84	-29.69	8.82	-2.95	0.09
Tmin 13	18.68	3.49	-33.61	11.29	4.21	0.18

Table 4.5 Correlation percentages between Clone, Site, LAI (leaf area infected), LN (leaf necrosis) and Rain (rainfall), Temp (mean daily temperature), whether sampling stand was near a water course, TMax (max. temperature for the day), TMin (min. temperature for the day) up to 14 days prior to sampling l-p = *Melampsora larici-populina*, m = *M. medusae* and m-p = *M. medusae-populina*

Factor	Clone	Site	LAI	LN	LN (l-p)	LN (m)	LN (m-p)
Rain3	-21.1	10.7	-3.3	-17.3	-21.9	-14.5	60.1
Rain4	-21.1	10.7	-3.3	-17.3	-21.9	-14.5	60.1
Rain5	-21.1	10.7	-3.3	-17.3	-21.9	-14.5	60.1
Rain6	-21.1	10.7	-3.3	-17.3	-21.9	-14.5	60.1
Rain7	-21.1	10.7	-3.3	-17.3	-21.9	-14.5	60.1
Rain8	-21.1	10.7	-3.3	-17.3	-21.9	-14.5	60.1
Rain9	2	-4.9	3.3	-27.6	-9.5	-21.9	12.3
Rain10	-29.3	10.3	0.7	-25.7	-23.6	-18.9	50.8
Rain11	-13.1	-49.7	7	7.7	-10.3	9.2	22.4
Rain12	-21.1	10.7	-3.3	-17.3	-22	-14.5	60.1
Rain13	0.5	17.4	-14.8	29	18.5	7.5	30
Temp0	-31.2	-37.4	6.6	-6	-15.9	-0.6	27
Temp1	-23.2	-33.9	2.5	13.6	1.2	8.2	16
Temp2	-3.2	-38.7	3.6	33.7	20.6	16.2	4.3
Temp3	-10.1	-31.9	0.9	31.6	20.3	13.6	8
Temp4	-10.8	-36.1	1.2	26.8	11.7	12.8	17.6
Temp5	-11.4	-32.1	-1.2	29	13.1	15	13
Temp6	-9.8	-37.8	2.4	31.2	18.6	15.3	3.9
Temp7	-18.8	-26.5	-2.5	23.5	11.1	10.4	16.7
Temp8	-17.8	-22.1	-1.7	21.7	11	6.4	27.8
Temp9	-9.1	-26.1	-2	26.1	10.8	11	25.8
Temp10	-20.1	-25	0.3	19.4	8.3	7.6	21.3
Temp11	-18	-24.4	-0.3	23.7	15.3	7.9	15.9
Temp12	-15.1	-21.8	2.1	21.5	14.4	7.1	13.9
Temp13	-11.2	-41.3	1.9	25.1	7.9	16.8	4.8
Water source	21.2	49.8	29.1	-11.3	6.5	-13	-8.8
TMax0	-34.8	-1.7	-1.6	-22.7	-29.5	-10.1	41.7
TMax1	-26.5	-17.4	0.2	-18	-34	0	29.4
TMax2	-4.1	-4.4	-7.5	35.9	27.7	10.7	16.2
TMax3	-3.5	-14.5	-3	37.9	31.1	13	4
TMax4	2.7	-27	-5.5	40.6	17.1	24.7	4.7
TMax5	2	-24.7	-9.5	35.3	3.4	28.1	9.5
TMax6	9.6	-18.7	-10.6	45.8	19.6	31.1	-9.6
TMax7	-10.5	-22.6	-6.9	29.8	10.9	17.4	12.1
TMax8	-16.8	-17.6	-6.9	18.4	-1.5	10.8	32.2
TMax9	-37.3	-22.8	5.3	-12.9	-13.2	-12.2	41.7
TMax10	-17	21.1	-16.1	-8.9	-33.1	5.5	39.8
TMax11	-25.5	-7	-3.3	7.9	-1	2.1	27.1
TMax12	-15.7	3.7	-3.6	0.1	-12.2	2.4	27.5
TMax13	-7.5	-46.9	2.7	28.7	9.8	20.5	-2.9
TMin0	-27	-53.6	12.4	2	-5.4	3.3	10
TMin1	-9.2	-32.6	1.6	32.4	23.8	12.8	4.3
TMin2	-0.3	-59.3	12.6	22.4	8	16.9	-7.7
TMin3	-12.6	-46.2	4	22.2	5.8	13.7	12.8
TMin4	-20	-39.9	5.8	15.8	7.5	4.2	23.9
TMin5	-15.8	-33.8	1.9	25.8	16.7	9.4	14.1
TMin6	-16.3	-41	5.7	21.9	13.1	8.3	13.5
TMin7	-24.6	-32.3	3.9	19.1	15.7	3.4	15.9
TMin8	-12.7	-20.6	-0.4	24.8	18.2	5.3	23.4
TMin9	-6.9	-30.4	2	29.2	17.6	12.2	12.5
TMin10	-12.4	-36.9	5	28.8	23.4	9.5	5.5
TMin11	-16.1	-31.4	3.8	27.5	24.8	7.7	3.8
TMin12	-8.2	-30	2.1	32.4	25.2	11.1	7.2
TMin13	-13.9	-43.9	5.6	24.8	13.8	11.6	8.9

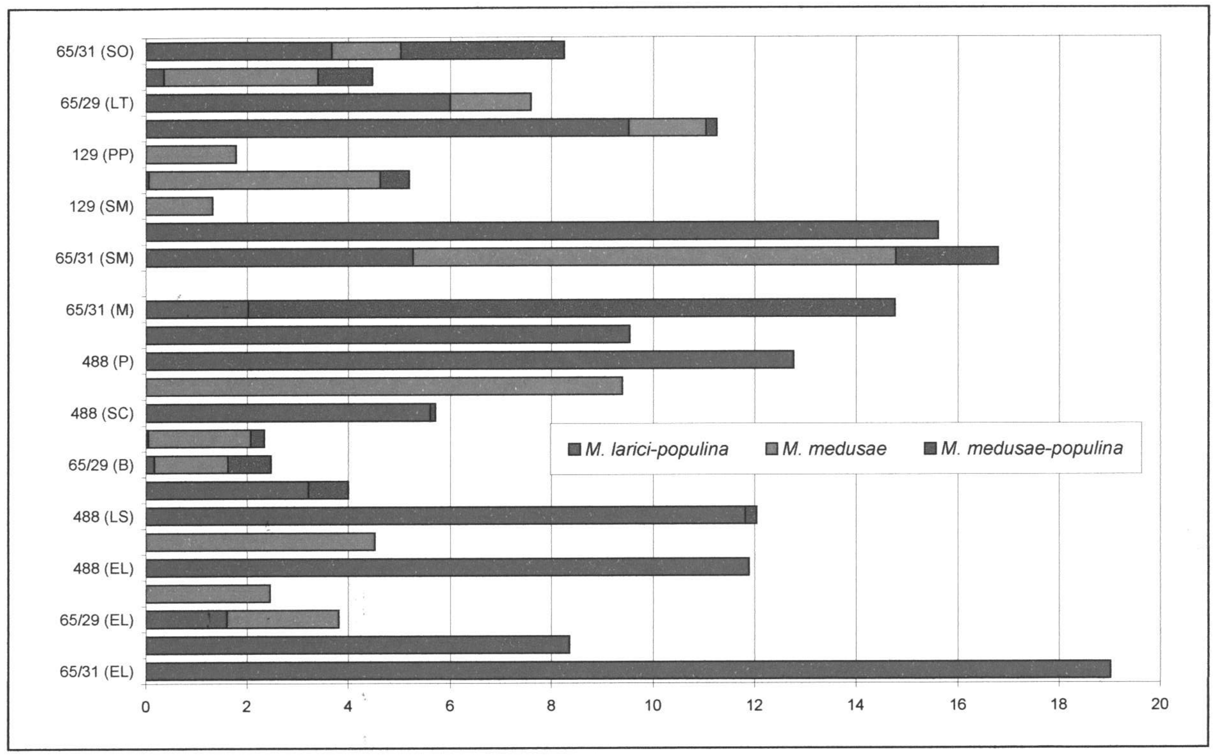

Fig 4.3 Composition of *Melampsora* species infecting poplar clones at different sites in South Africa. SO - Seven Oaks, KwaZulu-Natal; LT - Loui Trichardt, Mpumalanga; PP - Paulpietersburg, Mpumalanga; SM - Sheepmoor, Mpumalanga; M - Mtubatuba, KwaZulu-Natal; P - SC - Winterton (SW), B - Winterton (NE), KwaZulu-Natal; LS - Ladysmith, KwaZulu-Natal; EL - Elandslaagte, KwaZulu-Natal; ELC - colder site on the same farm (EL).

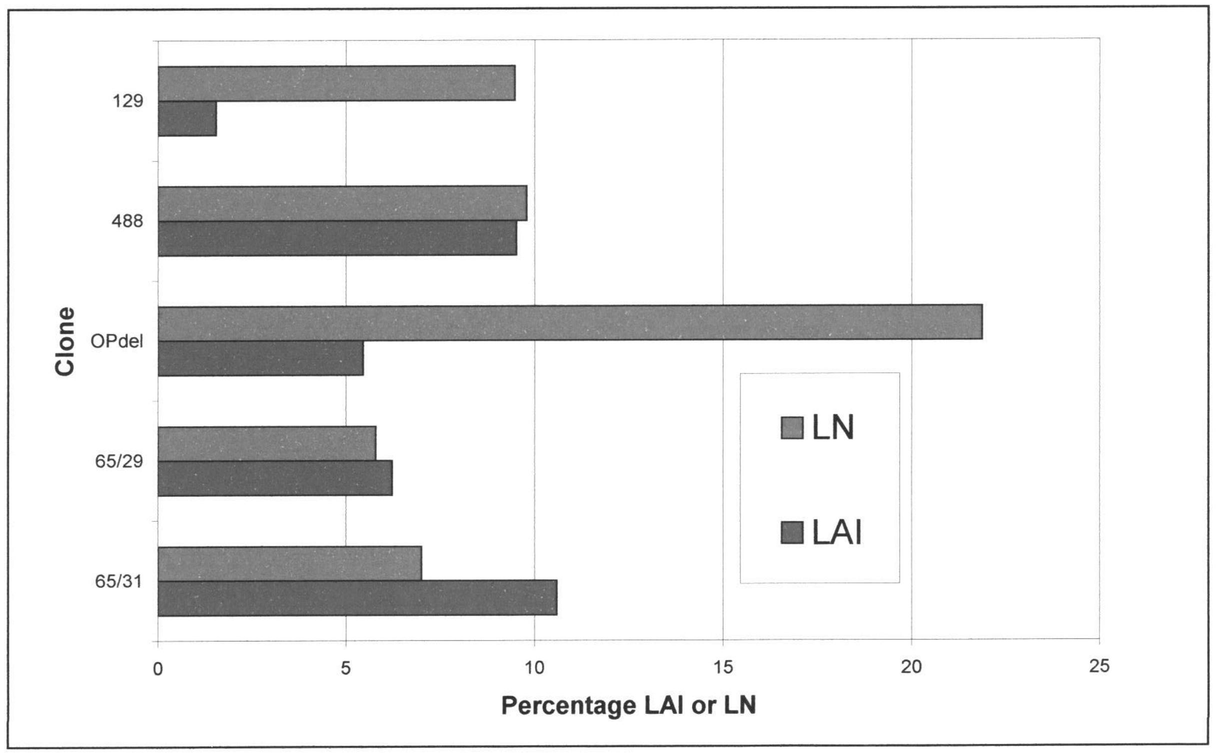

Fig. 4.4 Percentage leaf necrosis (LN) and leaf area infected (LAI) of different poplar clones sampled over South Africa 129 - Clone 129, 488 - Clone I488, OPdel -Clone *Populus deltoides* var *missouriensis*, 65/29 - Clone 65/29, 65/31 - Clone 65/31.

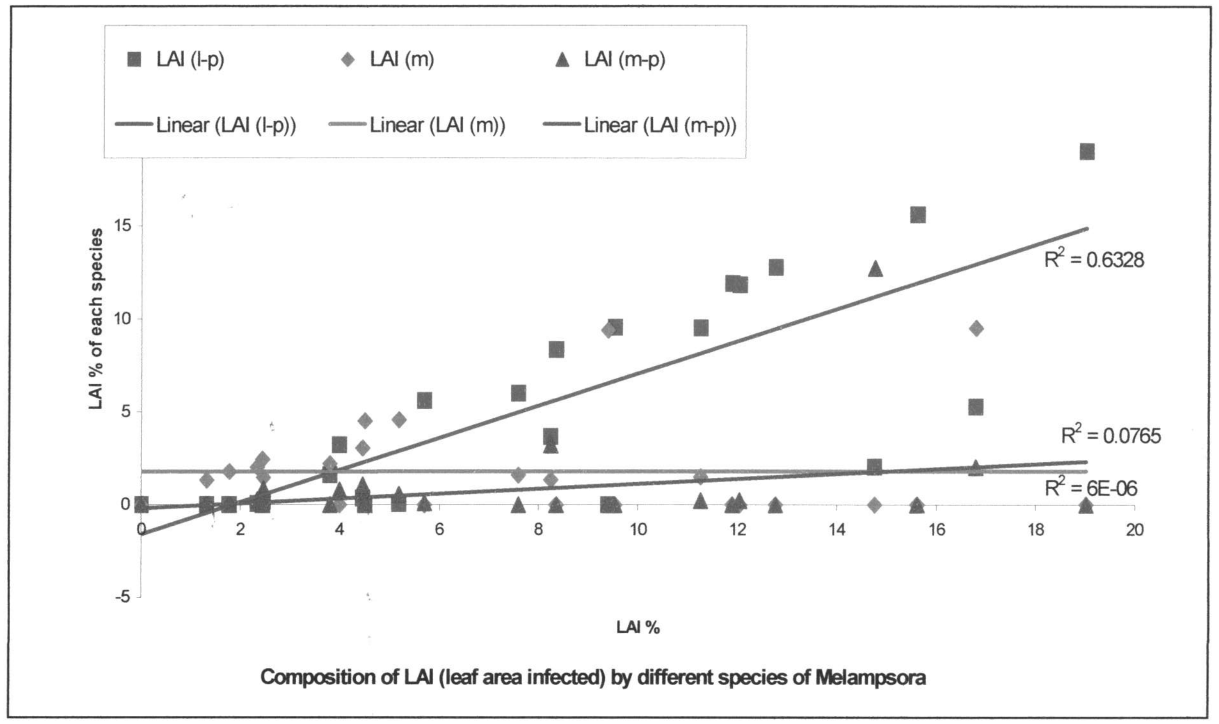

Fig. 4.5 Linear regression analysis of different levels of *Melampsora* spp. infection making up total leaf area infected (LAI).

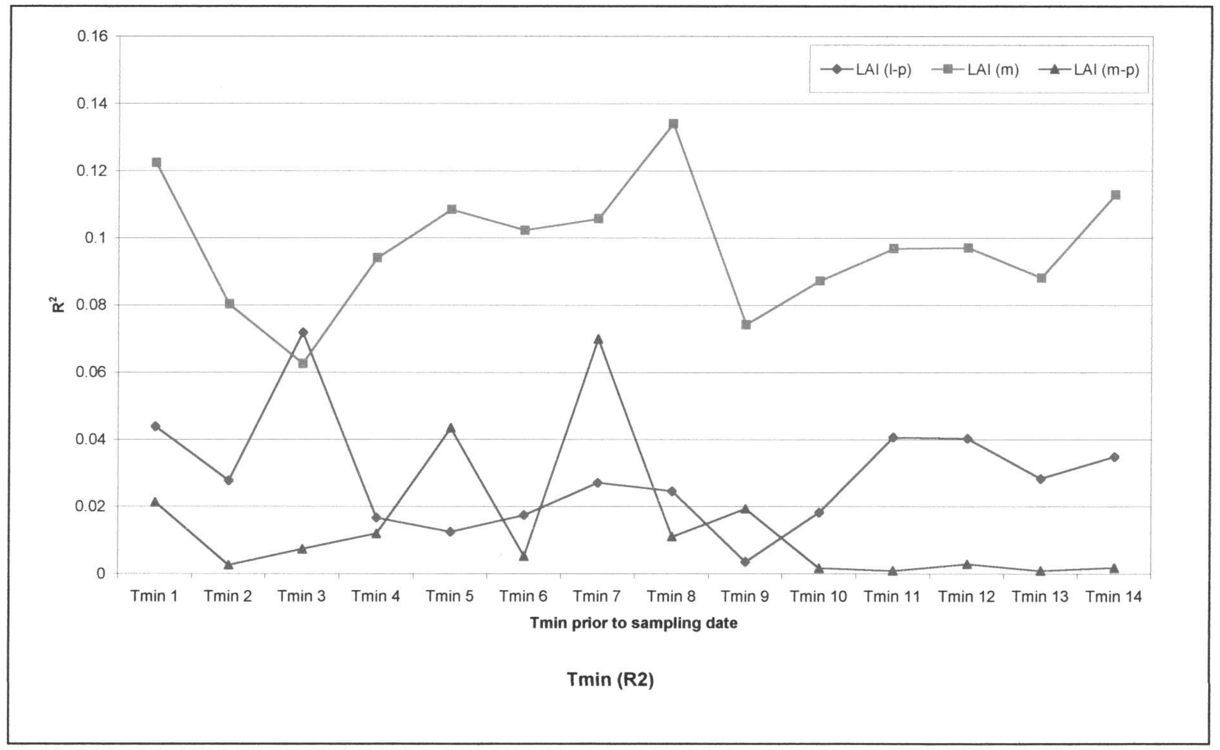

Fig. 4.6 R^2 values obtained for min. temperature (TMin) in days after sampling as affected by the different disease levels to determine a relationship between disease levels, TMin and time.

Linear regression of total LAI versus the respective different species of *Melampsora* LAI showed the total LAI correlating with *M. larici-populina* LAI (R = 79.5%). Similarly the leaf necrosis (LN) of *M. larici-populina* correlated with LAI (R = 45.2%)and LAI of *M. larici-populina* (R = 73.7%). Negative correlations were obtained for LAI of *M. larici-populina* with LAI of *M. medusae* and LN of *M. medusae* (R = -43.0% and -45.0%, respectively). The LAI of *M. medusae* correlated negatively with the LN of *M. larici-populina* (R = -53.0%). Overall LN as well as LN of *M. medusae* correlated with Clone (R = 44.4% and 56.2%, respectively). Leaf necrosis of *M. medusae* correlated with overall LN (R = 73.5%). Overall LAI as well as LN of *M. medusae-populina* correlated with rainfall Day 3 to Day 9 before sampling (R = 64% and 60.1% respectively). The LAI of *M. medusae-populina* correlated with max. temperature of Day 0, Day 1, Day 9 and Day 10 prior to sampling (R = 48.9%, 40.6%, 44.4% and 44.0%, respectively).

From Fig. 4.8 and 4.9 it is evident that when considering temperature, the South African climate is not limiting to development of *Melampsora* spp. epidemics. Optimal temperature conditions occur on the coastal regions where high humidity would also be conducive to disease development.

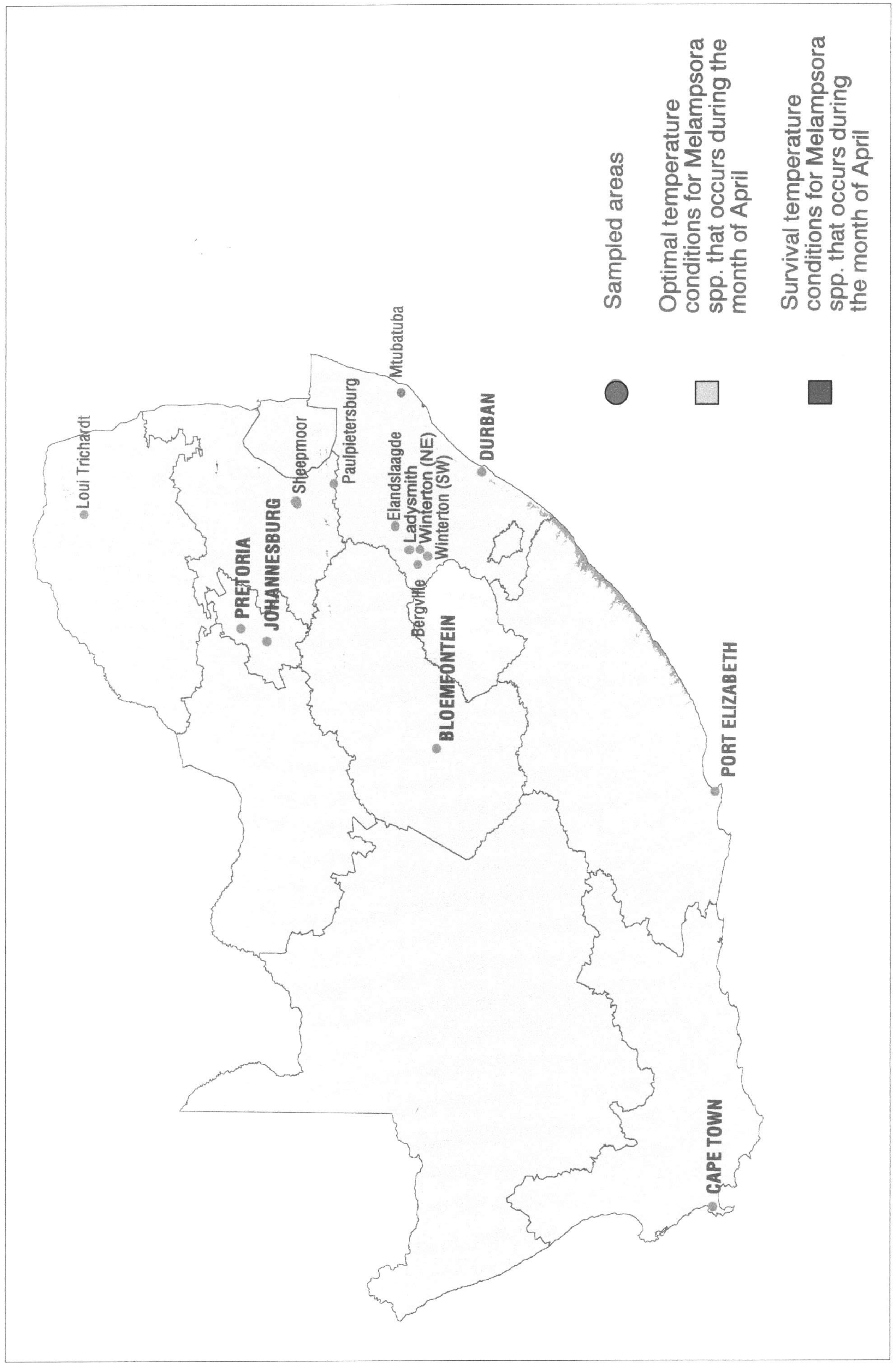

Fig. 4.8 Grid Map of optimal and survival temperatures for *Melampsora* spp. for the month of April generated from 50 year means.

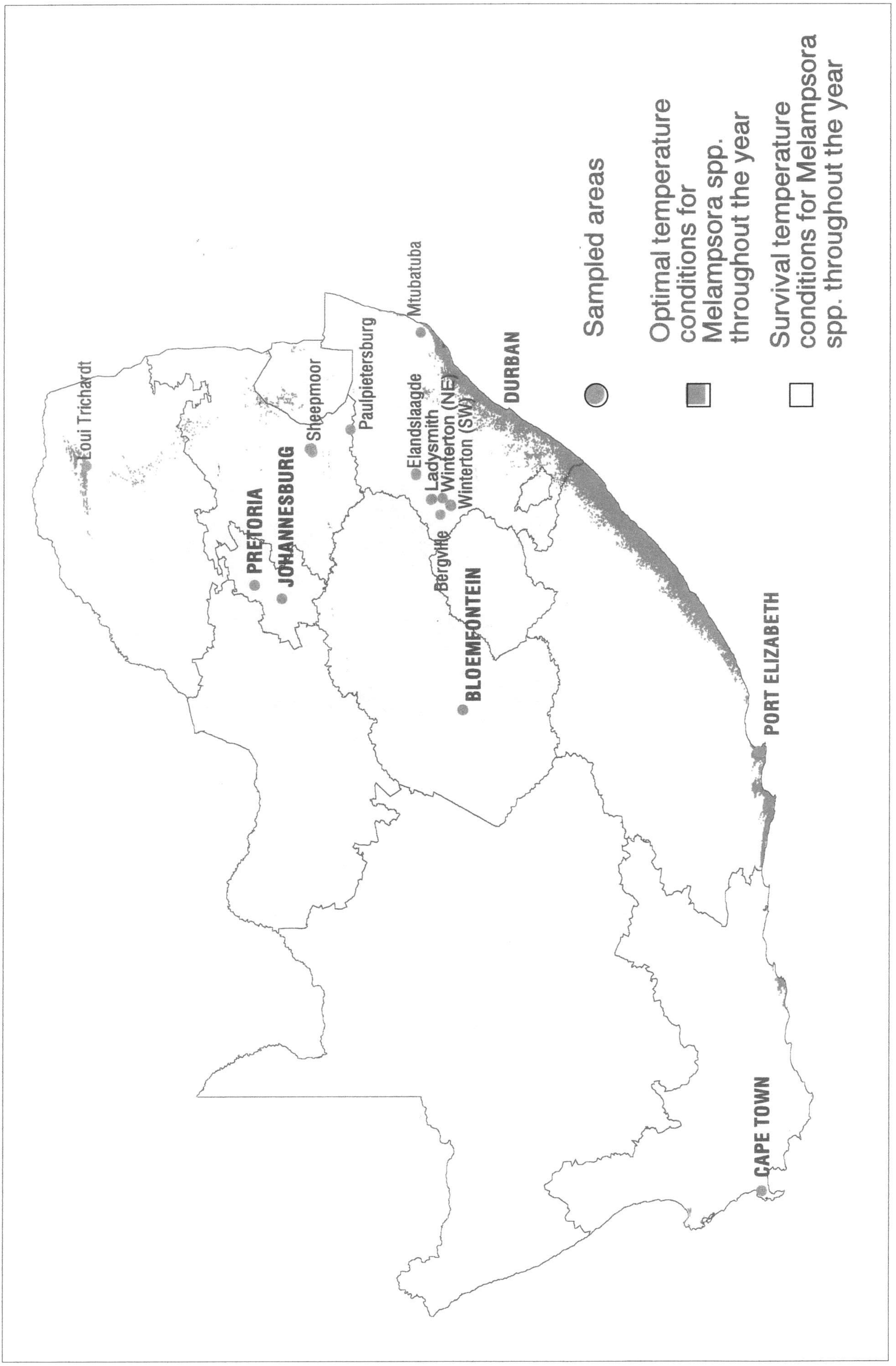

Fig. 4.9 Grid Map of annual optimal and survival temperatures for *Melampsora* spp. generated from 50 year means.

4.4 DISCUSSION

For the correlation analysis, only correlations greater than 40% are mentioned. The LAI for each site correlated positively, at 79.5% with the LAI caused by *M. larici-populina*. Although this species dominates infection on poplars in South Africa, a 79.5% correlation with LAI also indicates that this species has best acclimatized to the South African climate and that clones susceptible to this species are widely planted. One should also take note that of the three species observed, *M. larici-populina* has been present in South Africa for the greatest length of time. Hence, the other two species may still be acclimatizing. A correlation between LN and clone type would be easy to obtain and could be extrapolated as an indication point at which each clone abscises its leaf. Once certain levels of infection on a leaf have occurred the leaf will be rejected. Disease tolerance will vary between clones. Therefore clones with a high LN (Clone *P. deltoides* var *missouriensis*) are more tolerant than clones with a low LN (Clone 65/29). Unfortunately, this point of abscission has not been established for each clone and in all probability, environment would affect this factor.

In conducting this survey anticipated results were negative correlations of disease with temperatures outside of the fungi's growing and reproductive capacities. In this way it may have been possible to determine areas limiting to the fungus, but in which the poplars could grow. No correlations above 38.3% were obtained for mean temperatures for the two week period leading up to the sampling dates. However, positive correlations were obtained of 48.9% and 40.6%, between the leaf area infected by *M. medusae-populina,* and max. temperatures on the day of sampling and the day preceding it, respectively (Table 4.4). The LAI of *M. medusae-populina* also correlated with max. temperatures Day 9 and Day 10 before the sampling date at 44.4% and 44% respectively. Leaf area infected and LN of this same species correlated positively (mean of 50.7% and 46.0% from Day 4 to Day 14, respectively) with rainfall up to two weeks prior to the sampling date (Table 4.5). No rainfall occurred on any of the sites three days prior to sampling. Neither LAI nor LN correlated with min. temperatures. This may indicate that *M. medusae-populina* is more sensitive to its environment than the other two species which would also be true of a hybrid.

For a specific species or race of *Melampsora* there is an important variation in the pathogenicity according to species-host, and for each species-host the origin, family and clone. For a particular clone, the susceptibility slowly increases in summer, then suddenly drops in autumn. This results in the resistant clones only showing significant infection at this final stage whereas the susceptible clones are subjected to earlier infection. The increase in host susceptibility at the end of the vegetative season seems to be linked to the modifications in the chemical composition of sugars in the leaves, namely the increase in the ratio of soluble sugars and reduced sugars, especially glucose (or glucose and sucrose in the case of *M. larici-populina*). This phenomenon is enhanced by a nutritional deficiency in potassium and toxicity in nitrogen (FAO, 1979). Because the survey was conducted towards the end of the summer season, trends in *Melampsora* spp. composing LAI may be affected by the changing concentration of sugars in the leaf. The elevated *M. larici-populina* ratio may be as a result of this. Ragonese and Rial Alberti (1973), in a survey conducted in Argentina, found that infections occurring in April were attributable to *M. larici-populina* whilst infections occurring in May were caused by *M. albertensis*. From the survey conducted at Seven Oaks, Kwa-Zulu, Natal, it is evident that the populations of *Melampsora* fluctuated in a similar fashion over time.

Abundant rain and mild temperatures were reported to be favorable for the development of poplar leaf rust epidemics (Hamelin *et al.*, 1992). The optimal temperature range for uredospore dissemination was 18-22^{O}C in France (Taris, 1968) and 15-20^{O}C in North America (Widin and Schipper, 1980). Peak infection using a leaf disc bioassay was reported to occur at 20^{O}C, with no infection taking place below 2^{O}C or above 30^{O}C (Spiers, 1978). Laboratory studies have also indicated that exposure of *M. medusae* uredospores to 32^{O}C for 1 to 5 h reduced germination by 50%, while exposure to 35^{O}C for over 3 h or 36^{O}C for 1 h was lethal (Spiers, 1978). Hamelin *et al.* (1992) frequently recorded temperatures greater than 30^{O}C during periods of greatest disease increase. These temperatures are also limiting for dissemination which for *M. larici-populina* is greatest at 20^{O}C. This suggests that exposure to high temperatures in the field is not limiting to *Melampsora* rust development and that the fungus may benefit from microclimates controlled by the poplar host.

Weather has a strong effect on the performance of the clone and this is what may affect the disease level rather than weather conditions suitable for *Melampsora* spp. In favorable weather conditions as well as good soils the Clone 65/31 will grow a very big and soft leaf. Under conditions of slow growth the leaves are stiffer and appear to have a thicker cuticle. Softer leaves appear to be conducive to bigger pustules and heavier infection. These leaves are abscised early under epidemic conditions. Thicker leaves have smaller pustules and a higher necrotic percentage, indicating that leaves have been retained by the tree for a longer period (personal observation). Clone I488 has soft young leaves that are infected at a younger age than other clones. Singh and Heather (1982) found that clones inoculated with a mixture of uredospores of six races of *M. medusae*, on a high temperature regime (28/20°C), were more resistant (as determined by uredinial number) to leaf rust than those raised and incubated on any other combination of the high and low (20/10° C) pre- and post-inoculation temperature regimes. Irrespective of the parameter employed to assess disease intensity, cultivar constitution and pre- and post-inoculation temperature were significant contributors to the level of disease induced in the cultivars by the isolate of *M. medusae* (Singh and Heather, 1982).

Rain was found to play an important mechanical role in the dissemination of uredospores in France (Taris, 1968). In north central North America the number of uredospores caught on traps increased one week after rainfalls of 15mm or more (Widin and Schipper, 1980). These authors also reported that fewer uredospores were trapped in 1976, a year with lower early season rainfall, as compared with 1975. The greatest rate of increase of poplar leaf rust in New Zealand occurred after rain fell on 10 out of 14 days (Sheridan *et al.*, 1975).

Maximum infection of artificially inoculated detached leaves of eastern cottonwood occurred after eight hours of continuous leaf wetness. These results suggest that rain deficit and high max. temperatures are not limiting factors for poplar leaf rust epidemics when night conditions frequently offer optimal temperature and leaf wetness for infection (Hamelin *et al.*, 1992).

Precipitation deficits might affect rust epidemics indirectly when trees become water stressed. Other forms of stress (e.g., phosphorus and nitrogen deficiencies) have been reported to decrease poplar susceptibility to rust (Suzuki, 1973). In a drought year it is possible that these and other nutrients become less available, thus decreasing susceptibility to the disease. Temperatures (>30°C) considered high enough to reduce uredospore viability (Spiers 1978) were recorded frequently during the periods of greatest disease increase (Hamelin *et al.*, 1992). In addition, temperatures recorded during the 1988 and 1989 epidemics were well above the range of temperatures found to be favorable for uredospore dissemination (Taris, 1968; Widin and Schipper, 1980). However, min. temperatures within the optimal range for germination (>90% germination between 15 and 21°C) were recorded throughout the exponential phase of disease increase in 1988 and 1989 (Toole, 1967). In conclusion, it appears that cool night temperatures and leaf wetness periods of eight hours or more provide favorable conditions for poplar leaf rust epidemics during periods of low precipitation and high daytime temperatures. Overall, these results suggest that low rainfall and high daytime temperatures are not factors limiting poplar leaf rust epidemics in the central United States.

Pinon and Frey (1997) found that the complexity of races was lower in natural stands of *P. nigra* than in cultivated *Populus* stands. Populations of *M. larici-populina* collected on *P.* X *euramericana* cv. Robusta and *P. nigra* cv. Italica (Lombardy Poplar) were of a high richness and evenness, confirming the value of these clones for describing race populations. Populations from Western USA were of very low diversity, and it is suggested that this was in accordance with the recent introduction of the pathogen in North America. It is suggested that the race populations of *M. larici-populina* are mainly influenced by the structure of the host populations (Pinon and Frey, 1997). Low rust resistance is not always associated with poor growth rates, and the clones *P.* 'Agathe F', *P.* 'I-214', *P.* 'Heidemij' and *P. simonii* 'Fastigiata' exhibit good rust tolerance. The incidence of rust infestation was closely correlated with local weather conditions (Bencheva, 1995).

Amplified fragment length polymorphism (AFLP) markers were identified that were tightly linked to the locus conferring resistance to the leaf rust *M. larici-populina* in *Populus*. The study was carried out using a hybrid progeny derived from an interspecific, controlled cross between a resistant *P. deltoides* female and a susceptible *P. nigra* male. The segregation ratio of resistant to susceptible plants suggested that a single, dominant locus defined this resistance. This locus, which was designated *Melampsora* resistance (Mer), confers resistance against E1, E2, and E3, three different races of *M. larici-populina*. Further identification of markers can be useful in breeding programmes and are the basis for future cloning of the resistance gene (Cervera *et al.*, 1996). Clonal material with the resistance gene to Race E3 would be beneficial to South Africa as this is the only race to have been identified to occur here (Hawke, 1997).

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Chapter 5 THE RESPONSE OF *MELAMPSORA* SPP. INFECTING A POPLAR HYBRID (CLONE 65/31) TO DIFFERENT FUNGICIDE APPLICATIONS

ABSTRACT

Four trials were conducted to determine the potential of fungicides to control rust infections on poplars. Sixteen fungicides were tested. Naturally infected poplar trees of the clone 65/31, grown in pots, were used as test material. The first trial had 16 fungicide treatments and an untreated control. Four treatments were significantly more effective than others: Alto (cyproconazole) (at 0.3ml/L) with and without the adjuvant Armoblen 600 (at 0.75ml/L), Anvil (hexaconazole) (at 0.2ml/L) and Early Impact (flutriafol + carbendazim) (at 0.6ml/L), respectively. Oxycarboxin appeared to have enhanced disease progression. Two experimental strobilurins, Stroby WG and Quadris FL (kresoxim-methyl, BASF and azoxystrobin, Zeneca) (at 0.12ml/L, and 0.4ml/L, respectively) and a new class of fungicide, Astor WG40 (experimental, Novartis) (at 2g/L) controlled rust poorly. Four treatments were used in the second trial: Quadris as a foliar spray (0.4ml/L), Impact applied on superphosphate granules ((1ml + 5g)/tree), and two controls; superphosphate alone (5g/tree) and untreated. The key finding of the second trial was that Impact gave complete control as a granular application over a 56 day period. Superphosphate alone enhanced rust development slightly. A third trial was conducted which corroborated results obtained in the first two trials: Alto plus Armoblen 600 was the best treatment, Early Impact the next best, then Alto, Anvil, superphosphate coated with Impact, Impact alone, the untreated control, Duett and lastly, superphosphate alone. The superphosphate treatment again slightly increased the disease percentages. A fourth trial was conducted with different rates of Alto (0.1, 0.2, 0.3, 0.5, 0.7ml/L, and an untreated control), applied with the standard rate of Armoblen 600. All rates of Alto gave control of the disease, the highest rate being the most effective.

5.1 INTRODUCTION

Of the many *Melampsora* species that cause leaf rust of poplars, *M. medusae* Thum. and *M. larici-populina* Kleb. are the two most destructive, because each has a wide host range that includes many species of interspecific hybrids (Newcombe and Chastagner, 1993). *Melampsora medusae* is native to North America but has been introduced throughout most of the world where poplars are grown (Van Kraayenoord *et al.*, 1974). Schipper and Dawson (1974) reported *M. medusae* as being one of the most serious diseases of the eastern cottonwood, *Populus deltoides* Bartr. In intensively managed plantations yield losses of up to 65% in volume were reported for hybrid poplars with eastern cottonwood parentage (Widin and Schipper, 1981).

Development of clones resistant to rusts is an essential component of a successful rust management strategy. However, there are difficulties in breeding resistant varieties. For example, it is not always possible to combine resistance with other factors required, such as desirable wood qualities and fast growth. Furthermore, new physiological races of rusts may appear which can successfully infect and reproduce on previously resistant varieties. This process may be slowed by the use of fungicides. A further issue is plant material becoming susceptible to new races before they are ready to be harvested. In such cases, fungicides may be used to protect trees until they are ready to be harvested (Buchenauer, 1968).

The aim of this series of trials was to evaluate the response of a commercially-grown poplar clone (Clone 65/31) to different fungicide treatments. Two trials were conducted to test the response of *Melampsora* rust to fungicides currently on the market. Fungicides used in the second trial were not available at the commencement of the first trial. A follow-up trial was conducted to retest the best fungicides and to confirm results of the first two trials. A final trial was carried out to determine the most effective concentration at which Alto should be applied.

5.2 MATERIALS AND METHODS

5.2.1 Site Descriptions and Experimental Design

Trials 1 and 2

Trials 1 and 2 were conducted at the University of Natal, Pietermaritzburg (80km North-West of Durban, KwaZulu-Natal, South Africa) under shade cloth to prevent hail damage (Fig. 5.1). Naturally infected young poplar trees (1.5 - 2m in height) that had been grown for a year at the Redclyff nursery site, Lion Match plantations, Seven Oaks were individually bagged and arranged in groups of three (Fig. 5.2). Each bag was fed water with a dripper twice a day for four minutes (Fig. 5.2). Nine trees were allocated to each treatment, with three replicates per treatment. Seventeen treatments were part of Trial 1 and four treatments made up Trial 2 (Table 5.1). Trial 2 included treatments that were not available when Trial 1 commenced and was essentially a parallel trial. A randomization program was used to randomize the treatments within the replicates and these were then labeled with numbers to avoid bias during visual ratings.

Trials 3 and 4

Trial 3 (the follow-up fungicide trial) and Trial 4 (examining different concentrations of Alto) were conducted at the Lion Match Redclyff Nursery, Lion Match plantations, Seven Oaks. Seven Oaks lies at an altitude of 1040m above sea level, 29°12'45" South and 30°29'30" East, about 120km North-West of Durban in KwaZulu-Natal, South Africa. This is a summer rainfall area (800mm/yr) with max. daily of 36°C, mean of 28°C and mean monthly temperature of 20°C. Winters are cool with early morning frost, a min. -8°C and a mean monthly temperature of 12°C. The soils are of the Oakleaf form (MacVicar, 1991) and are deep. A randomization program was used to randomize the treatments and replicates. Each treatment had a border of five trees between it and the adjacent treatment. Three trees were assigned to each replicate (i.e., nine trees per treatment). Trial 4 had five replicates as opposed to the three used in the previous trials.

5.2.2 Fungicide Applications

Fungicides were measured out using weigh balances (accurate to 1/100 of a gram) or 1ml pipettes (depending on the formulation) and mixed with 1L of tap water in 1.5L hand spray bottles (Table 5.1 and Table 5.2). Trials 1 and 2 had a second treatment application after 45 days and sprays were applied from the top of the trees to imitate aerial spraying as closely as possible. A plastic screen was placed around each replicate (i.e., group of three trees), to prevent drift from one treatment to the next during spraying.

5.2.3 Disease Assessments

The effect of the fungicides was evaluated by rating the tenth and eleventh leaf of each tree. In Trial 1, disease was assessed for rust at 0, 12, 29, 43, 57, and 76 days after spraying (DAS); Trial 2: at 0, 13, 27, 41, and 55 DAS; Trial 3: at 0, 5, 19, 24, 34 DAS; and Trial 4: at 0, 16, 26, 34, 39, and 87 DAS. A visual rating scale (Fig. 4.1) (Fleming, 1996) was used as a basis for evaluating the percentage leaf area infected.

Fig. 5.1 Layout of fungicide Trials 1 and 2 conducted at the University of Natal, Pietermaritzburg, KwaZulu-Natal, South Africa. Yellow leaves indicate the presence of *Melampsora* rust.

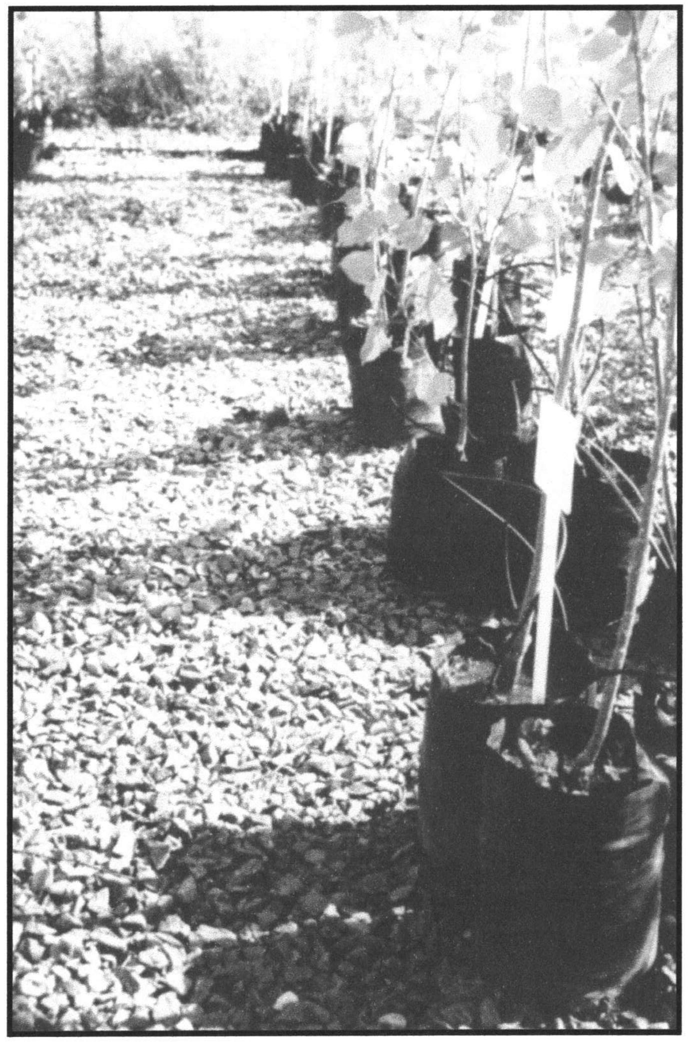

Fig. 5.2 Photograph showing irrigation system used for watering the trees and arrangement of replicates (R) within treatments in Trials 1 and 2.

Table 5.1 Fungicidal treatments used in Trials 1, 2, and 3; their rates, groups and active ingredient (a.i.)

Trial	Group	a.i.	Company	Trade Name	Rate/L
a,b,c	control	N/A	N/A	N/A	N/A
a	dithiocarbamate	mancozeb	Rohm and Haas Co.	DithaneWG	2g/L
a	piperazine	triforine	American Cyanamid Co.	Denarin	2ml
a,c	conazol	flutriafol	Zeneca	Impact	1ml
a,c	triazole	cyproconazole	Novartis	Alto	0.3ml
a	triazole	difenoconazole	Novartis	Score	0.2ml
a,c	triazole	hexaconazole	Zeneca	Anvil	0.2ml
a	triazole	propiconazole	Novartis	Tilt	0.2ml
a	anilinopyrimidine + protectant	cyprodinil+ fludioxonil	Novartis	Switch	0.4g
a	plant activator	acibenzolar-S-methyl	Novartis	Bion	0.15g
a	resistance stimulator	not known		Astor WG 40	2 g
a	morpholine	tridemorph	BASF	Calixin	0.6ml
a	strobilurin	kresoxim-methyl	BASF	Stroby WG	0.12ml
b	strobilurin	kresoxim-methyl	Zeneca	Quadris	0.4ml
a	carboxin	oxycarboxin	Uniroyal	Plantvax	0.6ml
b,c	fertilizer	single superphosphate		single superphosphate	5g/tree
c	triazole	epoxyconazol	BASF	Duett	0.4ml
a,c	triazole/ benzimidazole	flutriafol/ carbendazim	Zeneca	Early Impact	0.6ml
a	triazole/ benzimidazole	flusilazole/ carbendazim	Du Pont	Punch C	0.2ml
b,c	fertilizer + triazole	single superphosphate + flutriafol	Nitrochem + Zeneca	single superphosphate + Impact	5g+1ml per tree
a,c	triazole + adjuvant	cyproconazole + armoblen	Novartis + Akzo Nobel	Alto + Armoblen 600	0.3ml+ 0.75ml

a -Treatments used in Trial 1

b -Treatments used in Trial 2

c -Treatments used in Trial 3

Table 5.2 Treatments and rates of chemicals used in Trial 4

Treatment No.	Rate of Alto (ml/L)	Rate of Armoblen (ml/L)
1	0.1	0.75
2	0.2	0.75
3	0.3	0.75
4	0.5	0.75
5	0.7	0.75
6	0	0

5.2.4 Statistical Analysis

The disease progress curve for each treatment was used to give an Area Under Disease Progress Curve (AUDPC) value by using a trapezoidal integration program (Berger, 1981). The apparent infection rate was also calculated, using Vanderplank's (1963) logistic equation:

$$X_1 = X_0 \cdot e^{rt} \cdot (1-x)$$

where X_1 is final disease level

X_0 is initial disease level

e is the mathematical contsant

r is the apparent infection rate

t is the period between disease ratings

and $1-x$ is the correction factor for excluding already infected tissue

and **r** is calculated by:

$$r = 1/(t_1-t_0)*[\ln(X_1/\{1-X_1\})-\ln(X_0/\{1-X_0\})]$$

For this purpose 0.01% was added to all disease values before conversion to proportions (/100), so that a small natural logarithm could be obtained for ratings of zero disease. Statistical analysis of AUDPC values, final disease percentages (FD%) and apparent infection rates (**r**) were conducted using analysis of variance (ANOVA) and means separations were based on Fisher's LSD at the 5% confidence limit using Genstat 5.2 (Dicks, 2000). Correlation analysis was conducted on each of the means of treatments for each of the trials. A linear regression was performed on the results of Trial 4.

5.3 RESULTS

Early Impact and Alto with Armoblen and Alto (alone) ranked in the top four best fungicide treatments used in this trial to control rust infection in all parameters tested. Oxycarboxin was among the least effective of the fungicides used and plants treated with this fungicide had more disease than the control.

Table 5.3 Mean values of poplar rust levels, measured as the area under the disease progress curve (AUDPC), the apparent infection rate (**r**) and the final disease percentage (FD%) for Trial 1

Treatment	AUDPC	(*)	Rank	r	(*)	Rank	FD%	(*)	Rank
Early Impact	42.8	a	1	-0.0148	abc	4	0.26	a	3
Alto+Armoblen	52.9	ab	2	-0.0266	a	2	0.13	a	1
Alto	59.1	ab	3	-0.0308	a	1	0.13	a	2
Anvil	67.4	abc	4	0.0156	de	12	1.41	abc	5
Punch C	147.6	abcd	5	0.0157	de	13	1.12	ab	4
Astor	171.3	abcd	6	0.0230	e	16	5.18	abcd	11
Stroby	209.8	abcd	7	0.0035	cde	6	3.93	abcd	7
Denarin	268.4	abcde	8	0.0139	de	9	5.03	abcd	10
Impact	272.1	abcde	9	-0.0177	ab	3	4.83	abcd	9
Tilt	296.6	bcde	10	0.0198	de	15	6.95	cd	13
Dithane	312.1	cde	11	-0.0005	bcd	5	3.29	abcd	6
Bion	317.6	de	12	0.0171	de	14	6.67	bcd	12
Control	325.7	de	13	0.0098	de	7	4.65	abcd	8
Score	359.6	de	14	0.0249	e	17	7.47	d	14
Switch	370.8	de	15	0.0153	de	11	7.73	d	16
Calixin	385.3	de	16	0.0150	de	10	7.68	d	15
Oxycarboxin	483.5	e	17	0.0102	de	8	7.94	d	17

(*) Figures which have similar letters are not significantly different from each other as tested by Fisher's LSD Test at the 5% confidence limit.

Table 5.4 ANOVA table of AUDPC values (AUDPC), apparent infection rate (**r**) and final disease percentage (FD%) for Trial 1 (CV% based on an inverse logit data transformation)

Stratum	d. f.	Mean Square			F value			Probability		
		AUDPC	r	FD%	AUDPC	r	FD%	AUDPC	r	FD%
Treatment	16	53595	0.00091	24.7	2.38	4.62	2.20	0.018	<0.001	0.03
Replicate	2	138138	0.00004	43.7	6.13	0.21	3.91	0.006	0.809	0.03
Residual	32	22539	0.00020	11.2						
CV%		0.3	0.7	14.1						

Table 5.5 Mean values of poplar rust levels, measured as the area under the disease progress curve (AUDPC), the apparent infection rate (**r**) and the final disease percentage (FD%) for Trial 2

Treatment	AUDPC	(*)	Rank	r	(*)	Rank	FD%	(*)	Rank
Super P+Impact	73.3	a	1	-0.10112	a	1	0	a	1
Quadris	237.3	b	2	0.02978	b	4	4.58	b	3
Control	259.0	b	3	0.00440	b	2	2.81	b	2
Superphosphate	263.4	b	4	0.01946	b	3	4.64	b	4

(*) Figures which have similar letters are not significantly different from each other as tested by Fisher's LSD Test at the 5% confidence limit.

Table 5.6 ANOVA table of AUDPC values (AUDPC), apparent infection rate (**r**) and final disease percentage (FD%) for Trial 2 (CV% based on an inverse logit data transformation)

Stratum	d. f.	Mean Square			F value			Probability		
		AUDPC	r	FD%	AUDPC	r	FD%	AUDPC	r	FD%
Treatment	3	24667	0.0011	14.2	7.92	6.18	7.45	0.017	<0.001	0
Replicate	2	14760	0.0109	6.35	4.74	58.9	3.33			
Residual	6	3116	0.0002	1.91						
CV%		0	0.7	8.2						

The only significantly different treatment in this trial was superphosphate coated with Impact. It consistently ranked the best with all the data sets tested. Again, Early Impact and Alto with Armoblen and Alto (alone) ranked in the top four treatments for all the data sets. The level of control obtained by superphosphate coated with Impact in this trial did not attain the same level as Trial 2.

Table 5.7 Mean values of poplar rust levels, measured as the area under the disease progress curve (AUDPC), the apparent infection rate (**r**) and the final disease percentage (FD%) for Trial 3

Treatment	AUDPC	(*)	Rank	r	(*)	Rank	FD%	(*)	Rank
Alto + Armoblen	48.9	a	1	-0.0440	a	1	1.18	a	1
Alto	66.1	a	2	-0.0161	ab	2	1.88	a	2
Early Impact	66.8	a	3	0.0219	bc	4	2.75	ab	3
Anvil	78.1	ab	4	0.0253	bc	6	3.44	abc	4
Super P + Impact	89.7	ab	5	0.0226	bc	5	4.72	abc	6
Control	104.5	abc	6	0.0394	bc	7	6.75	bcd	7
Impact	106.8	abc	7	0.0039	abc	3	3.49	abc	5
Duett	139.3	bc	8	0.0533	c	9	7.67	cd	8
Superphosphate	159.7	c	9	0.0444	c	8	9.25	d	9

(*) Figures which have similar letters are not significantly different from each other as tested by Fisher's LSD Test at the 5% confidence limit.

Table 5.8 ANOVA table of AUDPC values (AUDPC), apparent infection rate (**r**) and final disease percentage (FD%) for Trial 3 (CV% based on an inverse logit data transformation)

Stratum	d. f.	Mean Square			F value			Probability		
		AUDPC	r	FD%	AUDPC	r	FD%	AUDPC	r	FD%
Treatment	8	3919	0.0000	22.8	2.71	2.6	3.35	0.043	0.05	0
Replicate	2	4749	0.0029	13.4	3.29	0	1.98			
Residual	26	1445	0.0011	6.8						
CV%		0	1.6	10.8						

Table 5.9 Mean values of poplar rust levels, measured as the area under the disease progress curve (AUDPC), the apparent infection rate (**r**) and the final disease percentage (FD%) for Trial 4

Treatment	AUDPC	(*)	Rank	r	(*)	Rank	FD%	(*)	Rank
0.7ml/L	127.1	a	1	0.0493	a	5	4.05	a	1
0.5ml/L	141.1	a	2	0.0510	a	6	4.53	a	2
0.3ml/L	257.0	a	3	0.0448	a	2	5.57	a	5
0.1ml/L	292.7	a	4	0.0456	a	3	5.13	a	3
0.2ml/L	331.2	a	5	0.0357	a	1	5.53	a	4
Control	720.9	b	6	0.0470	a	4	6.00	a	6

(*) Figures which have similar letters are not significantly different from each other as tested by Fisher's LSD Test at the 5% confidence limit.

Table 5.10 ANOVA table of AUDPC values (AUDPC), apparent infection rate (**r**) and final disease percentage (FD%) for Trial 4 (CV% based on an inverse logit data transformation)

Stratum	d. f.	Mean Square			F value			Probability		
		AUDPC	r	FD%	AUDPC	r	FD%	AUDPC	r	FD%
Treatment	5	237597	0.0003	9.4	6.47	0.27	0.90	<0.001	0.92	0.5
Replicate	4	51199	0.0016	8.5	1.40	1.66	0.81			
Residual	20	36695	0.0010	10.5						
CV%		0	1.5	11.1						

Trial 1

Correlation analysis showed that the AUDPC values correlated with the **r** values at 53.7% and the FD% levels at 91.4%. The **r** values correlated with the FD% values at 66.6%. Of the 17 treatments, eight were triazoles or triazole mixtures (Table 5.1). The superior mean values for AUDPC and FD%'s were all triazoles (Table 5.3 and Fig. 5.3-5.6). Use of Early Impact resulted in the lowest mean AUDPC value. Armoblen, a surfactant, increased the rust-inhibiting action of Alto. The probability levels are significant for all three variates (Table 5.4). Score, a triazole with a.i. difenoconazole, had the greatest AUDPC and **r** as well as a high FD% (Fig. 5.3-5.5). Figure 5.6 shows the disease progress curve means for each of the treatments used in this trial.

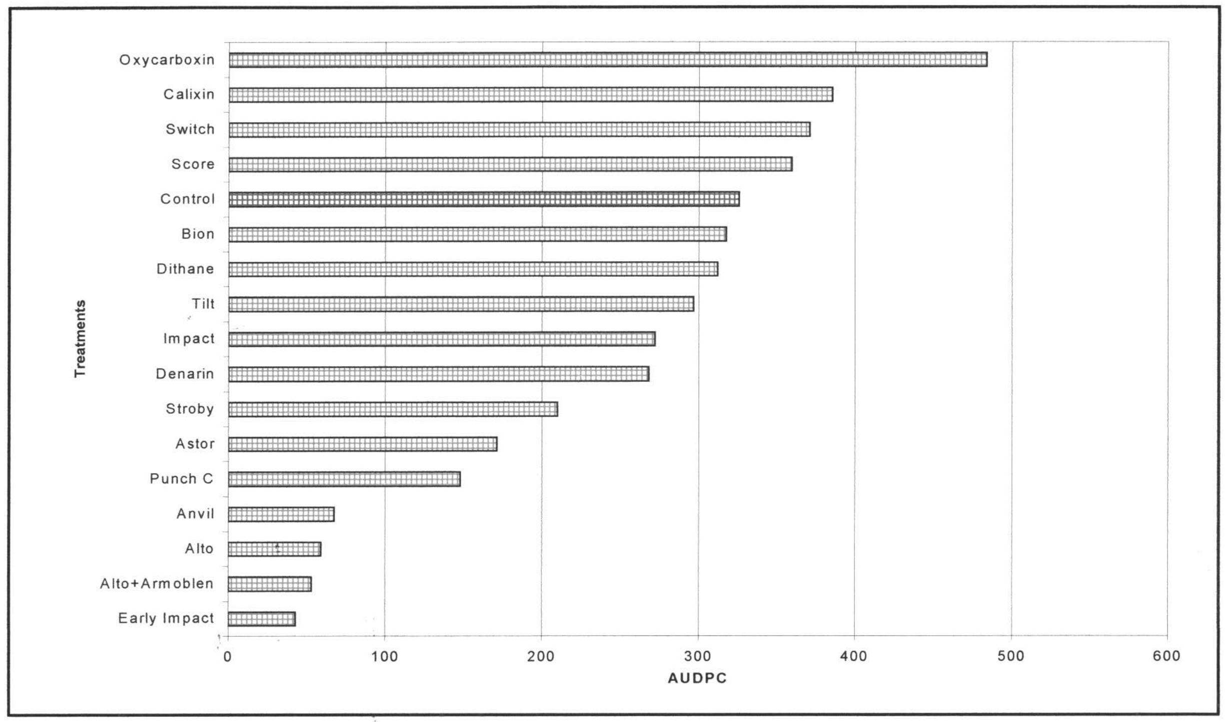

Fig. 5.3 Area Under Disease Progress Curve (AUDPC) means for different fungicide treatments used in Trial 1 which were applied to drip irrigated, bagged poplar plants (Clone 65/31) at the University of Natal, Pietermaritzburg.

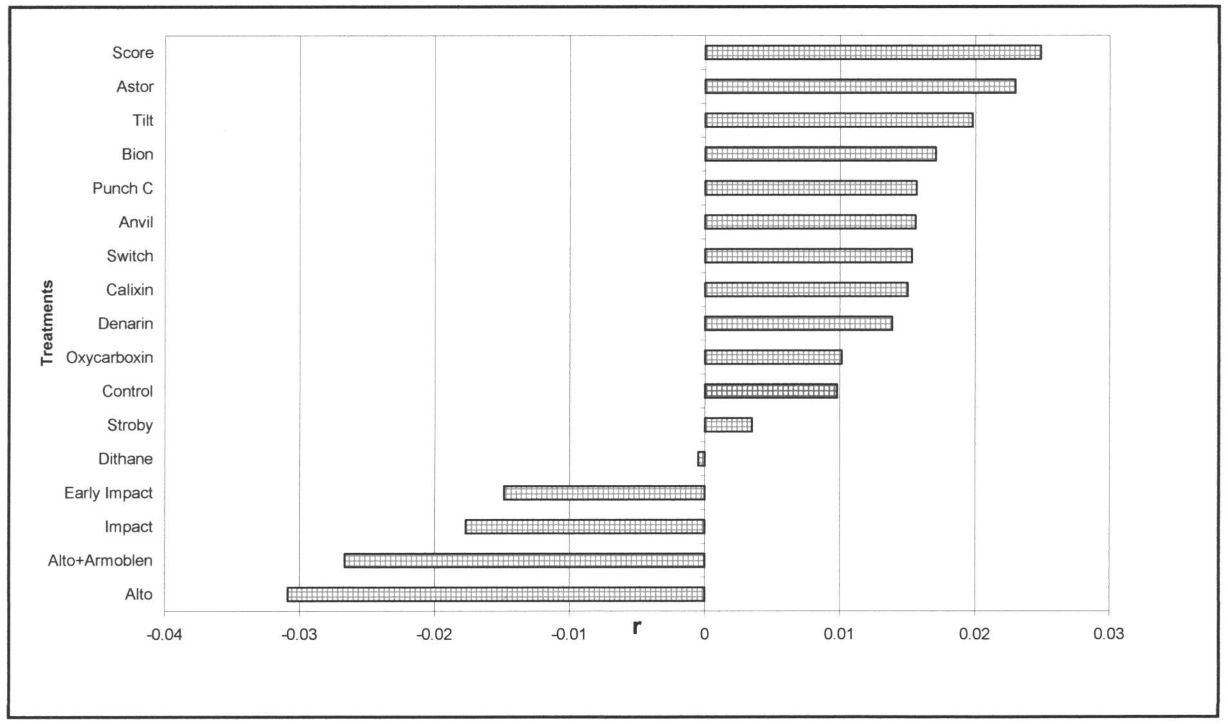

Fig. 5.4 Rate of disease progress (**r**) means for different fungicide treatments used in Trial 1 which were applied to drip irrigated, bagged poplar plants (Clone 65/31) at the University of Natal, Pietermaritzburg.

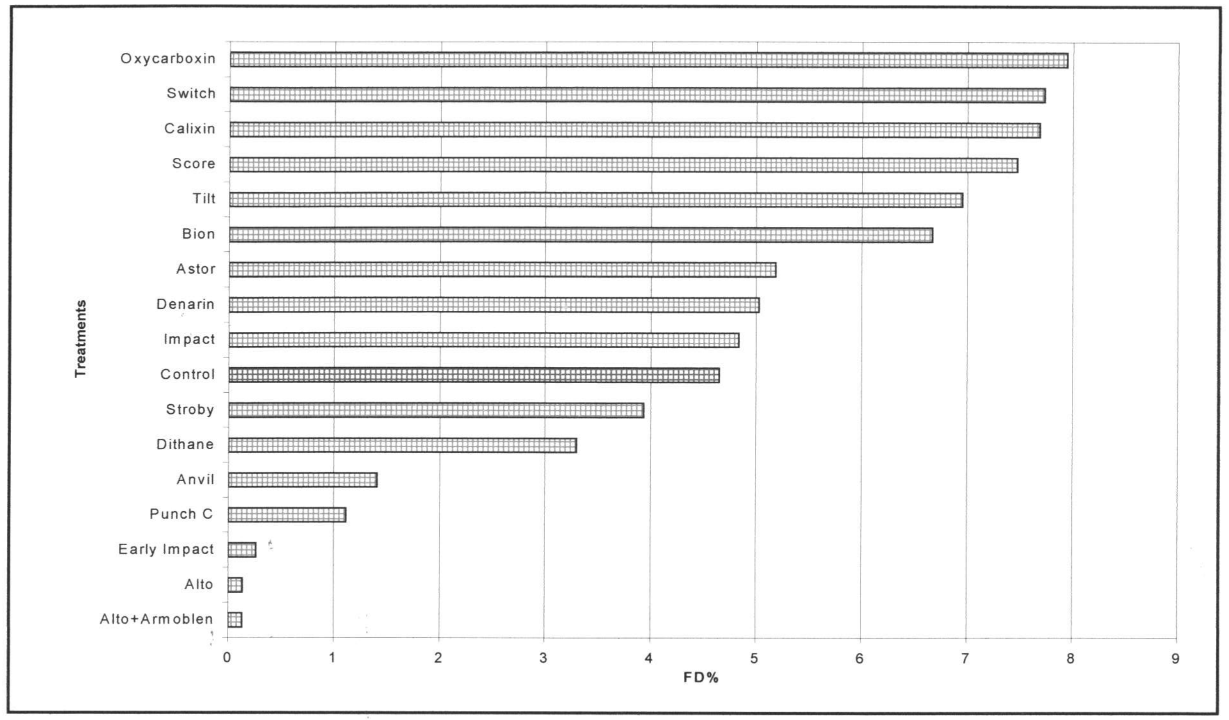

Fig. 5.5 Final Disease Percentage (FD%) means for different fungicide treatments used in Trial 1 which were applied to drip irrigated, bagged poplar plants (Clone 65/31) at the University of Natal, Pietermaritzburg.

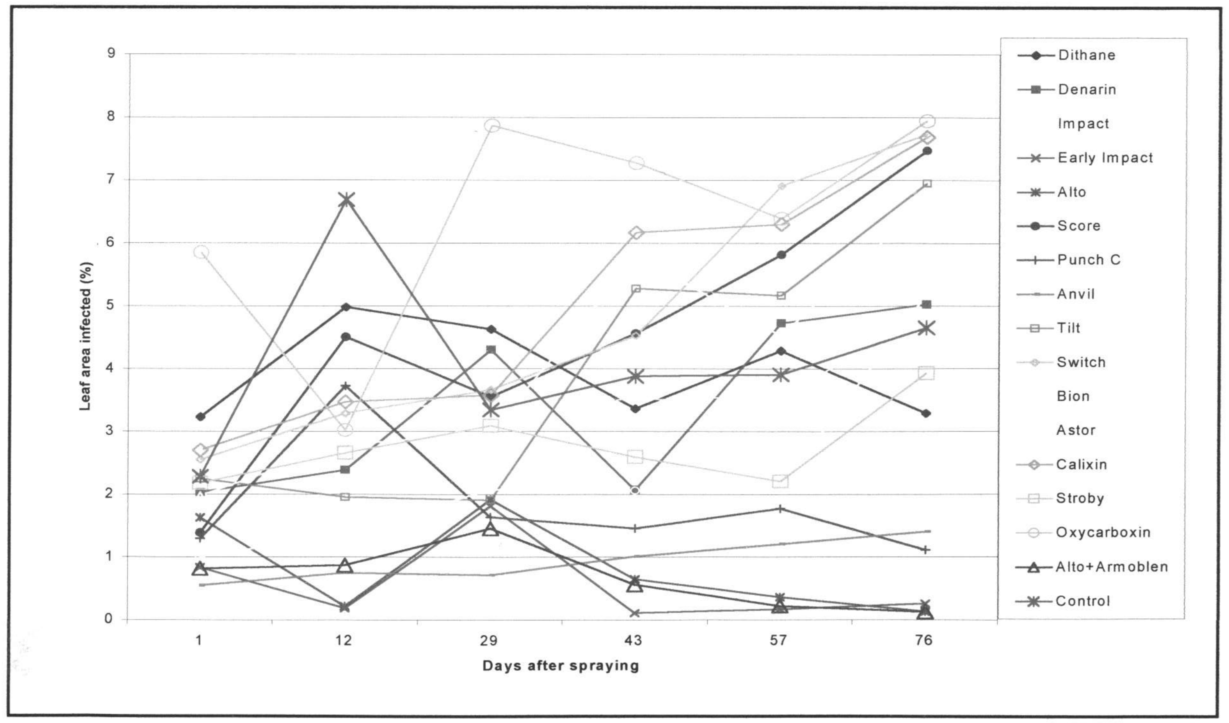

Fig. 5.6 Disease progress curves for different fungicide treatments used in Trial 1 which were applied to drip irrigated, bagged poplar plants (Clone 65/31) at the University of Natal, Pietermaritzburg.

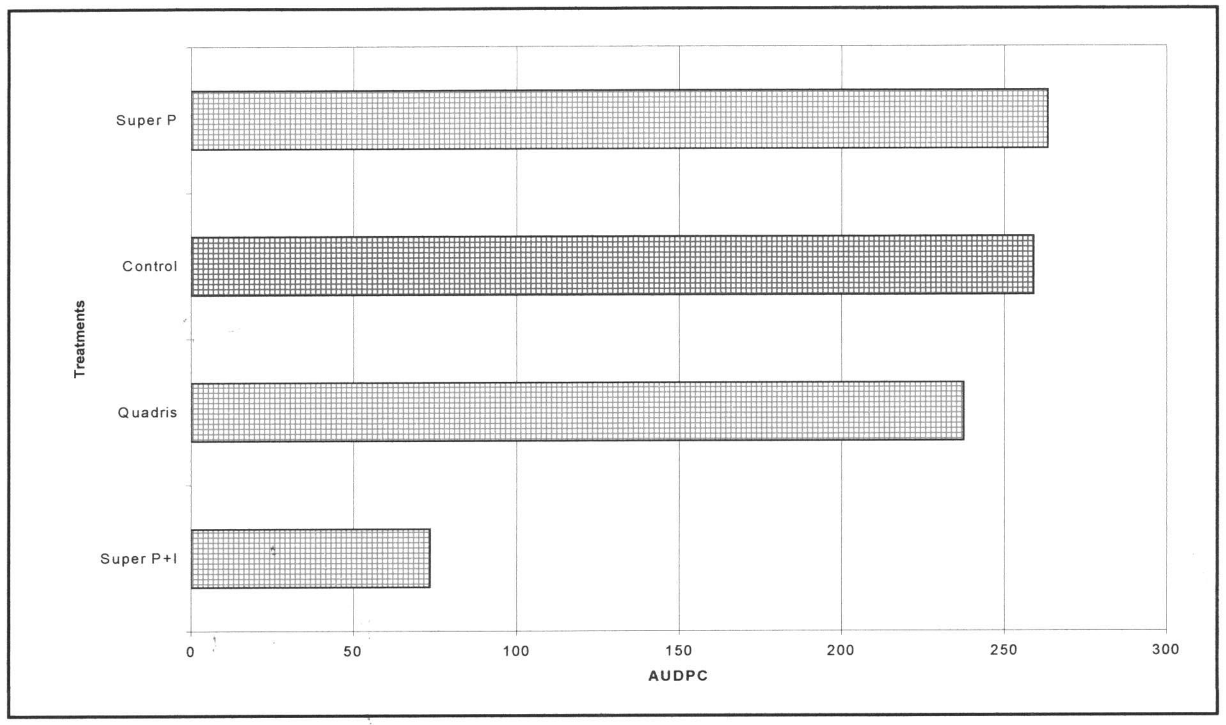

Fig. 5.7 Area Under Disease Progress Curve (AUDPC) means for different fungicide treatments used in Trial 2 which were applied to drip irrigated, bagged poplar plants (Clone 65/31) at the University of Natal, Pietermaritzburg.

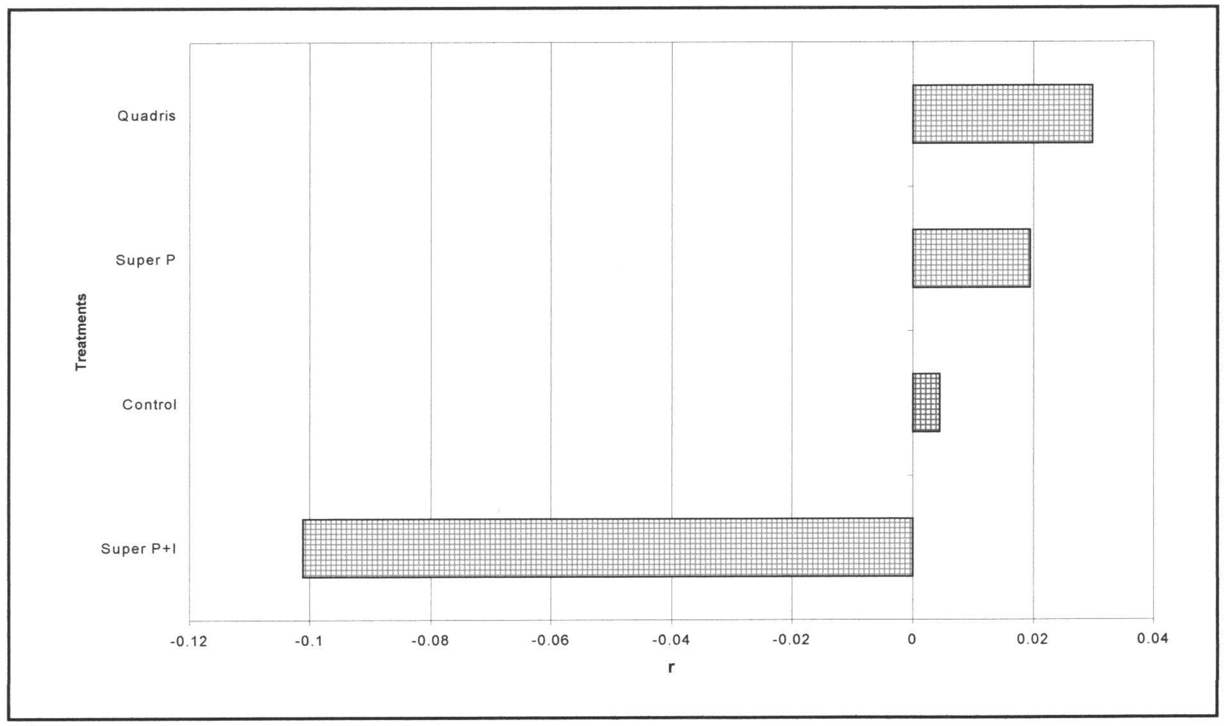

Fig. 5.8 Rate of disease progress (**r**) means for different fungicide treatments used in Trial 2 which were applied to drip irrigated, bagged poplar plants (Clone 65/31) at the University of Natal, Pietermaritzburg.

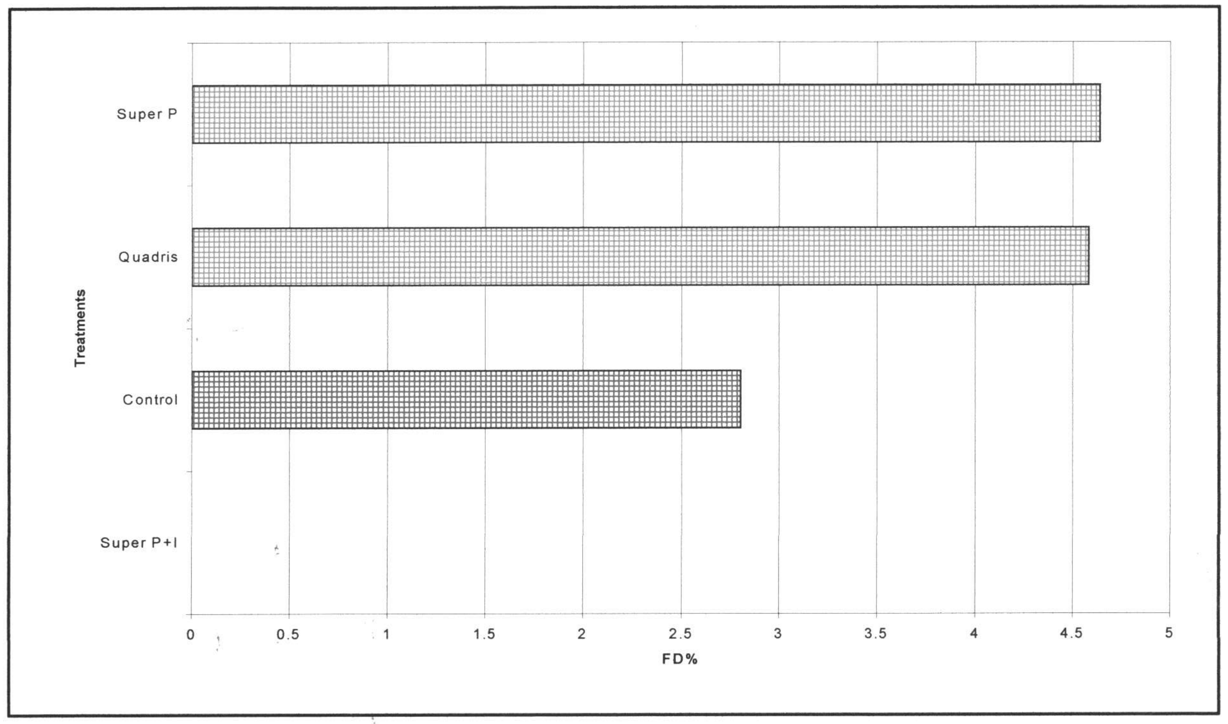

Fig. 5.9 Final Disease Percentage (FD%) means for different fungicide treatments used in Trial 2 which were applied to drip irrigated, bagged poplar plants (Clone 65/31) at the University of Natal, Pietermaritzburg.

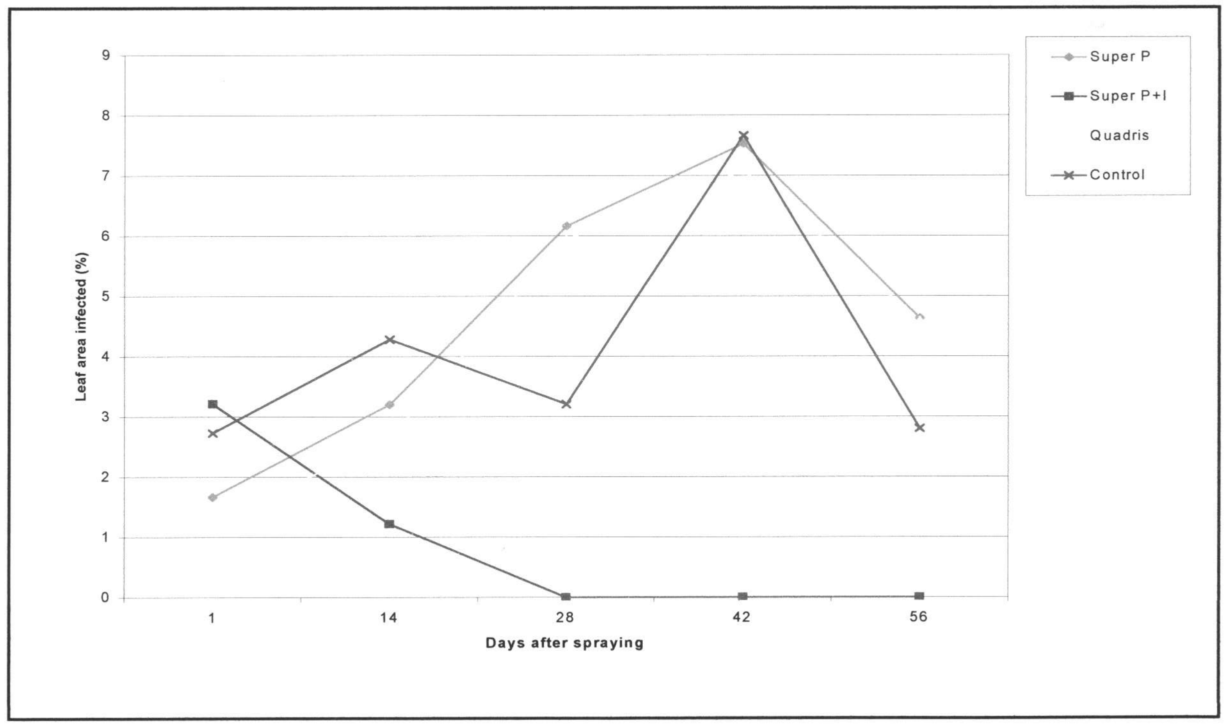

Fig. 5.10 Disease progress curves for different fungicide treatments used in Trial 2 which were applied to drip irrigated, bagged poplar plants (Clone 65/31) at the University of Natal, Pietermaritzburg.

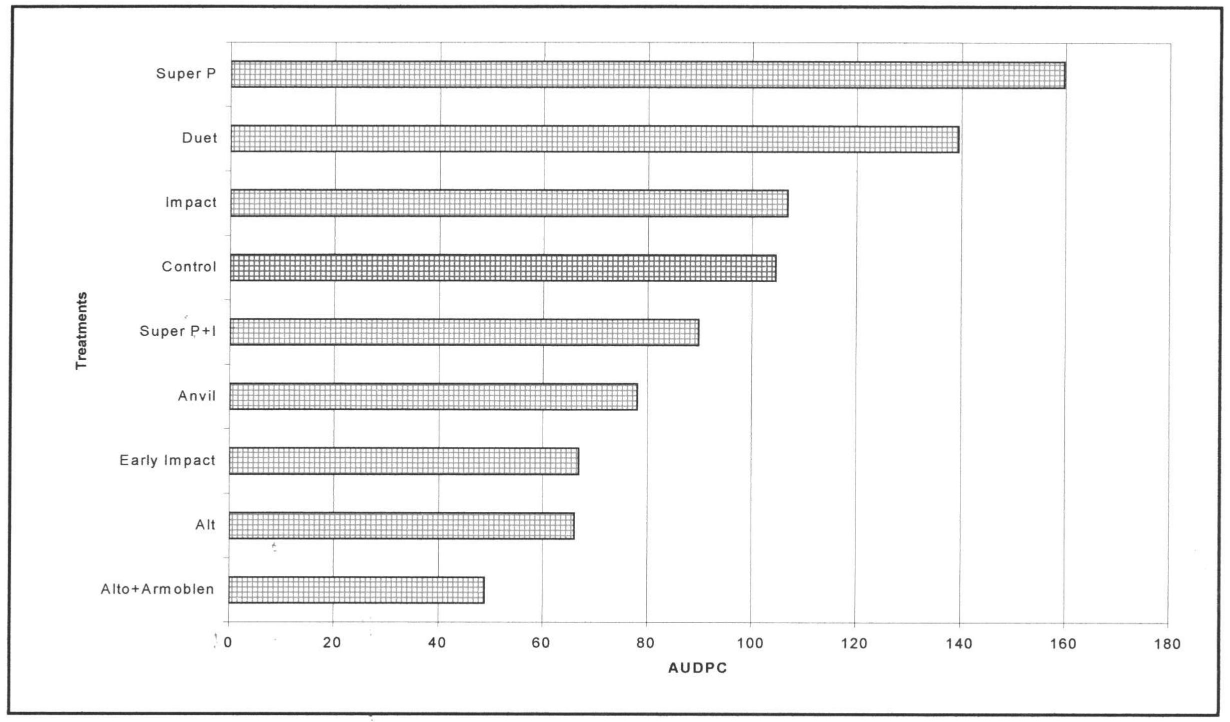

Fig. 5.11 Area Under Disease Progress Curve (AUDPC) means for different fungicide treatments used in Trial 3 which were applied to poplar plants (Clone 65/31) at Lion Match Redclyff Nursery, Seven Oaks, KwaZulu-Natal.

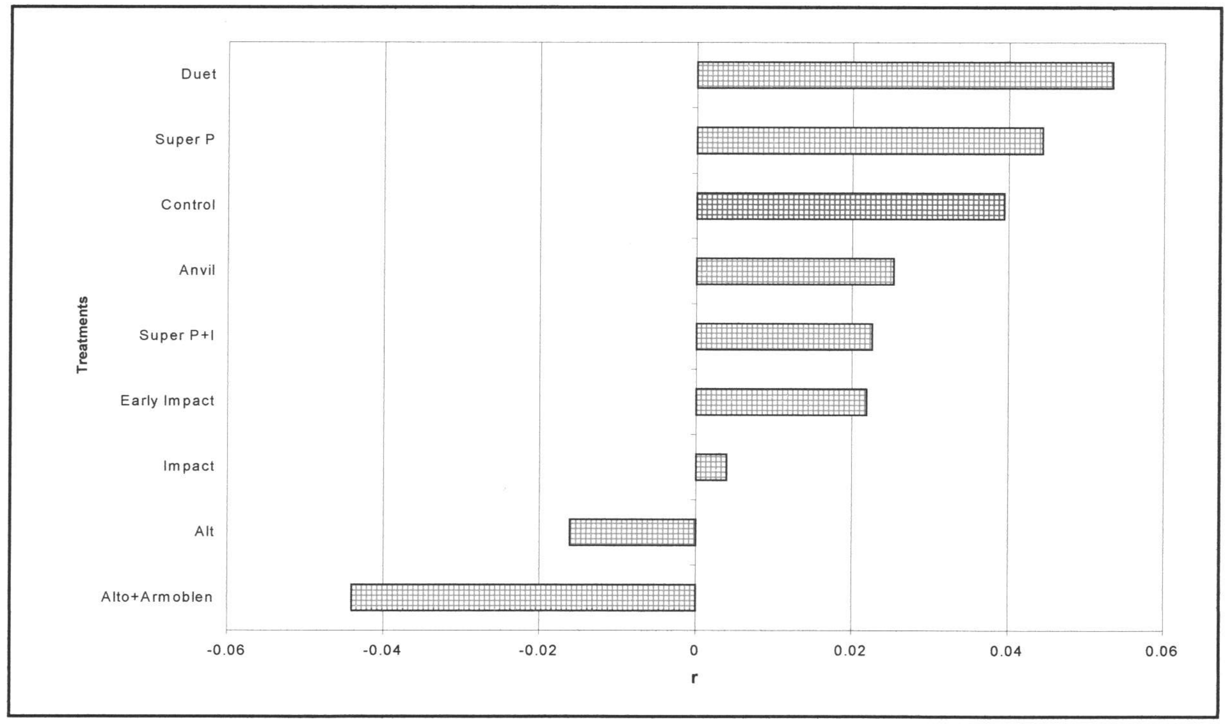

Fig. 5.12 Rate of disease progress (**r**) means for different fungicide treatments used in Trial 3 which were applied to poplar plants (Clone 65/31) at Lion Match Redclyff Nursery, Seven Oaks, KwaZulu-Natal.

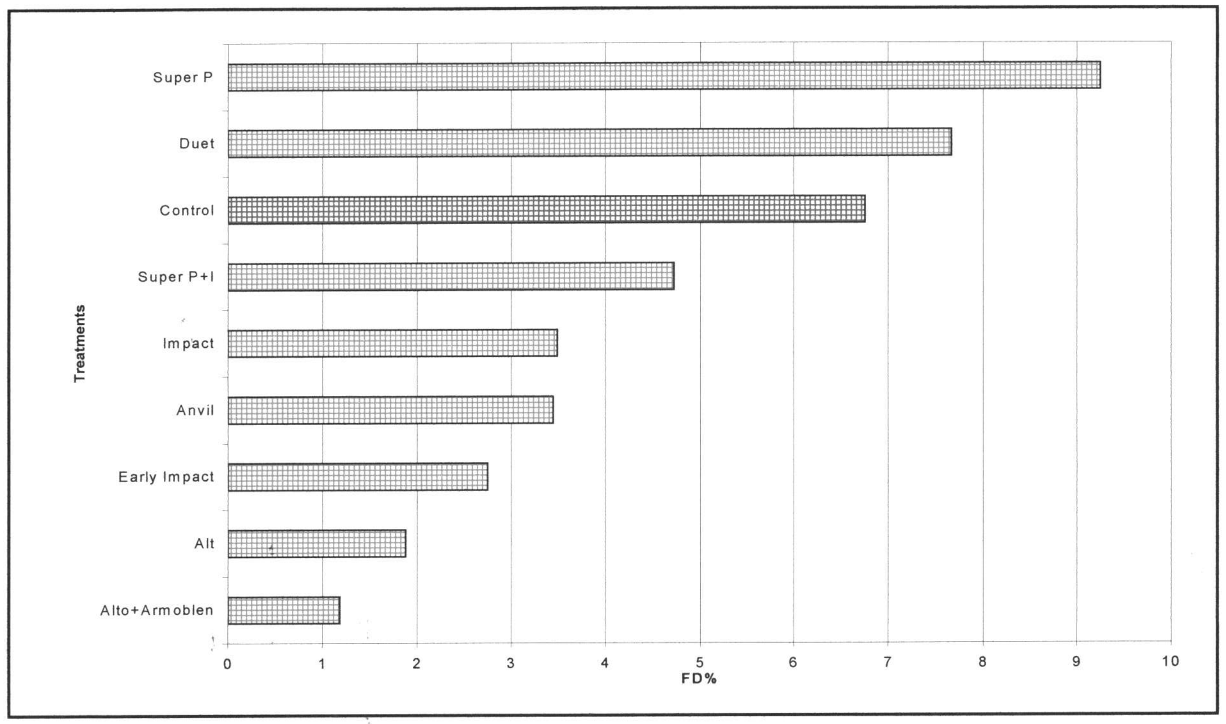

Fig. 5.13 Final Disease Percentage (FD%) means for different fungicide treatments used in Trial 3 which were applied to poplar plants (Clone 65/31) at Lion Match Redclyff Nursery, Seven Oaks, KwaZulu-Natal.

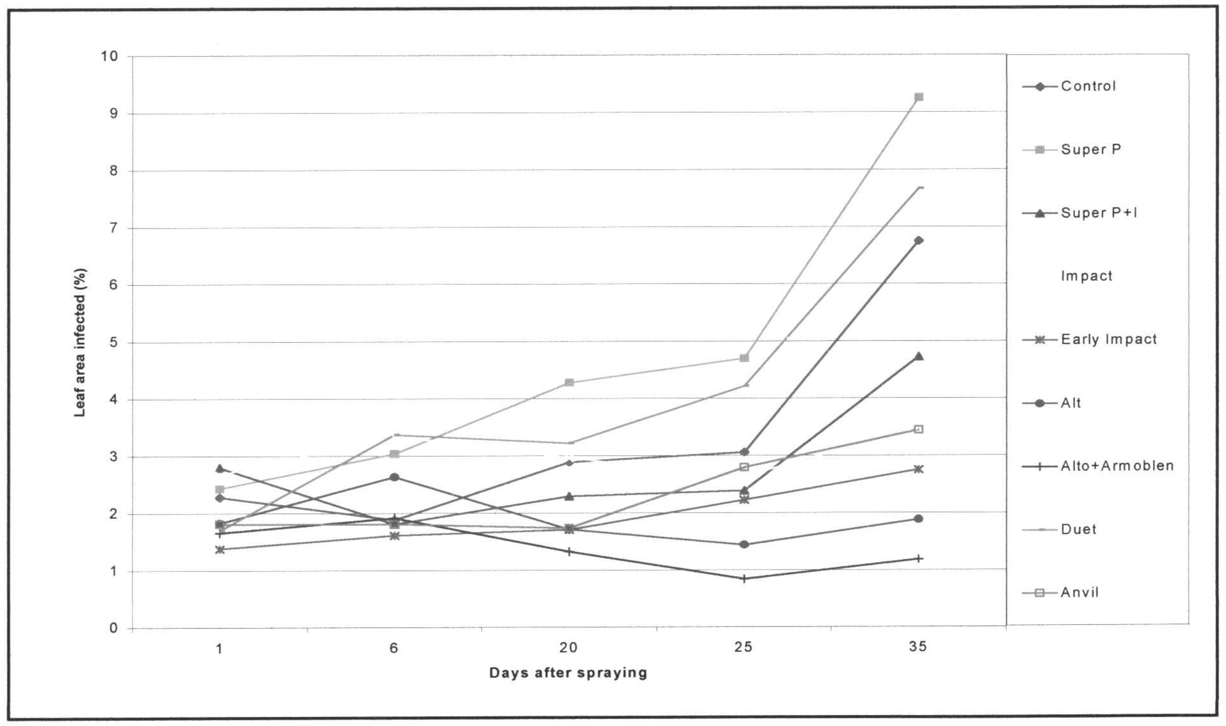

Fig. 5.14 Disease progress curves for different fungicide treatments used in Trial 3 which were applied to poplar plants (Clone 65/31) at Lion Match Redclyff Nursery, Seven Oaks, KwaZulu-Natal.

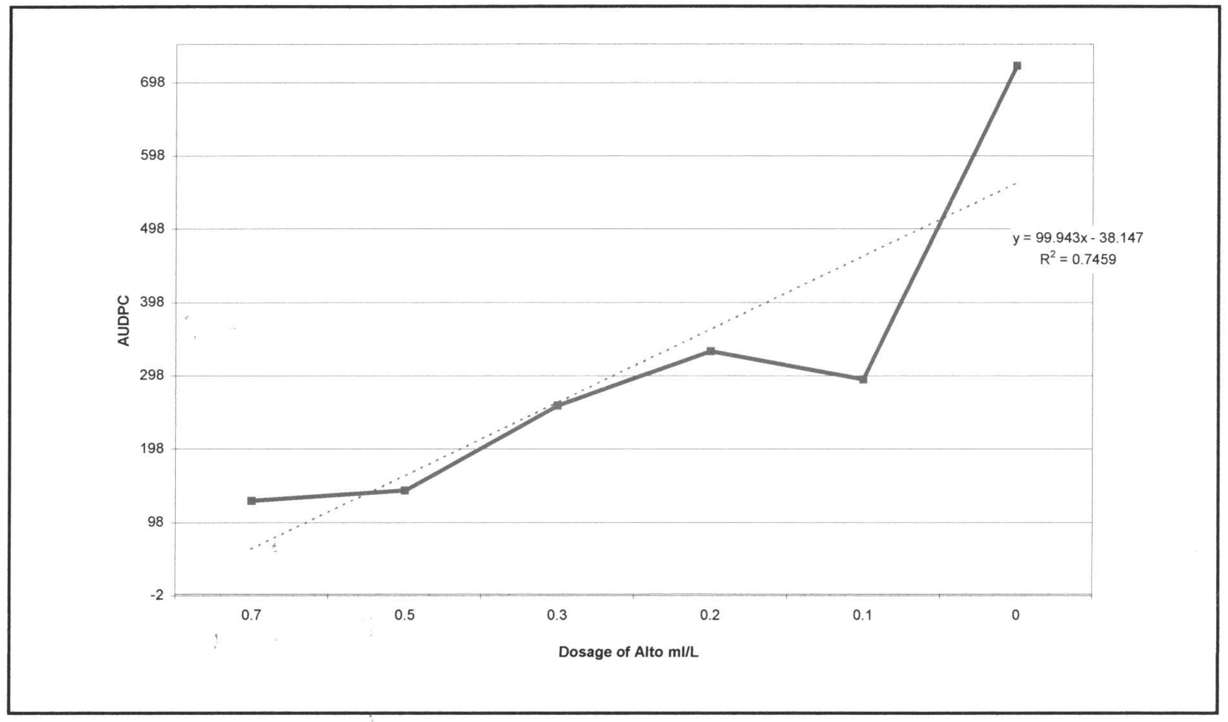

Fig. 5.15 Area Under Disease Progress Curve (AUDPC) means for different fungicide treatments used in Trial 4 which were applied to poplar plants (Clone 65/31) at Lion Match Redclyff Nursery, Seven Oaks, KwaZulu-Natal. Linear regression has been conducted on Alto treatments.

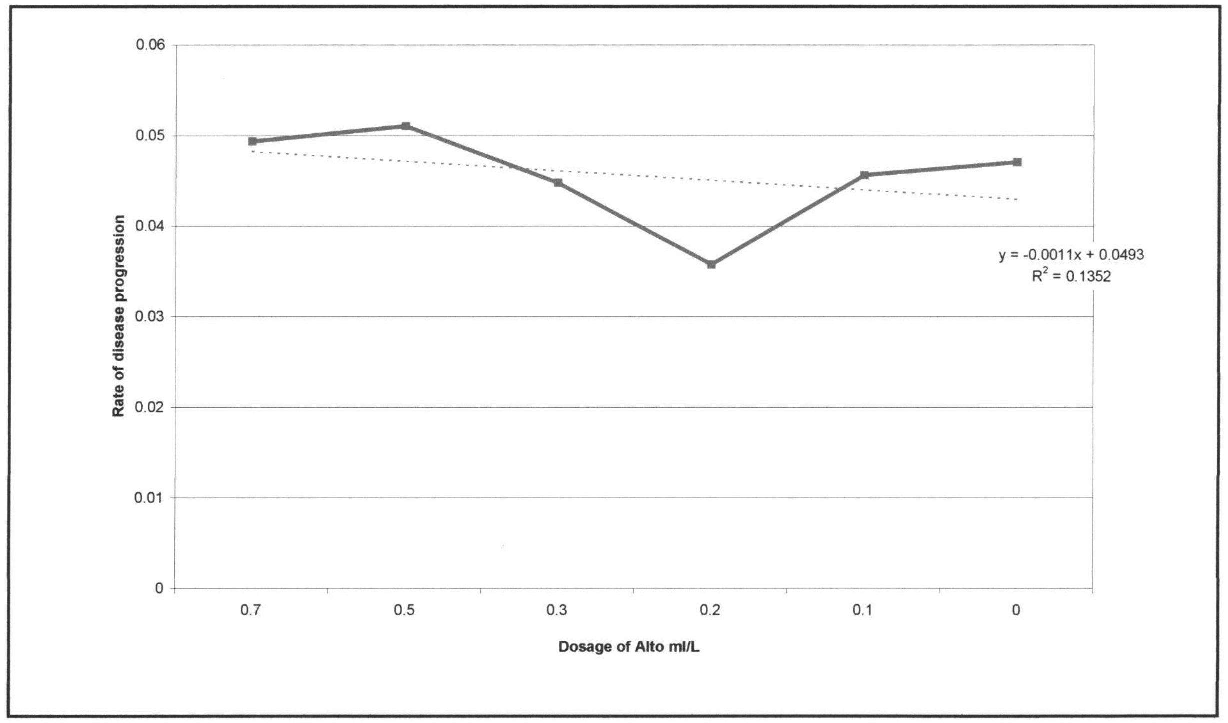

Fig. 5.16 Rate of disease progress (**r**) means for different fungicide treatments used in Trial 4 which were applied to poplar plants (Clone 65/31) at Lion Match Redclyff Nursery, Seven Oaks, KwaZulu-Natal. Linear regression has been conducted on Alto treatments.

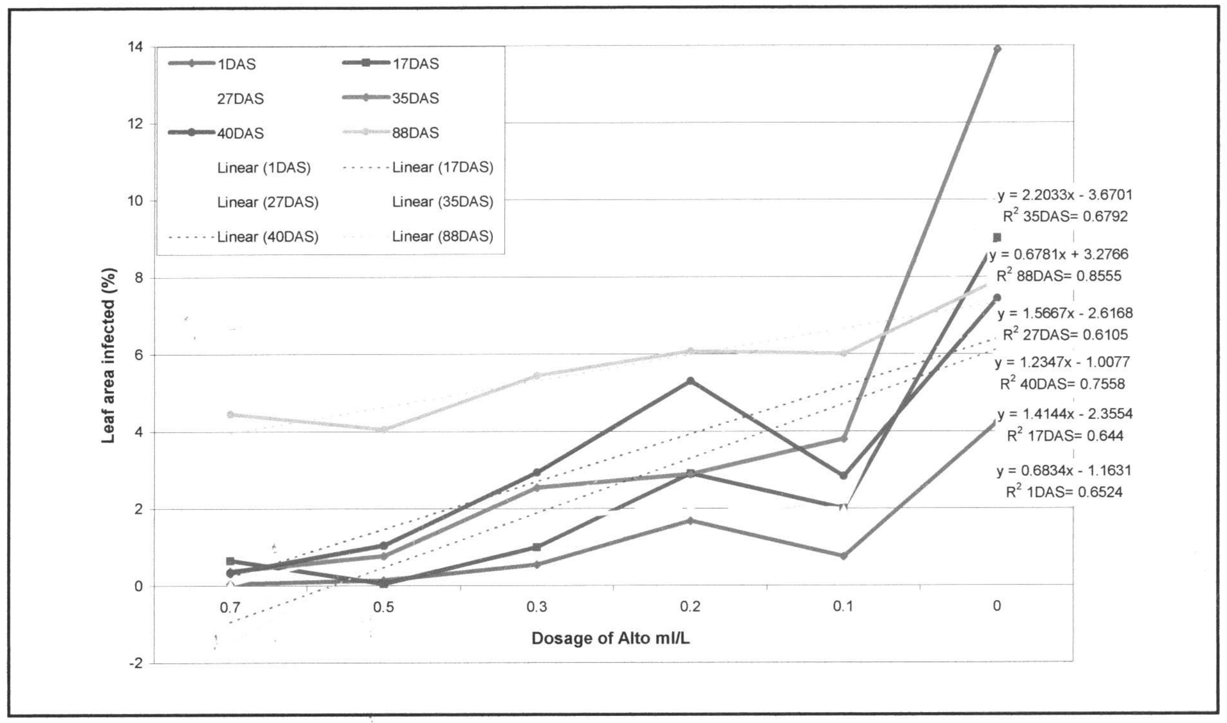

Fig. 5.17 Final Disease Percentage (FD%) means for different fungicide treatments used in Trial 4 which were applied to poplar plants (Clone 65/31) at Lion Match Redclyff Nursery, Seven Oaks, KwaZulu-Natal. Linear regression has been conducted on Alto treatments.

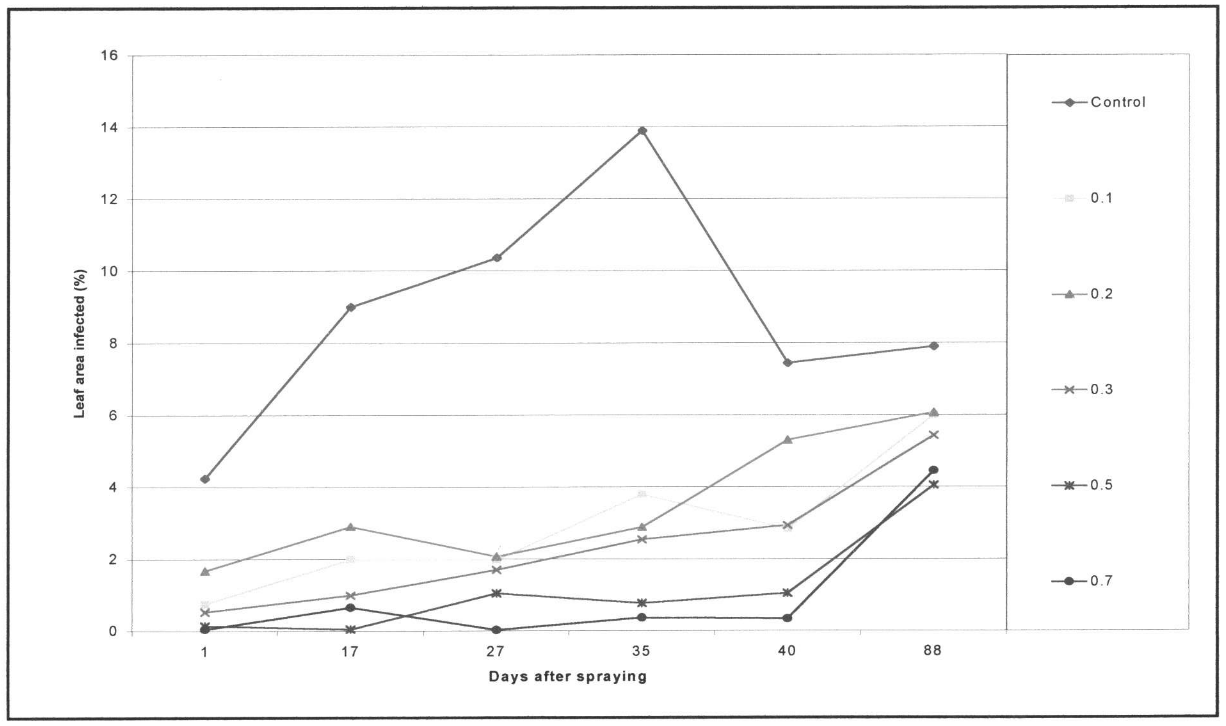

Fig. 5.18 Disease progress curves for different fungicide treatments used in Trial 4 which were applied to poplar plants (Clone 65/31) at Lion Match Redclyff Nursery, Seven Oaks, KwaZulu-Natal.

The incorporation of carbendazim with flutriafol in the formulation of Early Impact increased the action of the fungicide in comparison to Impact (flutriafol only) but the differences in the AUDPC, **r** or FD% were not significant.

Trial 2

Correlation analysis showed that the AUDPC data correlated with the **r** at 96.2% and the FD% at 89.7%. The **r** correlated with FD% at 96.8%. Superphosphate coated with Impact was significantly different from all other treatments at the 5% confidence limit (Table 5.5). Superphosphate increased the percentage leaf area infected but not significantly (Fig. 5.7-5.9). The FD% was taken after 56 days and no disease was recorded on trees treated with superphosphate coated with Impact (Fig. 5.10). The F-Tests were significant for all three variates (Table 5.6). Trees treated with superphosphate coated with Impact had no disease on the leaves when rated on the 28th day after spraying.

Trial 3

Correlation analysis showed that the AUDPC data correlated with **r** at 77.0% and the FD% at 94.3%. The **r** correlated with FD% at 85.2%. Similar trends were obtained in this trial as in Trials 1 and 2. Superphosphate coated with Impact did not give complete control as it did in Trial 2. Duett, a triazole, was not effective in controlling rust infection. Alto with Armoblen 600, and Alto alone, outperformed Early Impact which was the best treatment in Trial 1. As in Trial 2, superphosphate slightly enhanced disease progression (Fig. 5.11-5.13). Superphosphate coated with Impact and Impact (Fig. 5.14) both started at slightly higher disease percentages compared to other treatments but disease increase was retarded significantly with these treatments. Early Impact also retarded disease increase. Anvil maintained control until the third week and then the treatment appeared to weaken slightly in its action. Duett increased disease development.

Trial 4

Correlation analysis showed that AUDPC correlates with **r** at 63.9% and the FD% at -85.4%. The **r** correlated with FD% at -53.3%. All concentrations of Alto contained the rust development significantly better than the control, when looking at the AUDPC means (Table 5.9). The general trend was better control with higher concentrations of fungicide (Fig. 5.15). Table 5.9 showed no significant differences between all treatments when looking at the **r** and the FD% and this is reflected in the high probability levels in Table 5.10. Figure 5.16 illustrates that Alto at 0.2ml/L had the slowest **r** and Alto at 0.5ml/L had the fastest. Figure 5.18 shows the disease progress curve means of the different treatments used in this trial. By Day 88 the percentage leaf area infected of treated plots was still less than the control.

5.4 DISCUSSION

AUDPC correlated well with the FD% in all four trials (91.4% in Trial 1; 89.7% in Trial 2; 94.3% in Trial 3 and -85.4% in Trial 4).

In Trial 1 the untreated control showed less disease than would have been expected and this was attributed to its positioning within the trial. In the trial layout each replicate of the control was coincidentally positioned on the outer edges where conditions were less conducive to disease development than within the trial.

Rating for leaf area infected is a time-consuming process. Results using FD% were compared with results using the more accurate AUDPC method. In all the trials conducted the FD% reflected the ranking of the AUDPC values. Use of FD% instead of AUDPC results in a major reduction in the time and effort of data collection and data manipulation. However, in many cases when FD% is used, smaller significant differences between treatments are obtained than when AUDPC is used.

5.4.1 Dithiocarbamates

These are organic, immobile protectant fungicides that act generally in the disruption of cell function. The action of this group of fungicides is broad-spectrum. Zinc in mancozeb may cause zinc-sensitive plants to show phytotoxic symptoms (Hewitt, 1998). Dithane (mancozeb) showed very little potential to reduce rust infections on poplars, when considering the AUDPC value (it ranked 11th out of 17 treatments). However, for **r** and FD%, dithane ranked fifth and sixth respectively (Table 5.3). This would indicate that disease levels were probably high at the beginning of the trial but were maintained or slightly reduced. This is reflected in Fig. 5.6 where disease levels actually increased directly after application (possibly reflecting infection before application) and then tapered off gradually to Day 76.

5.4.2 The C14 Demethylation Inhibitors (DMIs)

Sterols are functional components in the maintenance of membrane integrity and are present in all eukaryotes. The principal sterol in most fungi is ergosterol which plays a unique role in the maintenance of membrane function:a reduction in ergosterol results in membrane disruption and electrolyte leakage (Carlisle, 1988) causing inhibition of hyphal growth and eventual death of the fungus (Carlisle, 1988; Lonsdale and Kotze, 1993; Hewitt, 1998). Little interference is associated with other metabolic functions (Carlisle, 1988). The synthetic pathway to ergosterol is a feature of most fungi (Basidiomycetes included) but is absent in the lower orders (Phycomycetes) which satisfy their sterol requirements directly from their hosts through mycelial uptake. Sterol biosynthesis inhibitors cannot be used to inhibit spore germination, which relies on stored products and can proceed in the absence of biosynthesis (Hewitt, 1998).

All the DMIs can penetrate the plant cuticle or seed coat and are often further translocated up the xylem. The risk of the development of resistant fungi is low and the main problems to date have been confined to the powdery mildews. However, the compounds have remained fully effective against other fungal pathogens. Resistant strains were generally cross-resistant to other DMI fungicides, consistent with the concept of a common mode of action (Lyr, 1995).

Variation in performance between DMIs may reflect differences in their binding affinities to the haem moiety of the demethylase enzyme. Cyproconazole, for example, exists in four isomeric forms, all of which show very high and almost equal fungicidal activity as a result of their similarity in affinity for the active site of inhibition (Gisi *et al.*, 1986). This may be why Deprez-Loustau *et al.* (1992) found cyproconazol to be much more fungitoxic toward seven ectomycorrhizal fungi than triadimefon.

Cyproconazole

Penetration into the leaf by Alto occurs within 30 minutes of application (Thomson, 1991) and the action is long lasting. Alto (alone) and Alto + Armoblen 600 were still decreasing the disease severity in Trial 1 at Day 76 (Fig. 5.6). These two treatments ranked highest in Trial 3 for all three data sets. Figure 5.14 shows that disease never attained a level greater than 3% LAI for both these treatments. The FD% in Trial 4 was taken 88 days after spraying. A linear regression (gradient of 0.68) of the rate of Alto and the percentage leaf area infected gave a correlation of 85.6% with LAI increasing as the rate of Alto decreased. This close correlation confirms that some control of the disease is still taking place at this late stage, nearly three months after the application. Correlation of prior disease ratings range between 61.1% and 75.6%, showing a strong response to rate of Alto and disease presence. The results of Trial 4 indicated the strong action of Alto, even at low concentrations (0.1ml/L). For economical purposes this product could be used at low concentrations and good control would still be obtained.

Difenoconazole

In all instances (Table 5.3) the untreated control had less disease than trees treated with Score. The disease progress curve (Fig. 5.6) shows that disease increased consistently and no disease control was apparent.

Epoxyconazol

The action of Duett was disappointing as this fungicide was the top wheat rust fungicide for aerial spraying at the time of application. No significant differences were obtained between any of the treatments and the control. This may be due to the short duration of the trial and the low disease pressure at the time the trial was conducted.

Flusilazole

Punch C is a formulation of flusilazole and carbendazim. This treatment ranked fifth and fourth for AUDPC and FD% respectively, and from the disease progress curve (Fig. 5.6) control of disease was long-acting.

Flutriafol

Impact is a broad spectrum systemic fungicide that is active in the vapor stage especially against powdery mildews. It moves systemically upward in the plant only and is inactive against Oomycetes and bacteria. It is also compatible with other pesticides and fertilizers and gives up to seven weeks of control (Thomson, 1991). These modes of action would take time to occur in the field and could possibly explain the delayed action witnessed with Early Impact in Trial 1. Disease levels fluctuated up to Day 43 (Fig. 5.6) and from then on, to Day 76, control appeared to be consistently good, not reaching more than 0.5% LAI.

Superphosphate coated onto Impact, a treatment in Trial 2, gave complete control four weeks after application (Fig. 5.10). These results could have a significant impact on other agricultural or silvicultural practices. This treatment was used by Ballinger *et al.* (1988) to control blackleg (*Leptosphaeria maculans* (Desm.) Ces. et de Not.) in rapeseed where they found it to be the most effective treatment, giving appreciable disease control and markedly increased yields. The treatment was applied as an in-furrow treatment at 250g a.i./ha. Ballinger and Kollmorgen (1986) and Brown, *et al.* (1985) also found this treatment to be effective against take-all and stripe rust of wheat, respectively.

Impact is a fungicide that moves acropetally through the plant (Lyr, 1995). A spray application to the leaves moves at most in a translaminar direction with very little lateral movement and no movement down the leaf towards the stem. As such, there can be no downward or upward movement of the fungicide to other parts of the plant once it has been applied to the leaf. However, when it is taken up by the roots, it is evenly distributed throughout the growing parts of the plant. Granular application of fungicides could be very useful for combating poplar rust as no costly aerial spraying would be involved. Application would be inexpensive, using labor to put down a granular application as is commonly practiced with top-up fertilizer applications. Comprehensive tests would need to be conducted to determine the residual effect of this type of application and its effectivity in the field.

Impact coated onto superphosphate did not perform as well as was expected in Trial 3 (Fig. 5.11-5.14). This was probably because the treatment was not applied in a confined environment (as it was previously, where it was placed within a bag) but to the ground where it would have been diluted in a greater area as soon as a rain event occurred. However, this treatment may still have potential when applied on a large scale.

Hexaconazole

Anvil had the fourth lowest AUDPC value and the fifth lowest FD% but because disease slowly, but consistently, increased (Fig. 5.6) it ranked twelfth for **r**.

Propiconazole

Tilt may have had some action against poplar rust up to Day 29 (Fig. 5.6). However, control was not significant (Table 5.3) and its action appeared to have ceased by Day 43.

5.4.3 $\triangle^{8,7}$ Isomerase and $\triangle^{14}$ Reductase Inhibitors

The spectrum of disease control of the $\triangle^{8,7}$ isomerase and $\triangle^{14}$ reductase inhibitors is limited compared with the C14-demythylation inhibitors, their major use being against the Erysiphales. However, they are effective against *Puccinia* spp. of the Basidiomycetes. The commercial performance of the morpholines and the piperadines relies on their use at high application rates (750g a.i./ha for fenpropimorph), suggesting that fungicidal activity is likely to be the sum of the inhibitory effects at all biochemically sensitive sites (Hewitt, 1998).

In Trial 1, Calixin appeared to have enhanced disease progression (Fig. 5.3). This may have been caused by the elimination of phylloplane competitors.

5.4.4 Inhibition of Tubulin Biosynthesis

Popularity of the benzimidazoles in the market place is based on their practical performance in the control of a wide range of Ascomycetes, Deuteromycetes and Basidiomycetes. However, they lack activity against the Oomycetes (Delp, 1995). Microtubules are alternating helices of α- and β-tubulins which form an essential part of the cytoskeleton and are active in spindle formation and the segregation of chromosomes in cell division. Benzimidazoles disrupt mitosis during cell division at metaphase. The mitotic spindle is distorted and daughter nuclei fail to separate, resulting in cell death. Benzimidazoles are highly selective despite the highly conserved nature of β-tubulins in all eukaryotic organisms. Oomycete fungi and all plants are insensitive to the benzimidazoles. The basis of selectivity is unknown but probably depends on structural differences at the binding sites of the microtubules. Minor changes in the amino acid complement, which may underlie selectivity between species, can also confer resistance within species. Complete resistance to the benzimidazoles soon appears and therefore severely limits the value and use of benzimidazoles, although not all fungi are affected. Of the five benzimidazole compounds on the market, only one is effective against *Tilletia* and *Ustilago* spp. and none are effective against *Puccinia* spp. (Hewitt, 1998).

Benzimidazoles used in these trials were formulated with flutriafol (Early Impact) and flusilazole (Punch C) which are both DMIs. Therefore, the specific action of carbendazim could not be ascertained. However, Early Impact outperformed Impact (flutriafol alone) in both trials where these two fungicides were tested (Fig. 5.3 and Fig. 5.11).

5.4.5 Inhibition of the Succinate Dehydrogenase Complex

Succinate dehydrogenase occurs in the respiratory chain as part of the succinate dehydrogenase complex. The complex contains non-haem iron-sulfur proteins that act in the transfer of electrons from reduced flavin adenine dinucleotide (FAD) to coenzyme-Q. Inhibitors of succinate dehydrogenase are specific Basidiomycete fungicides, including efficacy against *Rhizoctonia* spp. (Hewitt, 1998). The specificity of carboxamide fungicides is thought to be controlled by the structural configuration of the target site, which in *Basidiomycetes* may be unique (Buchenauer, 1990). Carboxin and oxycarboxin are systemically active against Basidiomycete fungi (Hewitt, 1998).

Oxycarboxin seemed to have enhanced disease progression (Fig. 5.6) and was not effective in controlling rust infection on poplars.

5.4.6 Inhibition of Complex III (Strobilurins)

Development of this group of fungicides is at a comparatively early stage but some compounds have already demonstrated the presence of potent and comprehensive activity against fungal pathogens. Strobilurins are active in the inhibition of electron transfer in Complex III (bc_1 complex) of the mitochondrial electron transport chain (Becker *et al.*, 1981). Spore germination is the developmental stage of target fungi with most sensitivity to strobilurins, and the activity spectrum is unusually extensive. In addition, the strobilurins have strong eradicant activity against powdery mildews. Generally, the compounds possess slow acting systemic properties and can provide long-term disease control (Hewitt, 1998). Redistribution within the crop is achieved through a continuous mechanism of absorption from the waxy cuticular layer of leaves into the plant and through movement via the vapor phase and readsorption into cuticular waxes (Sauter *et al.*, 1995).

The strobilurin (Stroby WG) was not highly ranked as an effective fungicide for the control of poplar rust (Fig. 5.3-5.5). However, its disease progress curve (Fig. 5.6) showed that the disease level was maintained up to Day 57 after which it increased, showing a limited period of control.

5.4.7 Plant Activators

Plant activators can be defined as substances that induce natural defense mechanisms in plants which enable them to protect themselves to varying degrees from the damaging effects that may be caused by fungi, bacteria, viruses, nematodes or other microorganisms. These substances have no direct effect on these microorganisms. Bion stimulates or induces the development of systemic acquired resistance in plants which gives rise to natural protection mechanisms against invading pathogens (Bosoff, 2000).

This product showed little effect on reducing the disease progression of *Melampsora* rust (Fig. 5.3-5.6).

5.4.8 Undefined Modes of Action

The anilinopyrimidines (cyprodinil) are broad-spectrum fungicides and have a potential use in a wide variety of crops. The major effect of this group is mediated by either a reduction in the biosynthesis of lytic fungal enzymes involved in cell lysis, or either their secretion at the point of fungal penetration (Daniels and Lucas, 1995; Milling and Richardson, 1995). These enzymes include pectinases, cellulases, proteinases and laccase. Pectinase and invertase secretion was demonstrated to be reduced by mepanipyrim, with an associated increase in their intracellular accumulation (Miura *et al.*, 1994a and b). This is thought to be the key mechanism of action of the anilinopyrimidines but the biochemical basis of the effect is not known. There is evidence that suggests the involvement of methionine biosynthesis inhibition.

Pseudomonas spp. are antagonistic to the growth and development of many fungi. Pyrrolnitrin is a secondary metabolite formed by *P. pyrrocina* which has anti-fungal properties but is unsuitable for use in practical disease control because of its instability in light. The optimization of the photostability of pyrrolnitrin led to the development of the commercial fungicides fenpiclonil and fludioxonil which are classed as phenylpyrroles. These have broad disease control spectra but are inactive against

Phycomycete fungi. The phenylpyrroles cause several changes in metabolism and the primary target is uncertain, although the inhibition of glucose phosphorylation may be the biochemical site of action (Hewitt, 1998).

Switch is a formulation of both cyprodonil and fludioxonil but was not effective in controlling *Melamspora* rust infection (Fig. 5.3-5.5). The final disease percentage was almost the highest of all the fungicides tested (Fig. 5.6).

In conclusion, from the various range of chemicals tested, DMIs proved to be the most effective group. Attention should be placed on this group for further development of a chemical control of poplar leaf rust. Formulation with a benzimidazole may further improve the action of DMIs in controlling rust poplar infections. The dithiocarbamates were effective as protectants but were significantly less effective than the DMIs. Fungicide groups targeting inhibition of tubulin biosynthesis, succinate dehydrogenase complex, Complex III and $\triangle^{8,7}$ Isomerase and $\triangle^{14}$ reducatase should not be considered as effective chemicals for the control of this rust.

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Chapter 6 RESPONSE OF A POPLAR HYBRID (CLONE 65/31) TO FERTILIZATION AT SEVEN OAKS, KWAZULU-NATAL, SOUTH AFRICA

ABSTRACT

A 3 x 3 x 3 factorial design was used to determine the effect of nitrogen (N), phosphorus (P) and potassium (K) on the growth of poplar trees and development of rust infection. Nitrogen (limestone ammonium nitrate (LAN) at 28% N) was applied at 0, 15.5 and 31kg/ha, K (KCl at 50% K) was applied at 0, 16.7 and 33.3 kg/ha and P (single superphosphate at 10% P) at 0, 5.3 and 10.6kg/ha. Over one year the single best tree grew 4.1 m, having received a treatment of 31kg N/ha, 10.6kg P/ha and 16.7kg K/ha. This same treatment gave the best mean growth of 3.1m. The poorest treatment was 15.5kg N/ha, 5.3kg P/ha and 33.3kg K/ha, with a mean of 1.7m in growth. The treatment of 15.5kg N/ha, 0kg P/ha and 33.3kg K/ha resulted in the lowest disease level with a mean of 23.5% leaf area infected (LAI). Treatment with 31kg N/ha, 0kg P/ha and 33.3kg K/ha resulted in the highest disease level with 39.2% LAI. The results suggested that higher N applications increased disease susceptibility, although this trend was not significant.

6.1 INTRODUCTION

Most species of poplar grown at present are fast growing, and therefore, demanding of nutrients and water and are highly responsive to every improvement of growing conditions (Baule and Fricker, 1970). Short-rotation intensively cultured (SRIC) hardwood plantations have generated much interest because they yield much more fibre than natural hardwood stands under similar rotation lengths (Wood *et al.*, 1976; Wittwer *et al.*, 1978; Hansen and Baker, 1979). One concern about these plantations is the accelerated loss of nutrients from the site due to the removal of large amounts of nutrient-rich biomass on short harvest cycles (Hansen and Baker, 1979). As rotation lengths shorten and management intensifies, tree plantations become similar to conventional agronomic systems and require fertilization to replace nutrients removed in harvesting (White, 1974; Boyle, 1975; Hansen *et al.*, 1983). Nitrogen is the nutrient most likely to limit growth of SRIC plantations, and N fertilization has been effective in increasing yields (Heilman *et al.*, 1972; Blackmon, 1977b; Wittwer *et al.*, 1978; Hansen and Tolsted, 1985). However, N fertilizer is expensive, and recovery of applied fertilizer N in plantation trees rarely exceeds 20% (Baker *et al.*, 1974). The remaining 80% can be immobilized in the soil, absorbed by vegetation other than the trees, leached below the rooting zone, or converted to gaseous forms and lost (Hansen *et al.*, 1988).

On the basis of the close connections between nutrient absorption, transport of ions, transpiration and the intercellular surrounding of ions, the water economy of trees can be influenced to a considerable extent by fertilizer treatment. In particular, the potassium (K^+) and calcium (Ca^{++}) ions act antagonistically. They have a distinct effect on the intensity of transpiration, and consequently, also on the water content of the trees. The K^+-ion causes swelling and hydration, thus increasing the turgidity of cells and tissues. Under conditions of turgidity, guard cells will open and more water is transpired. The Ca^{++}-ion has the opposite effect by closing the stomata (Baule and Fricker, 1970; Ridolfi *et al.*, 1996).

According to Schönnamsgruber (1961, 1965 In Baule and Fricker, 1970) and van der Meiden (1959, 1964 In Baule and Fricker, 1970), a good supply of K to the trees has been shown to make them more resistant to numerous injurious factors such as frost, drought, incidence of blight and insect attack. The same applies to *Marssonina*, a foliar fungus occurring on poplars. In Holland, Van der Meiden (1959 In Baule and Fricker, 1970) showed that the epidemic development of poplar rust was correlated with K deficiency. Leaves collected with a K content of 0.9% were severely, those with 1.0% moderately and those with 1.3% only slightly attacked by the rust. Another experiment laid out in the tree nursery at the Wageningen Experimental Station showed that the rust attack was much more severe on the plots with N applications than on plots which had received K, Mg, or both. The N treatment lead to a marked fall of the K content in the leaves, and this is thought to be the cause of the increased disease levels. Schönhar (1957,1962 In Baule and Fricker, 1970) reports that an attack on poplars by *Dothichiza populea* (European Poplar Canker) can, to a degree, be prevented by suitable measures of cultivation and fertilizer treatment. A balanced treatment with K, P and N is recommended, since investigations have shown that "a one-sided N supply may considerably increase the disease incidence". Nitrogen deficiency contributed significantly to larger *Cytospora chrysosperma* cankers. Although very high N treatments also had large cankers, these were not significantly different from other N treatments (Burks *et al.*, 1998).

This trial was conducted to determine the effect of soil fertility on growth and levels of rust infection on a match poplar plantation (*Populus deltoides X P. nigra*) in the midlands of KwaZulu-Natal.

6.2 MATERIALS AND METHOD

6.2.1 Description of Experimental Site

Seven Oaks lies at an altitude of 1040m above sea level, 29°12'45" South and 30°29'30" East, about 120km North West of Durban in KwaZulu-Natal, South Africa. This is a summer rainfall area (800mm/annum) with max. daily of 36°C, mean of 28°C and mean monthly temperature of 20°C, in summer. Winters are cool with early morning frost, a min. -8°C and a mean monthly temperature of 12°C. The soil type is of the Kranskop form, and the Fourdoun type (MacVicar, 1991). The soil depth is 800-1200mm and is described as being a dark to greyish brown, apedal, fine sandy clay loam topsoil (150-300mm thick) on dark to strong brown, apedal, fine sandy clay (500-700mm thick) on red to dark reddish brown, apedal, fine clay subsoil (800-1200mm deep) on saprolite.

Preparation of land, which was previously under pine, included de-stumping, discing three times and applying 15t of lime to compensate for the high acid saturation (64-87%). A drip irrigation system, with emitters 0.75m apart and delivering 2.3L/hr was installed at a row spacing of 3m to supply supplementary water in summer (Fig. 6.2). Spraying of weeds with Roundup® (glyphosate) was conducted regularly using a back-pack sprayer.

6.2.2 Experimental Design

An area of 1458m^2 (0.1458ha) was used to lay-out a 3x3x3 factorial N, P and K trial (Fig. 6.1). Nitrogen (LAN at 28% N) and K (KCl at 50% K) were to be applied at 0, 150 and 300kg/ha, and P (single superphosphate at 10% P) at 0, 50 and 100kg/ha. These rates were obtained from establishing the quantity of nutrients absorbed by a ton of poplar dry woody biomass (stem and branches). This was measured for the clone I-214 (*P.* X *euramericana*) in a cycle of 13 years with densities of 280 trees/ha (Bisoffi, 1997). An error in calculation was made and actual levels of fertilizer applied were approximately 10% of the planned levels (Table 6.2). Nine one year old, semi-evergreen clones (Clone 65/31) susceptible to *Melampsora* rust were allocated to each of the 27 treatments. This planting material was obtained from the Redclyff nursery site at the Lion Match plantations, Seven Oaks. After being removed from the ground they were tied in bundles of 25 trees and left in a cool stream until the site was ready for planting. The trees were bare-rooted and planted in holes 300x300x600mm deep at a 3x3m spacing. By the time of planting (28th August, 1998) some trees had commenced flushing. The fertilizers, LAN (28% N), single supers (10.5%P) and KCl (50%) were measured out per tree and applied to an area of 450mm radius (the dripper area) around the tree. The first fertilizer application was made after planting at the end of August, 1998 and the second at the beginning of September, 1999.

6.2.3 Collection of Data

A comprehensive initial soil survey (Table 6.1) of the plot determining sand, silt and clay percentages, organic carbon, cation exchange capacity (CEC), sodium (Na), P, K, calcium (Ca), magnesium (Mg), acidity, exchangeable aluminum, pH (water), pH (KCl) and electrical conductivity (EC) was conducted (Table 6.1) by the Cedara Fertilizer Advisory Service using methods outlined by Farina and Channon (1998). By following a serpentine pattern within each plot, data collected on one day could be compared with data collected on subsequent days. The height of each tree was measured 19 times over 19 months using a 5m Surveyors' staff. A Mututoyo digital vernier caliper (accurate to the 1/100mm) was used to measure the diameter at breast height (DBH) which was measured four times. As soon as rust appeared on the site, trees were assessed for presence and level of disease during each visit until disease had reached epidemic proportions on all trees. Zero was assigned to trees with no disease, one to those with a few pustules and 10 to those with pustules prolific on the leaves. During the second season of growth 18 disease ratings were taken for each plot using a visual rating scale of different rust levels for obtaining a percent leaf area infected (LAI). Leaves that had recently dropped were collected from around the central tree (in a 3m diameter) area and their dry weight obtained in order to get a measure of defoliation from each plot. Leaf samples were obtained during midsummer of the last season of the trial. Mature leaves were collected for this purpose and were analysed for N, protein, P, K, zinc (Zn), Ca, Mg, Na, copper (Cu) and manganese (Mn) (App. 1).

Soil samples were collected at the end of the second season using a β soil sampler and collecting numerous samples which were then mixed on a sheet of plastic and poured into a sample box. These were analysed for density, total carbon, sulfur (S) and N, nitrates, ammonium, P, K, Ca, Mg, exchangeable acidity, total cations, acid saturation, pH (KCl), Zn, Mn, organic carbon and clay percentage (App. 1).

6.2.4 Manipulation of Data and Statistical Analysis

In a single plot at least seven trees survived. Each subsequent height and diameter at breast height (DBH) reading was deducted from the first reading to obtain the growth at that stage. From these figures growth curves were obtained. Each growth curve was submitted into a program calculating the area under the growth curve (Area under height growth curve (AUHGC) and Area under diameter at breast height growth curve (AUDBHGC), respectively) (Berger, 1981). The height, DBH, AUHGC, AUDBHGC, LAI, leaf drop weight (LDW), Disease Presence and Foliar N, P and K percentages were tabulated and statistically analysed using analysis of variance (ANOVA). As the trial was not replicated, the N:P:K interaction (eight degrees of freedom) was used as the residual.

Table 6.1 Soil specifications of the trial site at Lion Match plantations, Seven Oaks, KwaZulu-Natal before the trial

Horizon	A1	B1	B2
Depth (mm)	270	800	1400
course Sand (%)	3	3	3.8
medium Sand (%)	5.4	4.3	4.3
fine Sand (%)	6.3	5.9	6.6
very fine Sand (%)	3.8	5.5	4.3
course Silt (%)	10.4	12.6	11.3
fine Silt (%)	17.6	15	9.9
Clay (%)	51	51.1	57.4
Org Carbon (%)	4.74	1.1	0.7
-33kPa (%)	33.71	27.15	29.13
-80kPa (%)	27.67	25.15	25.6
-500kPa (%)	24.85	22.78	24.6
-1 500kPa (%)	24.07	22.23	23.3
CEC (cmol (+)/kg)	12.46	12.05	12.67
Na (cmol (+)/kg)	0.06	0.09	0.1
K (cmol (+)/kg)	0.12	0.05	0.11
Ca (cmol (+)/kg)	0.13	0.21	0.17
Mg (cmol (+)/kg)	0.1	0.28	0.38
Tot (cmol (+)/kg)	0.41	0.63	0.76
Acidity (cmol (+)/kg)	1.49	0.83	0.41
Exch Al (cmol (+)/kg)	1.1	0.7	0.31
pH (water)	1.04	4.69	5.11
pH (KCl)	3.98	4.53	4.51
E.C. (mS/m)	46	20	6
P status (mg/kg)	0.8	0.6	1.4

Table 6.2 Table of fertilizer treatments made per tree for a fertilizer trial conducted at Lion Match plantations, Seven Oaks, KwaZulu-Natal

N				P				K			
kg N/ha		g LAN/tree		kg P/ha		g single supers/ tree		kg K/ha		g KCl/tree	
a	b	a	b	a	b	a	b	a	b	a	b
0	0	0	0	0	0	0	0	0	0	0	0
150	15.5	482	53.6	50	5.3	429	47.7	150	16.7	270	30
300	31	964	107.1	100	10.6	857	95.3	300	33.3	540	60

a-intended application

b-actual application

15.5N 5.3P 33.3K	15.5N 10.6P 0K			
15.5N 0P 16.7K	0N 0P 33.3K	31N 5.3P 16.7K	0N 5.3P 0K	31N 10.6P 33.3K
31N 0P 0K	0N 10.6P 16.7K	0N 5.3P 33.3K	0N 0P 16.7K	31N 10.6P 16.7K
31N 15.3P 0K	0N 10.6P 0K	31N 0P 33.3K	15.5N 10.6P 33.3K	15.5N 0P 0K
15.5N 5.3P 16.7K	15.5N 10.6P 16.7K	0N 10.6P 33.3K	31N 5.3P 33.3K	15.5N 5.3P 0K
0N 5.3P 16.7K	15.5N 0P 33.3K	31N 0P 16.7K	0N 0P 0K	31N 10.6P 0K

Fig. 6.1 Plan diagram of trial design with values corresponding to kg/ha (scale width of one plot = 9m and length = 9 m, i.e., trees were planted on a 3m x 3m spacing) for a fertilizer trial conducted at Lion Match plantations, Seven Oaks, KwaZulu-Natal.

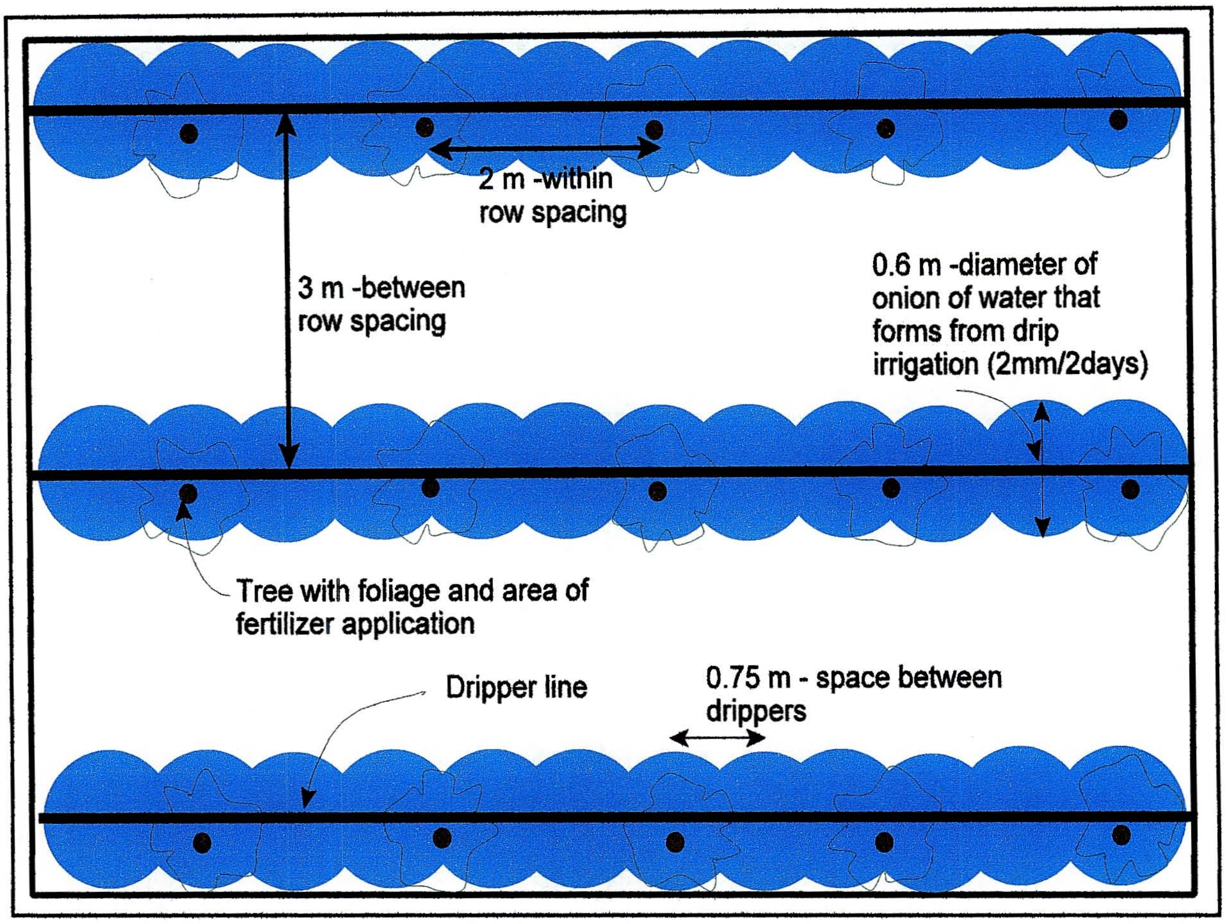


Fig. 6.2 Aerial plan of part of the trial showing layout of irrigation system and tree spacing as well as dispersion of water

6.3 RESULTS

No significant differences were obtained for any of the variates tested in this trial (Table 6.4, 6.5, 6.7, 6.9, 6.10, 6.12 and 6.13). As can be seen in Table 6.2 the amounts of fertilizers were incorrectly calculated and roughly one tenth of the actual amounts were applied. However, the fertilizers were applied in the correct proportions.

6.3.1 Effect of Fertilizer Applications on Soil Nutrient Levels

Analysis of soil samples taken at the end of the second season, showed a slight increase in soil N levels in response to max. (31kg/ha) N fertilizer applications, but the response was not significant (Table 6.3 and 6.4 and App. 1A). No trends could be seen in the soil nitrate levels with respect to N fertilizer applications. Although ammonium levels showed no trends with respect to N fertilizer applications, the F probability that the ammonium levels would increase with increasing N applications was 0.087. The interaction effects showed max. soil N levels with 0 and 5.3kg P/ha and 0 and 16.7kg K/ha. Throughout the main and interaction effects, P soil levels increased with increasing P applications but not at the same rate of application (App. 1B). Potassium soil levels increased with increasing K applications and around the same rate of application (i.e., at the 16.7kg/ha treatment there was a 17.7mg/L increase from the 0kg K/ha treatment and at the 33.3kg K/ha treatment there was a 41.9mg/L increase from the 0kg K/ha treatment which is just over double the 17.7mg/L) (App. 1C). Total carbon percentage and organic carbon percentages showed an upward trend with increasing N fertilization. Total soil S percentage, Ca and Mn levels showed no relation to fertilizer nutrient applications. Magnesium, on the other hand, showed a possible linear trend with increasing N fertilizer applications and inverse trend with increasing K fertilization (App. 1F) as did Zn with increasing P fertilizer applications, shown in the main and interaction effects (App. 1G).

Table 6.3 Soil nutrient levels from samples taken from a fertilizer trial at the Lion Match plantations, Seven Oaks, KwaZulu-Natal in April, 2000 (after 1.5 years) which received annual fertilizer applications

N	P	K	Total N	Nitrate	Ammonium	P	K	Acid Sat.	pH (KCl)
	kg/ha		%		mg/l			%	
0	0	0	0.283	4.73	4.13	3	138	1	4.70
0	0	16.7	0.278	5.82	4.56	3	238	1	5.08
0	0	33.3	0.312	8.48	4.92	4	154	0	5.06
0	5.3	0	0.276	5.96	4.94	5	224	7	4.47
0	5.3	16.7	0.359	8.22	4.76	23	159	1	4.71
0	5.3	33.3	0.292	2.94	6.35	5	153	2	4.78
0	10.6	0	0.319	4.62	5.35	10	141	3	4.75
0	10.6	16.7	0.270	4.19	4.56	24	243	0	5.09
0	10.6	33.3	0.327	5.04	4.40	34	207	0	5.23
15.5	0	0	0.285	7.31	4.08	4	131	1	5.63
15.5	0	16.7	0.309	5.32	5.27	4	146	2	4.68
15.5	0	33.3	0.277	6.73	5.49	5	202	3	4.59
15.5	5.3	0	0.277	6.86	4.93	6	122	0	6.13
15.5	5.3	16.7	0.300	4.79	5.01	17	100	1	5.25
15.5	5.3	33.3	0.280	5.48	5.93	12	219	7	4.48
15.5	10.6	0	0.311	5.27	5.81	20	149	12	4.29
15.5	10.6	16.7	0.331	4.97	6.27	6	136	0	5.55
15.5	10.6	33.3	0.308	7.87	5.08	8	210	1	5.37
31	0	0	0.312	3.84	5.02	3	174	1	4.86
31	0	16.7	0.344	6.86	5.20	4	122	12	4.32
31	0	33.3	0.310	5.51	4.16	4	166	1	4.93
31	5.3	0	0.340	6.28	4.45	5	120	0	5.26
31	5.3	16.7	0.314	5.74	5.20	6	170	1	4.86
31	5.3	33.3	0.292	5.46	3.98	28	182	1	5.71
31	10.6	0	0.267	6.27	5.24	33	155	0	5.34
31	10.6	16.7	0.303	4.48	4.27	5	199	1	4.91
31	10.6	33.3	0.324	6.48	4.45	6	238	1	5.25
	Mean		0.304	5.76	4.96	10.6	170.3	2.2	5.01

Table 6.4 ANOVA table of total soil nitrogen, nitrate and ammonium levels, as affected by nitrogen, phosphorus and potassium fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Rep N.P.K stratum	d.f.	Mean Square			F Value			Probability		
		N %	NO_3^-	NH_4^+	N %	NO_3^-	NH_4^+	N %	NO_3^-	NH_4^+
N	2	0.000	0.66	1.00	0.56	0.3	3.36	0.59 (NS)	0.75 (NS)	0.09 (NS)
P	2	0.000	0.81	0.26	0.11	0.37	0.88	0.90 (NS)	0.70 (NS)	0.45 (NS)
K	2	0.001	0.4	0.00	0.72	0.18	0.13	0.52 (NS)	0.84 (NS)	0.88 (NS)
N:P	4	0.001	1.02	0.38	0.90	0.46	1.28	0.51 (NS)	0.76 (NS)	0.35 (NS)
N:K	4	0.000	0.44	0.53	0.23	0.65	1.77	0.92 (NS)	0.64 (NS)	0.23 (NS)
P:K	4	0.001	3.58	0.54	0.62	1.62	1.83	0.66 (NS)	0.26 (NS)	0.22 (NS)
N:P:K (residual)	8	0.001	2.21	0.30						

CV% (N%)=9.5 CV% (NO_3^-)=25.8 CV% (NH^{4+})=11

NS = non-significant (P=>0.05);* = significant ($P \leq 0.05$);** = highly significant ($P \leq 0.01$);*** = very highly significant ($P \leq 0.001$)

Table 6.5 ANOVA table of soil phosphorus and potassium levels, as affected by nitrogen, phosphorus and potassium fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Rep N.P.K stratum	d.f.	Mean Square		F Value		Probability	
		P	K	P	K	P	K
N	2	23.6	1630	0.21	1.09	0.814 (NS)	0.381 (NS)
P	2	359.1	1774	3.22	0.19	0.094 (NS)	0.354 (NS)
K	2	9.1	3980	0.08	2.66	0.922 (NS)	0.130 (NS)
N:P	4	41	302	0.37	0.20	0.825 (NS)	0.930 (NS)
N:K	4	77.4	2987	0.69	2.00	0.616 (NS)	0.188 (NS)
P:K	4	76.9	869	0.69	0.58	0.619 (NS)	0.685 (NS)
N:P:K (residual)	8	111.4	1495				

CV% (P)= 99.3 CV% (K)=22.7

NS = non-significant (P=>0.05);* = significant ($P \leq 0.05$);** = highly significant ($P \leq 0.01$);*** = very highly significant ($P \leq 0.001$)

6.3.2 Effect of Fertilizer Applications on Foliar Nutrient Levels

Analysis of leaf samples showed that the treatment with the highest N percentage (4.22%) had the highest protein and P percentages and among the highest K percentages. The ratio N:P:K is very close to values obtained from the USA (Coleman, 2000) from healthy, fast growing trees (Table 6.6). The lowest N percentage (3.08%) had the lowest protein and P percentages and among the lowest K percentage. With the exception of the K percentage, all values were less than the values obtained from the USA. The ratio N:P:K was high for both N and K.

Foliar N levels increased slightly with increasing P fertilizer applications (App. 1H). At 33.3 K kg/ha increasing N fertilizer applications increased the foliar N percentage.

An increasing foliar P percentage corresponded to increasing P fertilizer applications (App. 1I). At 16.7 and 33.3 K kg/ha increasing N fertilizer applications appeared to increase the foliar P percentage. At 33.3 K kg/ha increasing P fertilizer applications increased the foliar P percentage.

Foliar K percentages increased with increasing K and P fertilizer application (App. 1J). At 31 N and 10.6 P kg/ha increasing K fertilizer applications increased the foliar K percentage. At 0 K kg/ha foliar K percentages decreased with increasing N fertilizer applications. However, at 33.3 K kg/ha foliar K, percentages increased with increasing N fertilizer applications.

Table 6.6 Foliar nutrient levels from samples taken in January, 2000 after two fertilizer applications

N	P	K	N	Protein	P	K	N:P:K		
	kg/ha			(%)					
0	0	0	3.46	21.60	0.28	2.30	12.4	1	8.2
0	0	16.7	3.39	21.19	0.32	2.45	10.6	1	7.7
0	0	33.3	3.18	19.86	0.25	1.67	12.7	1	6.7
0	5.3	0	3.26	20.35	0.28	2.22	11.6	1	7.9
0	5.3	16.7	3.14	19.65	0.25	2.03	12.6	1	8.1
0	5.3	33.3	3.19	19.94	0.30	2.19	10.6	1	7.3
0	10.6	0	3.62	22.62	0.32	2.12	11.3	1	6.6
0	10.6	16.7	3.13	19.53	0.26	2.50	12.0	1	9.6
0	10.6	33.3	3.49	21.80	0.33	2.69	10.6	1	8.2
15.5	0	0	3.74	23.37	0.34	2.02	11.0	1	5.9
15.5	0	16.7	3.17	19.84	0.27	1.88	11.7	1	7.0
15.5	0	33.3	3.40	21.23	0.28	2.41	12.1	1	8.6
15.5	5.3	0	4.22	26.36	0.41	2.69	10.3	1	6.6
15.5	5.3	16.7	3.51	21.96	0.30	1.98	11.7	1	6.6
15.5	5.3	33.3	3.23	20.21	0.28	2.02	11.5	1	7.2
15.5	10.6	0	3.23	20.22	0.28	1.82	11.5	1	6.5
15.5	10.6	16.7	3.81	23.84	0.32	2.29	11.9	1	7.2
15.5	10.6	33.3	3.51	21.91	0.31	2.50	11.3	1	8.1
31	0	0	3.08	19.26	0.24	1.93	12.8	1	8.0
31	0	16.7	3.43	21.41	0.29	2.42	11.8	1	8.3
31	0	33.3	3.69	23.06	0.30	2.43	12.3	1	8.1
31	5.3	0	3.24	20.22	0.29	1.88	11.2	1	6.5
31	5.3	16.7	3.24	20.22	0.28	2.44	11.6	1	8.7
31	5.3	33.3	3.59	22.43	0.34	2.87	10.6	1	8.4
31	10.6	0	3.51	21.95	0.31	2.31	11.3	1	7.5
31	10.6	16.7	3.29	20.54	0.33	1.99	10.0	1	6.0
31	10.6	33.3	3.41	21.33	0.32	2.63	10.7	1	8.2
Minima			3.08	19.26	0.24	1.67			
Maxima			4.22	26.36	0.41	2.87			
Means			3.41	21.33	0.30	2.25	11.5	1	7.5
USA (Coleman, 2000)			3.19		0.31	1.85	10.3	1	6.0

Table 6.7 ANOVA table of foliar nitrogen, phosphorus and potassium percentages, as affected by nitrogen, phosphorus and potassium fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Rep N.P.K stratum	**d.f.**	**Mean Square**			**F Value**			**Probability**		
		N	**P**	**K**	**N**	**P**	**K**	**N**	**P**	**K**
N	2	0.112	0	0.050	1.06	0.70	0.33	0.390 (NS)	0.522 (NS)	0.731 (NS)
P	2	0.000	0	0.050	0.10	0.85	0.35	0.939 (NS)	0.464 (NS)	0.712 (NS)
K	2	0.040	0	0.130	0.41	0.31	0.91	0.674 (NS)	0.741 (NS)	0.441 (NS)
N:P	4	0.030	0	0.030	0.32	0.51	0.22	0.854 (NS)	0.732 (NS)	0.919 (NS)
N:K	4	0.080	0	0.107	0.78	1.11	0.75	0.567 (NS)	0.414 (NS)	0.585 (NS)
P:K	4	0.020	0	0.070	0.17	0.60	0.48	0.948 (NS)	0.674 (NS)	0.751 (NS)
N:P:K (residual)	8	0.105	0	0.143						

CV% (N)= 9.5 CV% (P)=13.3 CV% (K)=16.8

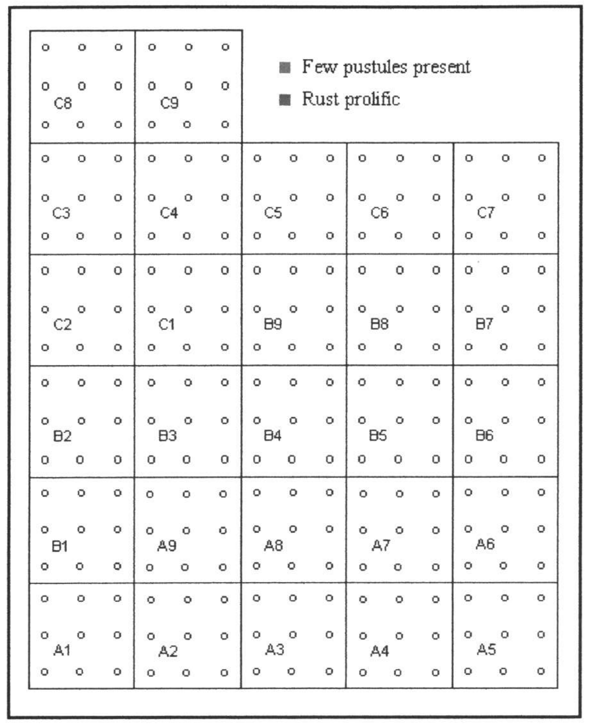
NS = non-significant (P=>0.05);* = significant ($P \leq 0.05$);** = highly significant ($P \leq 0.01$);*** = very highly significant ($P \leq 0.001$)

6.3.3 Effect of Fertilizer Applications on Disease Levels

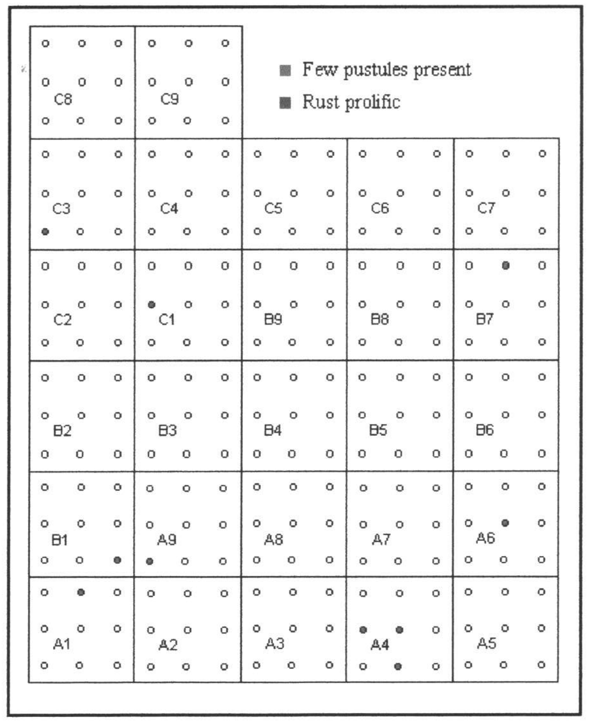
Rust first appeared on the site 55 days after planting (DAP) and reached epidemic proportions on all trees by 96 DAP (Fig. 6.3). Increasing N fertilizer applications were associated with a decrease in rust severity 55 DAP (App. 1K). At 16.7kg K/ha increasing P fertilizer application was associated with an increase in rust severity 55 DAP. At 0kg N/ha and 0kg P/ha rust severity decreases with increasing K fertilizer application 55 and 68 DAP. For 31kg N/ha and 0kg P/ha rust severity also decreases 68 and 83 DAP, respectively. Increasing K fertilizer applications were associated with a decrease in rust severity 68 DAP. At 0 and 16.7kg K/ha disease severity decreased with increasing P fertilizer applications, 83 DAP (App. 1K).

At 5.3 P kg/ha LAI increased with increasing N fertilizer applications (App. 1L). At 15.5 N kg/ha LAI increased with increasing P fertilizer applications. At 0 K kg/ha LAI increased with increasing N fertilizer applications, and decreased with increasing P applications. At 10.6 P kg/ha LAI increased with increasing K fertilizer applications (App. 1L).

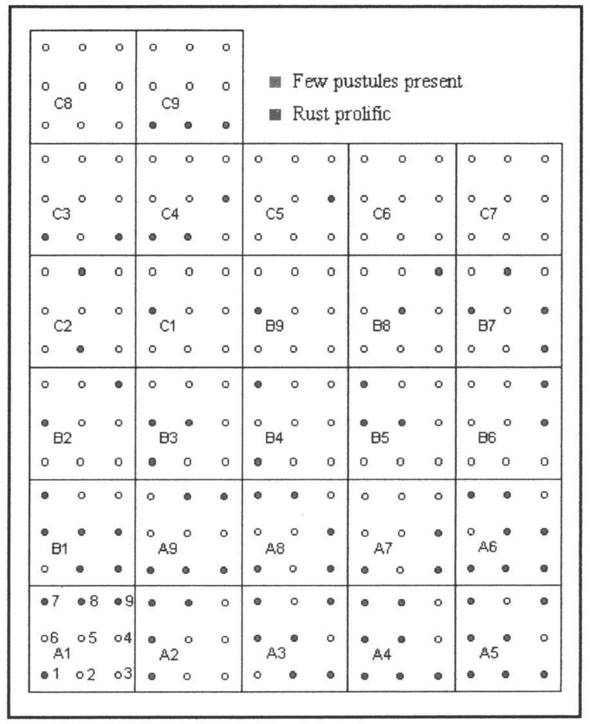
Fig. 6.3 Aerial plans of trial showing spread of rust over time at a fertilizer trial site at the Lion Match Company plantations, Seven Oaks, KwaZulu-Natal.



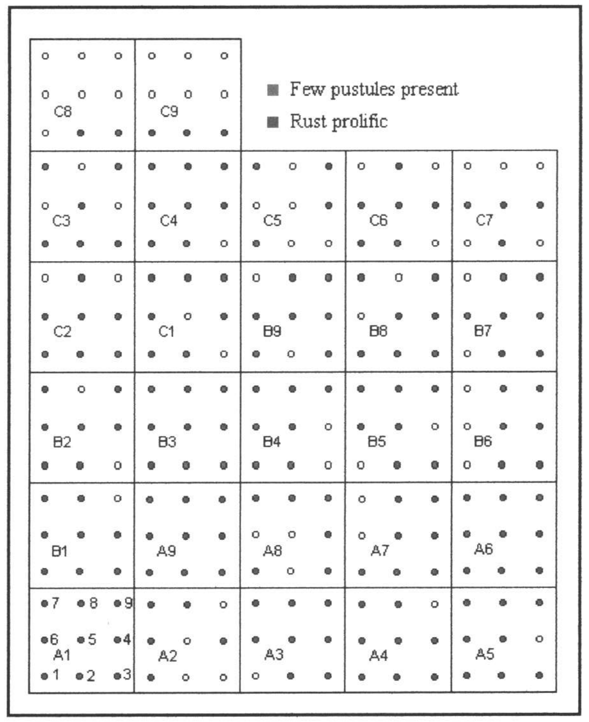
1. Plot plan as on the 7 October, 1998 (40 DAP) showing no incidence of rust



2. Rust as it first appeared on the 22 October, (55 DAP) on specific trees within plots



3. Rust incidence on the 4 November (68 DAP), on specific trees within plots



4. Rust incidence on the 19 November (83 DAP), on specific trees within plots

Although LDW fluctuated from 2.005g to 11.412g (Table 6.8) no significant differences were found. At 10.6 P kg/ha leaf drop weight increased with increasing N fertilizer applications (App. 1M). At 16.7 K kg/ha leaf drop weight decreased with increasing N fertilizer applications. At 31 N kg/ha leaf drop weight increased with increasing P and K fertilizer application. However, at 15.5 N kg/ha leaf drop weight decreased with increasing P fertilizer applications.

Table 6.8 Mean leaf area infected (LAI) and leaf drop weight to evaluate effect of fertilizer treatments on the levels of disease at Lion Match Company plantations experimental irrigation trial, Seven Oaks, KwaZulu-Natal, South Africa

N	P	K	LAI	Leaf drop weight
	kg/ha		%	g
0	0	0	33.83	6.186
0	0	16.7	35.39	6.427
0	0	33.3	36.89	5.896
0	5.3	0	29.88	5.936
0	5.3	16.7	25.28	10.623
0	5.3	33.3	30.17	2.143
0	10.6	0	30.67	3.873
0	10.6	16.7	33.00	3.094
0	10.6	33.3	36.94	3.467
15.5	0	0	33.44	4.253
15.5	0	16.7	27.00	3.707
15.5	0	33.3	23.50	11.412
15.5	5.3	0	31.06	9.780
15.5	5.3	16.7	35.89	5.461
15.5	5.3	33.3	34.60	3.414
15.5	10.6	0	32.60	3.533
15.5	10.6	16.7	38.11	4.384
15.5	10.6	33.3	32.94	3.097
31	0	0	38.50	2.776
31	0	16.7	26.19	2.005
31	0	33.3	39.18	2.419
31	5.3	0	36.94	2.889
31	5.3	16.7	38.67	4.944
31	5.3	33.3	34.61	3.743
31	10.6	0	31.28	3.438
31	10.6	16.7	28.72	3.782
31	10.6	33.3	31.71	8.211
Means			32.85	4.848

Table 6.9 ANOVA table of rust appearance 55, 68 and 83 days after planting (DAP), as affected by nitrogen, phosphorus and potassium fertilizer applications at Lion Match Company plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season. Zero was assigned to trees with no disease, one to those with a few pustules and 10 to those with pustules prolific on the leaves

Rep N.P.K stratum	d.f.	Mean Square			F Value			Probability		
		55	68	83	55	68	83	55	68	83
			DAP			DAP			DAP	
N	2	0	8.8	0.98	0.83	1.43	0.10	0.471 (NS)	0.294 (NS)	0.926 (NS)
P	2	0	2.01	1.35	0.10	0.33	0.11	0.928 (NS)	0.730 (NS)	0.900 (NS)
K	2	0	2.18	9.41	1.69	0.35	0.74	0.244 (NS)	0.712 (NS)	0.505 (NS)
N:P	4	0	1.57	1.45	0.55	0.26	0.11	0.707 (NS)	0.899 (NS)	0.974 (NS)
N:K	4	0	0.58	3.37	0.52	0.10	0.27	0.726 (NS)	0.981 (NS)	0.891 (NS)
P:K	4	0	2.07	0.55	0.98	0.34	0.00	0.468 (NS)	0.846 (NS)	0.996 (NS)
N:P:K (residual)	8	0	6.14	12.6						

CV% (N)= 9.5 CV% (P)=13.3 CV% (K)=16.8

(NS) = non-significant (P=>0.05);* = significant ($P \leq 0.05$);** = highly significant ($P \leq 0.01$);*** = very highly significant ($P \leq 0.001$)

Table 6.10 ANOVA table of leaf area infected (LAI) and leaf drop weight (Leaf wt), as affected by nitrogen, phosphorus and potassium fertilizer applications at Lion Match Company plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Rep N.P.K stratum	d.f.	Mean Square		F Value		Probability	
		LAI	Leaf wt	LAI	Leaf wt	LAI	Leaf wt
N	2	8.80	7.455	0.60	1.17	0.573 (NS)	0.359 (NS)
P	2	0.29	4.211	0.02	0.66	0.981 (NS)	0.543 (NS)
K	2	4.73	0.089	0.32	0.01	0.734 (NS)	0.986 (NS)
N:P	4	53.45	8.009	3.63	1.25	0.057 (NS)	0.363 (NS)
N:K	4	16.17	5.262	1.10	0.82	0.420 (NS)	0.546 (NS)
P:K	4	12.68	9.931	0.86	1.55	0.526 (NS)	0.276 (NS)
N:P:K (residual)	8	14.73	6.392				

NS = non-significant (P=>0.05);* = significant ($P \leq 0.05$);** = highly significant ($P \leq 0.01$);*** = very highly significant ($P \leq 0.001$)

6.3.4 Effect of Fertilizer Applications on Growth

After the first year, a growth of 4.1m was obtained having received a treatment of 31kg N/ha, 10.6kg P/ha and 16.7kg K/ha (Table 6.11). This same treatment gave the best mean growth of 3.1m. After 19 months, a growth of 6.39m was obtained from a treatment receiving 15.5kg N /ha, 5.3kg P/ha and 16.7kg K/ha. The best mean growth was obtained from the treatment receiving the greatest amount of fertilizer (i.e., 31kg N/ha, 10.6kg P/ha and 33.3kg K/ha).

Height growth and AUHGC increased with increasing N fertilizer applications (App. 1N-O). At 0kg K/ha and 10.6kg P/ha height growth and AUHGC increased with increasing N fertilizer applications. At 15.5kg N/ha height growth and AUHGC decreased with increasing P fertilizer applications. At 0kg P/ha AUHGC increased with increasing K fertilizer application. However, at 5.3kg P/ha AUHGC decreased with increasing K fertilizer application.

Diameter at breast height (DBH) growth and AUDBHGC increased with increasing N fertilizer application (App. 1P-Q). At 0kg K/ha and 10.6kg P/ha DBH growth increased with increasing N fertilizer application. At 15.5kg N/ha DBH growth decreased with increasing P fertilizer application. At 33.3kg K/ha AUDBHGC increased with increasing N fertilizer application. At 31kg N/ha and 0kg P/ha AUDBHGC increased with increasing K fertilizer application. However, at 15.5kg N/ha and 5.3kg P/ha AUDBHGC decreased with increasing K fertilizer application.

Table 6.11 Growth variables :height, area under the height growth curve (AUHGC), diameter at breast height (DBH) and area under DBH growth curve (AUDBHGC) after 19 months of growth and having received two fertilizer applications

N	P	K	Height growth	AUHGC	DBH growth	AUDBHGC
	kg/ha		m		mm	
0	0	0	3.959	126581.6	19.9	3719.7
0	0	16.7	3.584	111931.6	16.8	3227.3
0	0	33.3	4.692	146815.9	22.8	4246.9
0	5.3	0	4.717	148726.1	22.4	4601.1
0	5.3	16.7	4.292	136028.0	22.9	4151.9
0	5.3	33.3	4.741	160593.4	20.7	3734.3
0	10.6	0	4.363	151997.0	17.9	3362.6
0	10.6	16.7	4.165	137497.7	18.0	3603.6
0	10.6	33.3	4.042	122207.6	20.2	3732.7
15.5	0	0	4.138	135415.1	19.3	3662.0
15.5	0	16.7	4.955	131396.1	27.8	5113.3
15.5	0	33.3	3.889	122487.3	21.8	4322.4
15.5	5.3	0	4.606	148528.0	24.0	4344.3
15.5	5.3	16.7	4.311	136283.6	22.5	3927.6
15.5	5.3	33.3	3.494	101853.3	18.7	3469.3
15.5	10.6	0	4.123	114641.1	23.1	4521.0
15.5	10.6	16.7	3.969	127048.1	20.5	3680.6
15.5	10.6	33.3	4.020	120805.3	20.2	3852.6
31	0	0	4.517	128673.1	25.0	4527.0
31	0	16.7	3.390	97511.4	19.9	3531.7
31	0	33.3	3.912	114082.3	20.9	3669.9
31	5.3	0	4.579	143847.9	22.3	3976.1
31	5.3	16.7	4.982	141148.6	28.7	5375.0
31	5.3	33.3	4.207	131896.7	21.1	3913.0
31	10.6	0	4.381	137706.1	21.9	3637.3
31	10.6	16.7	4.806	166270.0	19.7	3466.1
31	10.6	33.3	5.376	158090.6	26.6	4890.3
Mean			4.304	133335.7	21.8	4009.6

Table 6.12 ANOVA table of height and area under height growth curve (AUHGC), as affected by nitrogen, phosphorus and potassium fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Rep N.P.K stratum	**d.f.**	**Mean Square**		**F Value**		**Probability**	
		Height	**AUHGC**	**Height**	**AUHGC**	**Height**	**AUHGC**
N	2	6925	2.463 x 10^7	0.55	0.21	0.599 (NS)	0.817 (NS)
P	2	2760	7.138 x 10^7	0.22	0.60	0.809 (NS)	0.571 (NS)
K	2	121	2.442 x 10^7	0.01	0.21	0.991 (NS)	0.818 (NS)
N:P	4	3294	5.432 x 10^7	0.26	0.46	0.896 (NS)	0.765 (NS)
N:K	4	6776	12.660 x 10^7	0.53	1.07	0.715 (NS)	0.433 (NS)
P:K	4	9828	9.557 x 10^7	0.78	0.80	0.571 (NS)	0.555 (NS)
N:P:K (residual)	8	12676	11.870 x 10^7	8.55	4.99		

CV% (Height)= 19.8 CV% (AUHGC)= 12.6

NS = non-significant (P=>0.05);* = significant ($P \leq 0.05$);** = highly significant ($P \leq 0.01$);*** = very highly significant ($P \leq 0.001$)

Table 6.13 ANOVA table of diameter at breast height (DBH) and area under DBH growth curve (AUDBHGC), as affected by nitrogen, phosphorus and potassium fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Rep N.P.K stratum	**d.f.**	**Mean Square**		**F Value**		**Probability**	
		DBH	**AUDBHGC**	**DBH**	**AUDBHGC**	**DBH**	**AUDBHGC**
N	2	122.41	1.944 x 10^6	1.45	0.48	0.291 (NS)	0.636 (NS)
P	2	47.72	1.541 x 10^6	0.56	0.38	0.590 (NS)	0.696 (NS)
K	2	0.89	0.037 x 10^6	0.00	0.01	0.990 (NS)	0.991 (NS)
N:P	4	30.07	1.709 x 10^6	0.36	0.42	0.834 (NS)	0.790 (NS)
N:K	4	31.37	0.648 x 10^6	0.37	0.16	0.823 (NS)	0.953 (NS)
P:K	4	68.82	2.153 x 10^6	0.81	0.53	0.551 (NS)	0.718 (NS)
N:P:K (residual)	8	84.64	4.060 x 10^6	5.18	6.60		

CV% (DBH)= 16.3 CV% (AUDBHGC)= 19.3

NS = non-significant (P=>0.05);* = significant ($P \leq 0.05$);** = highly significant ($P \leq 0.01$);*** = very highly significant ($P \leq 0.001$)

6.4 DISCUSSION

Although no significant results were obtained for this trial, the applications of fertilizer macro nutrients did affect the variates tested. Many problems were associated with this trial which would have had large effects on the variates. The quantities of fertilizer were incorrectly calculated, resulting in the application of approximately one tenth of the fertilizer that should have been applied. The planting material was incorrectly used at planting. The one-year old nursery stock used in this trial had been pulled from the ground and bundled into groups and placed in a fresh perennial stream. Here they began shooting so that once they were planted out into the field, they had no root system to replace water lost to transpiration. This resulted in a delayed response to fertilizer applications and severe stunting of growth with other possible implications thereafter. Three months after planting, 16.5% of the planting material had either been replaced with cuttings of the same clone or were still showing establishment problems, and by March of the second season, a 6.6% fatality had occurred, with some plots having at least two dead trees.

Browsing antelopes (bushbuck and duiker) were also a problem, especially when the trees were small, as they fed on the shoots and leaves of the poplars. They have been known to break down substantial trees using their horns. An electric fence was erected that curbed this problem.

During the course of the second season it became apparent that certain trees in the plot were sharing a different response in growth and rust resistance than the rest of the material. They were of a darker green colour, were more prolific in terms of growth (height and DBH), with more dense foliage and possessed greater rust resistance than other material. It is suspected that this material was of another clone.

6.4.1 Effect of Fertilizer Applications on Soil Nutrient Levels

No significant results were obtained for any of the analyses conducted on the soil nutrient levels. This was due to the low application rate of the macronutrients applied, through an error in calculation. For further studies it is recommended that a soil analysis of the plots within a few weeks of applying the treatment fertilizers be conducted that would act as a check by reflecting fertilizer applications that had been made.

Nitrogen soil levels were low after the fertilizer applications. This was expected due to the uptake by the trees and leaching of N fertilizers from the soil since the soil sampling was conducted at the end of the summer season. Therefore, there was little or no carry over from the previous years fertilizer treatment as was found by Hansen *et al.* (1988). The fact that the carbon and organic carbon percentages increase with increasing N fertilization showed that N fertilization increased litter production and had a greater effect on litter production than disease. The increased P and K levels with increasing P fertilizer application indicated that the plants did not utilise all the available P and K in the soil. The P levels increased at a slower rate than was applied. At higher application rates P may have been more available to the plant and taken up in greater quantities by the trees. The K levels increased at the same rate of application. Increased applications of K had no effect on uptake. This also indicates that P may have been a limiting nutrient in the soil, but not K.

The apparent lack of soil nutrient uptake may be attributed to existing soil nutrients not being depleted or being a limiting factor at this young age. This has been substantiated by McLaughlin *et al.* (1985) who found that high concentrations of NO_3^- existed in the soil solution during the first two years of growth and these became greatly reduced after the second growing season, coinciding with a tree growth response to N fertilization. However, since such small quantities of fertilizer were applied in this trial, it was difficult to draw conclusive results.

The application of lime reduced the acidity of the soil from a KCl pH of 3.98 to 5.01 with an acid saturation of on average of 2.2%. The original high acidity of the soil was typical of an old pine site with a pH unfavourable for poplar growth. The application of lime returned the soil to a favourable one for growing poplar.

6.4.2 Effect of Fertilizer Applications on Foliar Nutrient Levels

No significant differences were obtained for the different treatments that were tested, although some trends were visible in the table of means. Nitrogen trends were only obtained at the highest K fertilizer application and increasing P and K foliar nutrient levels corresponded with their respective fertilizer applications. Foliar nutrient levels for all treatments were intermediate to optimum (Table 1.4) and in most cases higher than USA optimum levels (Table 6.6), with the exception of Ca (1.23mg/L) which was higher than the optimum level for poplars (0.8mg/L). The high level of Ca would be due to the application of lime to reduce the acid saturation of the soil.

Uptake of N was almost exclusively for the use of proteins since foliar N% correlated highly with the protein content of the leaves (99.99%). The foliar analyses showed the lowest N percentage to be in the treatment plot that received only 31kg N/ha. This may imply that in order for N to be taken up by the trees other nutrients are necessary. The plot with the highest percentage foliar N received only 15.5kg N/ha and 5.3kg P/ha. This substantiates the theory that soil N was not limiting. Uptake of N, P and K appears to be related. This is illustrated by the positive correlation between foliar N percentages with foliar P percentages (85%) and foliar K percentages (61.6%).

Hansen *et al.* (1988) applied four different levels of N (0, 56, 112 and 168kg N /ha). The highest application gave a foliar N% of approximately 3.8% on silt loam and the rest of the treatments averaged 3.5%. The high foliar N% obtained from applying little or no fertilizer was attributed to the moderate weed invasion not utilising entirely, the available soil N. Although low fertilizer applications were made in this trial, no deficiency was evident and foliar nutrient levels compare favourably to those obtained by Hansen *et al.* (1988). They also found that foliar N concentrations taken just before the annual application were unrelated to the fertilization rate, indicating a lack of N carry-over from the previous year's fertilization treatments. Only in the third year did foliar N correlate with fertilization amounts and these occurred with a simultaneous rapid increase in tree stem and branch biomass. The fifth year of growth brought about canopy closure with a simultaneous decline in herbaceous weed cover. During this period fertilization had little effect on foliar N, which was high in comparison to previous years. This was attributed to a shift in N pools from herbaceous vegetation to the trees.

Chen (1974) found rates of photosynthesis per unit leaf area to positively correlate with leaf N content (%). Hence, the relationship of poor growth performance with deficient N applications.

6.4.3 Effect of Fertilizer Applications on Disease Levels

Disease was first recorded in the site on the 22 October, 1998, almost two months after planting. Being a virgin site it was rather disconcerting to notice how rapidly the disease spread. From Fig. 6.3 it can be seen that the first infections took place at the lower portion of the site which was closest to other poplar plantations. The trial was situated at the top end of a valley which had a six year old poplar plantation situated approximately 1km below it. It was assumed that innoculum from this stand blew up the valley to initiate a new epidemic in this site.

Analysis of the relationship between leaf drop and LAI gave a correlation of -41% indicating that as LAI increased the leaf drop weight decreased. This was contrary to the expected result which would have been increased leaf drop weight, due to defoliation from increased levels of disease. However, these results were not significantly different.

6.4.4 Effect of Fertilizer Applications on Growth

No significant differences were obtained for growth. Trends in increased growth with increased N application were evident (App. 1N-Q), but not consistent. A response to fertilizer was evident in that the single best tree after one year received one of the highest fertilizer treatments (31kg N, 10.6kg P and 16.7kg K) and the best mean growth after 19 months was obtained from the highest fertilizer treatment (Table 6.11). Nigrogen has been found to increase poplar growth (Blackmon and White, 1972; DeBell *et al.*, 1975; Frison, 1976; Blackmon, 1977b; Frison, 1978; Heilman *et al.*, 1993). However, McLaughlin *et al.* (1987) found no significant difference in dry weight of hybrid poplar biomass between fertilized and unfertilized treatments after three years. Blackmon (1977a) suggested that root systems of young trees are insufficiently developed to use the added N, and that trees on silt loam, which would be more deeply rooted do not need it, thus accounting for the lack of response to N fertilization in the early years of growth.

Nitrogen deficiency results in slower diameter and height growth, a larger number of vessels, shorter fibres, and a lower percentage of ray cells (Cheng and Bensend, 1979), decreasing the quality of the wood for match making.

Frison (1976 and 1978) found little response to K fertilizer, with the exception that trees treated with K as well as N and P produced wood of higher basic density. Phosphorus, applied alone or with N, had no significant effect on growth. Another consideration when examining fertilizer uptake is soil acidity. Soil acidity and soil alkalinity have a negative effect on the uptake of P by young poplars (Liani, 1971).

White and Carter (1970) found a positive relation between height growth and extractable K to a depth of 300 mm in a study on natural poplar stands. This accounted for 94% of the variation in height growth in seven of the eight stands studied. There were indications that high soil Ca concentrations were also necessary for adequate growth (White and Carter, 1970). High levels of Ca are extracted from the soil and incorporated into woody biomass by poplars (Table 1.3). The macronutrients, Ca and

Mg are often overlooked and it is not surprising that Gelhaye *et al.* (1997) found an NPKCa treatment to be the most productive in increasing growth.

Fertilizers should be carefully selected as some fertilizers are not beneficial to increased production. Geyer (1974) applied granules (NPK + chlordane) at planting time and found that increased mortality occurred and pellets (NPK) had no beneficial effects (Geyer, 1974).

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Chapter 7 OVERVIEW

In the past, plantation forestry has satisfied needs within communities, often without due consideration to the cost to the environment. The approach of foresters has tended to be one-sided, with an emphasis being placed on single factors, such as fast growing planting stock, or routine fertilization. For example, fast growing planting stock will not necessarily be fast growing at high altitudes and may be the slowest growing variety in a high altitude zone, and routine fertilization may be unnecessary if the soil is already sufficiently fertile. Through continued abuse of resources such as soil structure, fertility and water, production has also suffered and the need for these resources to be protected has become evident. Also, damage to the environment which may take a short period to inflict can have lasting effects, taking years, if ever, to restore itself. However, approaches in forestry are moving towards a more sustainable model. The Concise Oxford Dictionary of Current English (1995) defines sustain as "support, bear the weight of, esp. for a long period" and sustainable as "which conserves an ecological balance by avoiding depletion of natural resources". Sustainable systems can be observed in wild, native forests which can be used as a reference of sustainable forestry as planted forests are essential to supply a growing demand in wood products (Boyle, 1999). Sustainability can be achieved, in part, by integrating all facets in the approach to forestry, and should be the goal of all successful farmers. The following can be used as a guideline when considering an integrated approach to poplar production.

7.1 POLITICS OF POPLAR GROWING IN SOUTH AFRICA

Production of poplars in South Africa is a relatively small industry. Most farmers only grow small pockets of poplars, with reasons for growing poplars varying greatly. Trees from a stand may be individually selected and felled for extra income when the need arises. Many farmers plant on land that cannot be used for other income generating purposes (non-arable land). Poplars are often planted as shade trees for livestock in pastures. This is possible due to the low leaf area index allowing sufficient light through, so that grass is able to grow under the shade trees. The farmers' approach is often low key in that a small stand of poplars is established with the recommended handful of 2:3:2 fertilizer but is then left until the trees are considered ready to be harvested.

Harold Churchill, chief forester of the Lion Match Company plantations, has estimated that their factory requires 6 000ha of poplars to be grown at current production levels in South Africa. Currently, little over 2 300ha are grown and this area is declining. This is due to The National Water Bill, 1998 which prevents foresters from planting commercial timber within 30m of a water course (Gildenhuys, 1999) and 50m of a wetland (Kotze, 2001). This is in the hopes of conserving South Africa's dwindling water resources.

In the poplar areas of South Africa, rainfall occurs during the summer months. During the winter months the need to conserve water increases. Conservation of water is a major concern in South Africa as there is insufficient water to sustain the growing human population. Semi-evergreen varieties would still be transpiring water over the winter months. However, deciduous varieties would not. This would amount to a substantial saving in water at a time in the year when water is most scarce. From the perspective of water conservation these would be useful clones to plant.

7.2 ENVIRONMENT

Suitability of the environment should be considered for the appropriateness of poplar production. Chapter One of this thesis discusses the environmental requirements of poplars. Site selection should be away from areas with normally extended periods of high humidity. Soils should be analysed for mineral status, organic carbon content, acidity and water holding and drainage capacity. The type of water supply is of fundamental importance, as poplars are a riparian genus and require a substantial amount of water.

7.3 PLANTING MATERIAL

Selection of suitable planting material is of paramount importance. Climate in South Africa is very much warmer than native climates for poplar species. The light intensity is higher and day length fluctuates less due to the lower latitude. Some poplar material, as a result, will not grow in this country (Churchill, 1998). The Lion Match Company have over the years, introduced many productive poplar clones into South Africa. Many new clones are currently being evaluated for their suitability to the South African environment. A database of the performance of available clones should be constructed so that farmers may be provided with the best available planting material for their specific environmental conditions.

Use of diverse planting material in a mixed clonal planting provides some genetic diversity within what would be a highly genetically uniform stand of trees. This stand type would be one step closer to a sustainable system, and has the added advantage of reduced disease incidence through the creation of 'barriers' of resistant trees around susceptible ones. This is discussed further under Point 7.5 Disease Management of this chapter.

A further consideration for a sustainable plantation would be a naturally regenerated stand. No land preparation is required with naturally regenerated stands (no soil disturbance takes place) and stand establishment costs can be reduced by up to 90%. With careful consideration to the parent stock in the stand, the naturally regenerated stand may be used as a cost effective breeding programme from which superior planting stock may be obtained. The advantage of this is the production of clones suitably adapted to the South African conditions.

7.3.1 Dry Land Production

Most poplar plantations are dry land plantations. Drought and frost resistance are the two main factors to consider, under this type of practice. Shoot die-back and burning of shoots of young trees, which occur under conditions of water stress and frost, respectively, can affect the form of the trunk of the mature tree and devalue it. Drought resistant clones are often typified by a thicker cuticle which reduces water loss through the stomata. Rapid responses, such as early closure of stomata under water stress conditions also aid in drought resistance. By using clones that bud-off early in the autumn and flush late in the spring, the likelihood of productive foliage experiencing a frost event can be minimised. Clones that bud-off early and flush late, i.e., have short growing seasons, are Clones I488 and 129, as well as Clone *P. deltoides* var *missouriensis*.

7.3.2 Intensive Production

Intensive cultivation of poplars to maximise biomass production for the supply of pulp in paper making is widely carried out in North America. Poplar pulp is of a desirable quality due to the pale, uniform colour of the wood. These 'fibre farms' are largely under drip irrigation. Yields under these intensive conditions have been tripled in half the dry land growing rotation. Fertilization, which is applied through the drippers, is continually monitored using foliar analyses. Breeding programmes provide a wide range of fast growing hybrid poplars which are grown in these 'fibre farms'. Under these conditions poplars are considered one of the fastest growing plants in the world.

The manipulation of water and nutrients in an intensively managed poplar plantation can increase plant resistance to frost damage and disease epidemics. High salt concentrations within the leaf cells decrease the freezing point of the cytoplasm. Chapter Six of this thesis covers nutritional effects on the development of *Melampsora* epidemics. The nutritional status of the tree affects the reaction of the tree to all diseases of poplars. However, further discussion of this is beyond the scope of this thesis.

Intensive cultivation takes many forms. One farmer (Boetiger, 2001) in South Africa, intensively cultivating poplars, is practising flood irrigation and alley-cropping with maize. The poplar trees are planted with a 1.8m in row spacing and 8m between row spacing. To date this has been running for 4.5 years. In the third year the rows were planted to soya beans as the trees were getting too large, and shading the maize. Soya beans also replenish soil N levels. Maize production yielded approximately 5t/ha per season. This is comparable to an average yield under a monocropping flood irrigated system. Coupled with the poplar production, current estimates of height and diameter are approximately 15-20m and 120-150mm respectively (at 4.5 years of age), and make this a highly profitable venture. Inter-cropping with grazing and other crops can also be conducted highly successfully.

Personal observations suggest that Clone 65/31 was one of the best performing clones in South Africa, with the capacity to produce high tonnages under the right conditions. These were observed in Mtubatuba and under irrigation in Seven Oaks and Winterton, all in KwaZulu-Natal. The poplars planted in Mtubatuba were planted on an aquifer, hence water was available year-round. Performance of this clone under dry land conditions was still competitive, in comparison to other clones.

Clones suitable for intensive production should have long growing seasons. Response to fertilizer applications should be rapid, through extensive root systems that have large surface areas (many root hairs).

7.4 LAND PREPARATION

Soil test results should be examined to determine the nutritional status of the soil. Chapter One of this thesis covers fertilization requirements of poplars. Phosphorus does not move in the soil and K has limited soil movement. Hence, if the soil is deficient in these elements, levels should be ameliorated through amendment during land preparation. Serious consideration should be given to the addition of micronutrients in the fertilization programme. The effect of high acid saturation, a common quality of South African soils, on poplar production is unexplored. Incorporation of lime during land preparation may be necessary to reduce high acid saturation levels. Land preparation conducted by The Lion Match Company includes discing three times. Cuttings can be planted directly into the soil, usually three per station. Bare-rooted trees are planted in pits, 0.3m x 0.3m x 0.6m deep.

Pre-emergence herbicides may be sprayed for the control of weeds. However, weed control has not necessarily been found to be beneficial to survival or growth of poplar (as Netzer and Noste (1978) found in irrigated short-rotation intensively cultured poplar) and weeds may even be beneficial as natural slow release fertilizers. Much of the nutrients are retained in the weed herbage and slowly made available to the trees through decomposition of the herbage. Little response to N fertilization takes place prior to canopy closure (Blackmon, 1977; McLaughlin *et al.*, 1987). Thereafter, response to N fertilization has been recorded. Blackmon (1977) explained that the root structure of young poplar trees is insufficiently developed to benefit from fertilizer applications. At the stage of canopy closure, root development is much more advanced and fertilization is very important for optimal production and healthy trees.

7.5 DISEASE MANAGEMENT

7.5.1 Yield Loss

From the preliminary national survey, it is clear that rust is present all over South Africa, and no poplar clone was found to be completely resistant. Since rust epidemics are complex, involving host, pathogen, weather, and human activities, all operating in time and space, determination of the resulting loss in biomass is also complex (Main, 1983). For foliar diseases the effect on yield is attributable to the visible symptoms, and is proportional to it (Shaw, 1997). Estimated yield (diameter^2 x height) of *P. deltoides* at 15 years of age in an Illinois plantation was inversely related to the average leaf rust infection scores (on a 5-point scale) (Jokela and Lovett, 1976). Schipper and Dawson (1974) reported *M. medusae* as being one of the most serious diseases of *P. deltoides* and in intensively managed plantations yield losses of up to 65% in volume were reported for hybrid poplars with *P. deltoides* parentage (Widin and Schipper, 1980). Churchill (1998) estimated a 50% depletion of yield resulting directly from *M. medusae* infections of previously uninfected clones. Other farmers estimated a 33% loss in yield. These estimated losses are substantial and would be heavily experienced at the factory, particularly as there is no access to alternative sources of poplar wood. Up to R24 500 per hectare loss of revenue is estimated to be lost due to *Melampsora* epidemics on poplars in South Africa (Table 7.1).

Table 7.1 Estimated per hectare loss in production and revenue of poplar from poplar rust caused by *Melampsora* spp. on different site qualities after a 17 year rotation

Site quality	**Tons/ hectare**	**Estimated production loss**		**Revenue/ ton**	**Estimated revenue loss**	
		50%	33%		50%	33%
Excellent	200	100	66.6	R 245	R 24 500.00	R 16 317.00
Good	150	75	50	R 245	R 18 375.00	R 12 250.00
Marginal	100	50	33.3	R 245	R 12 250.00	R 8 158.50
Very poor	85	42.5	28.3	R 245	R 10 412.50	R 6 933.50

Note: One farmer obtained yields of 428 tons/ha after a 35 year rotation at 4m by 4m spacing

7.5.2 Presence of a Virulent Hybrid Rust

During studies for this thesis came the discovery that the rust hybrid *M. medusae-populina* was found to occur in South Africa. The two parent species (*M. larici-populina* and *M. medusae*) and this hybrid were found in almost all commercial plantations. Pathogenicity testing of South African isolates of *M. medusae-populina* found this hybrid able to infect all but two of the previously “resistant” varieties that had been tested in Europe.

7.5.3 Resistant Plant Material

Use of resistant plant material is the most common and widely used method of disease control. An extensive network of poplar breeding programmes exists worldwide and most incorporate screening for disease resistance as a fundamental part of the programme. Attempting to obtain resistant varieties from these sources, i.e., European, New Zealand and North American breeding programs, may not be successful as the South African situation is unique. Resistant material obtained from Europe would probably be susceptible to *M. medusae* and resistant material from North America would probably be susceptible to *M. larici-populina*. The challenge is that even if poplar clones are resistant to both *M. larici-populina* and *M. medusae*, they may be susceptible to *M. medusae-populina*.

This necessitates the development of a poplar breeding programme in South Africa. Many of the host/pathogen interactions occurring in the *Populus/Melampsora* pathosystem appear to be driven by single gene relationships. These can be unstable as a single gene change can result in susceptibility. The development of a breeding program in South Africa, incorporating horizontal resistance breeding strategies, would be beneficial to the industry. However, breeding programs are costly, and it would be uneconomic for the small industry that is present in South Africa.

Farmers in South Africa have planted from a limited selection of clones, these being Clones 65/31, 65/29, I 488, 129 and *P. deltoides* var *missouriensis*. Churchill (1998) has imported many more new clones from Portugal, Italy and North America which are currently being screened. From the many clones previously received, very few have been selected as commercially viable. Hence, to obtain a broad genetic base, a continual importation and selection of new material needs to take place. Disease development and clone performance should be monitored strictly in an evaluation program. Table 7.2 illustrates a strategy for screening clone performance.

In Table 7.2, the nursery as well as the field are considered. As mentioned above, some situations require a clone that responds rapidly to optimal water and fertilizer supplies. This clone would be suitable for high quality sites and more intensive forestry practices and would perform well in the nursery, but not necessarily in the field. Clones that perform well in the field may be screened further for qualities such as low water usage, good wood quality and tolerance to drought, frost, flood, soil acidity and low soil nutrients.

Personal observation of clones already present in South Africa has found that Clone 65/31 appeared to have a high degree of rust tolerance, in that high levels of disease were sustained before leaf drop occurred. Clones 129 and 65/29 are two of the more resistant clones, that sustain mainly low levels of disease. Clone I 488 was one of the most susceptible clones with leaves being infected with large pustules at a very young age.

Table 7.2 Clone performance worksheet to evaluate and screen imported poplar material

Clone Details

Name:
Parentage:
Origin:
Date received:
Site of planting:
Date planted:

Performance Details

Nursery:	Date of first summer flush:
	Date of budding off:
	Height after first year:
	DBH after first year:
	Breadth of first fully expanded leaf:
	Leaf retention and canopy extent:
Field:	Date of first summer flush:
	Date of budding off:
	Height after first year:
	DBH after first year:
	Breadth of first fully expanded leaf:
	Leaf retention and canopy extent:

Disease Developments

Number of rust free days:
Date of first appearance of rust:
Date when rust became prolific (many pustules):
Defoliation date:

Wood Quality

Comments

7.5.4 Prevention of Disease Carry-Over Through Winter

Clone 65/31 is a semi-evergreen variety. This means that although budding-off occurs during the winter months, the leaves are not abscised and can be retained for more than one season. Under unfavourable conditions, such as a high rust infection, or drought, defoliation will take place. Semi-evergreen varieties also carry-over a far greater number of uredospores from the end of summer to spring through infection on the leaves. The mild winter experienced in South Africa (in comparison to those of north America and Europe) is not limiting to the pathogen and infection continues throughout the winter season on leaves still retained by semi-evergreen varieties. Conversely, deciduous trees have the potential to reduce *Melampsora* inoculum, through decomposition during the winter months of abscised *Melampsora* infected leaves that are dropped onto the ground. These are then decomposed over the winter months. In South Africa, the deciduous Clone 129 is slightly slower growing than Clones 65/29 and 65/31. However, Clone 129 is disease resistant.

7.5.5 Prevention of Further Disease Importation

Poplar rust was not an indigenous inhabitant of South Africa and was imported, most likely with poplar material. Quarantine measures are insufficient to prevent further importation of other diseases. Cuttings brought into the country are quarantined for six months in bags. During this period development of disease results in a fungicide spray application to maintain the cuttings. A fungal disease that is imported through this plant material is hence not eradicated with this procedure. The Lion Match Company have lost up to 80% of imported plant material through inadequate quarantine growing procedures. From these perspectives, there seems to be little point in quarantining plant material at all.

To prevent the opportunity of further disease importation, poplar plant material can be treated. Heat treatment in steam baths has been shown to eradicate disease causing agents (bacterial, fungal or viral pathogens) in sugarcane setts, a practice that could easily be replicated on poplars. Cuttings should be sealed dry in a plastic bag and placed in a cool environment (3-5°C). They can be stored for up to six months in this way.

7.5.6 Manipulation of Nutrients

In an integrated approach, the use of fertility as a means of controlling disease may be feasible. Fertilizer applied in the correct amounts can improve plant health and hence a plant's capacity to protect itself against disease. Poplar rust was present in the flood irrigated alley-cropping system where nutrients were generously applied, but was not observed to cause defoliation (personal observation). Fertilizers should be balanced and can be monitored using leaf analyses. Samples of harvested wood should be examined to determine what quantities of nutrients have been removed from the soil and should be replenished for a sustainable system. Since P does not readily move in the soil this should be incorporated at planting.

Potassium metasilicate, a soluble product, can be used as a drench or foliar spray and has the added advantage of silicon. Silicon has been shown (Kaiser, 2001) to increase fungal resistance by the deposition of silicon at the site of fungal infection. Silicon also reduces aluminium toxicity, a common problem with South African soils. Annual applications of other nutrients should be made. Large proportions of calcium have been incorporated in biomass (Table 1.3). There is a paucity of information on the effect of calcium applications on the growth and nutrient status of poplar stands. With the continuous removal of such large quantities of calcium that are not being replaced, the soil will become depleted and unable to sustain a productive poplar stand.

7.5.7 Fungicidal Control

Controlling rust infections on poplars using fungicides may be best achieved using fungicides from the triazole group. The cheapest option in Table 7.3 is using the lowest rate of Alto. Chapter Five of this thesis shows that this treatment gave significant control of poplar rust relative to an untreated control. Impact coated onto single super phosphate granules is one of the more innovative, effective ways for controlling poplar rust.

Table 7.3 Costs of the five best fungicide treatments from Trials 1-4 in Chapter Five of this thesis.

Application	Cost/L	Rate/L of Water	Rate/ha (based on 2 000L water/ha)	Cost/ha	Nett Present Value
Alto	R 269.71	0.1ml	0.2L	R 53.94	R 486.85
		0.2ml	0.4L	R 107.88	R 973.69
		0.3ml	0.5L	R 134.86	R 1 217.20
		0.5ml	1.0L	R 269.71	R 2 434.32
		0.7ml	1.4L	R 377.59	R 3 408.01
Alto + Armoblen	R 269.71 R 78.50	0.3ml + 0.75ml	0.5L + 1.5L	R 252.61	R 2 279.98
Impact	R 92.84	1ml	2.0L	R 185.68	R 1 675.89
Impact + single superphosphate	R 92.84 R 1 200.00/tonn	1ml + 5g/tree	1.1L + 5.55kg	R 108.73	R 981.36
Early Impact	R 89.14	0.6ml	1.2L	R 106.99	R 965.66
Anvil	R 263.31	0.2ml	0.4L	R 105.33	R 950.68

* Net present value calculated using equation $V_n = V_o(1 + i)^n$ (Fuller, 2001) with a 6% inflation rate and a 12% interest rate (Hawke, 2001) (Example of calculation - App. 3)

7.5.8 Biological Control

Biological control of disease causing organisms is an increasingly popular option of control. Microbes may act by direct parasitism of rust, indirect injurious effects on the hosts or by introducing antibiotics. They may also decrease the success of the rust by competition for nutrients and space. *Cladosporium* spp. are common leaf saprophytes. *Cladosporium tenuissimum* is a mycoparasite of uredia and germinating uredospores of *M. larici-populina in vitro.* It destroys all but the echinules. It also exhibits antibiosis towards germinating uredospores. In addition, this fungus penetrates the mycelium produced by germinating uredospores. Disintegration of the uredospore mass, adjacent to entry points, suggests at least partial involvement of enzymatic processes. The potential impact of *C. tenuissimum* on the epidemiology of *M. larici-populina* is two-fold, i.e., the presence of the mycoparasite can decrease the number of uredia produced on leaves, and there appears to be enhanced germination of conidia of *C. tenuissimum* when attached to a uredospore of *M. larici-populina.* The two appear to sporulate together in the laboratory (Sharma and Heather, 1988).

When species of *Alternaria* were applied to poplar leaves prior to inoculation with *M. larici-populina*, they too, significantly decreased uredospore germination and subsequent development of rust infection. They appear to send branches into the uredospores which destroy the spores. Three species of *Bacillus*, which occur commonly as saprophytes on needles of the Douglas fir, when applied as a mixture onto needles of glasshouse-grown seedlings, inhibited germination of basidiospore inoculum of *M. medusae* and controlled it (Littlefield, 1981).

During the course of this thesis another fungus was found to colonize rust pustules and appeared to be assimilating the uredospores of *Melampsora* species (Fig. 7.1). This fungus was found to be a species of *Sphaerellopsis*. Bruton and Rijkenberg (1986) made a light and electron microscopy study of *S. filum* (Biv.-Bern.: ex Fr.) Sutton. Its role as a potential biological control agent has been discussed by Hubbes *et al.* (1983). It is the most widespreadurediniculous fungus, existing on 362 rust species from 30 genera in more than 50 countries of the world (Krzan, 1981). The fungus develops dark fruiting bodies (pycnia) seated deep inside the erumpent pustules of the rust (Fig. 7.2 and 7.3). Conidiophores arise by blastic development from the inner walls of the pycnidium and form two-celled conidia (Fig. 7.3) which, encased in a mucous-like matrix, are exuded via the ostiole to emerge as a whitish cirrhus (Fig. 7.4). After the first wetting of the cirrhus the conidia are released from the matrix to interact with the rust fungus.

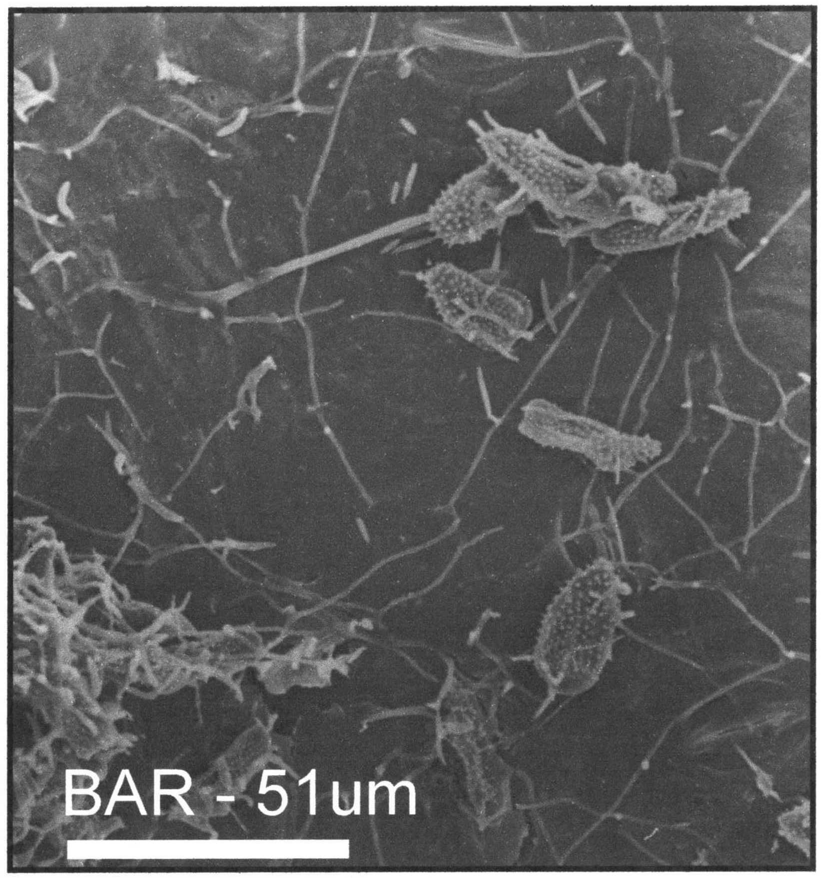

Fig. 7.1 Conidia and germ tubes of *Sphaerellopsis filum* hyperparasitising *Melampsora larici-populina* on a poplar leaf

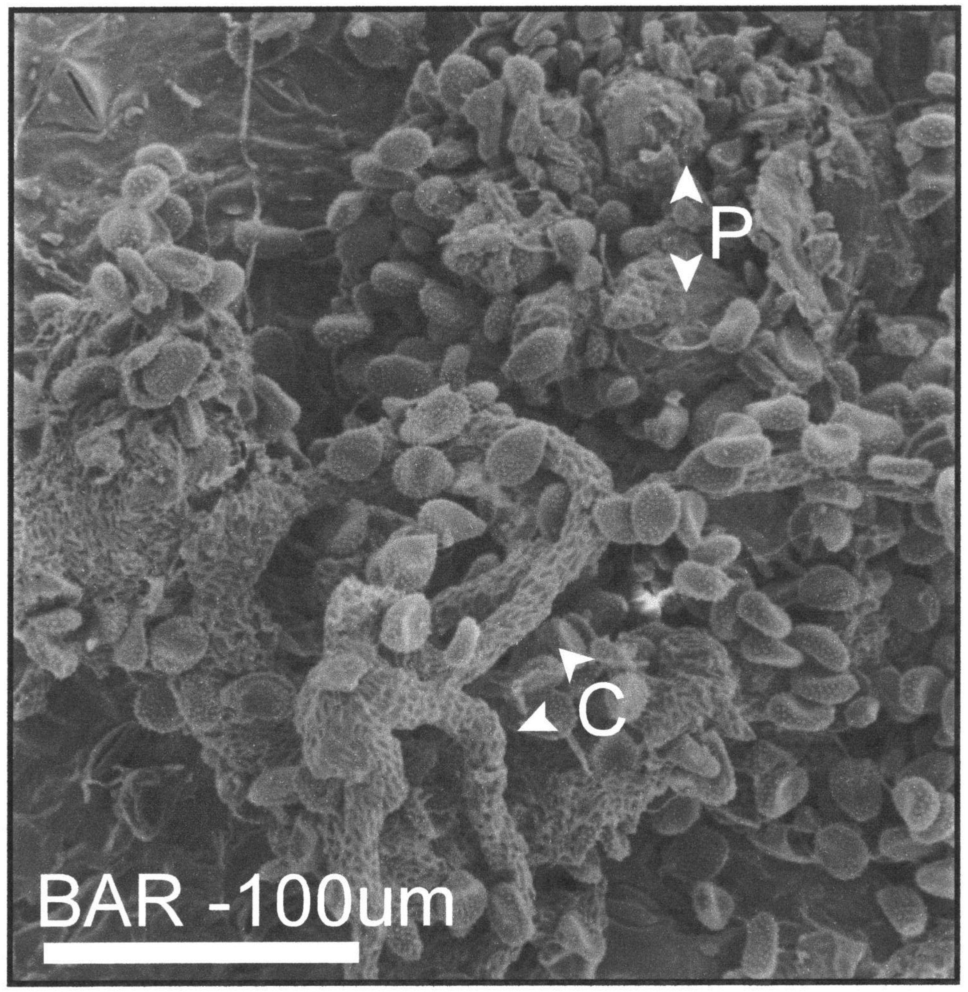

Fig. 7.2 Fruiting bodies (pycnia - P) of *Sphaerellopsis filum* that have exuded a cirrhus (C) of conidia seated deep within a *Melampsora medusae* pustule on a poplar leaf

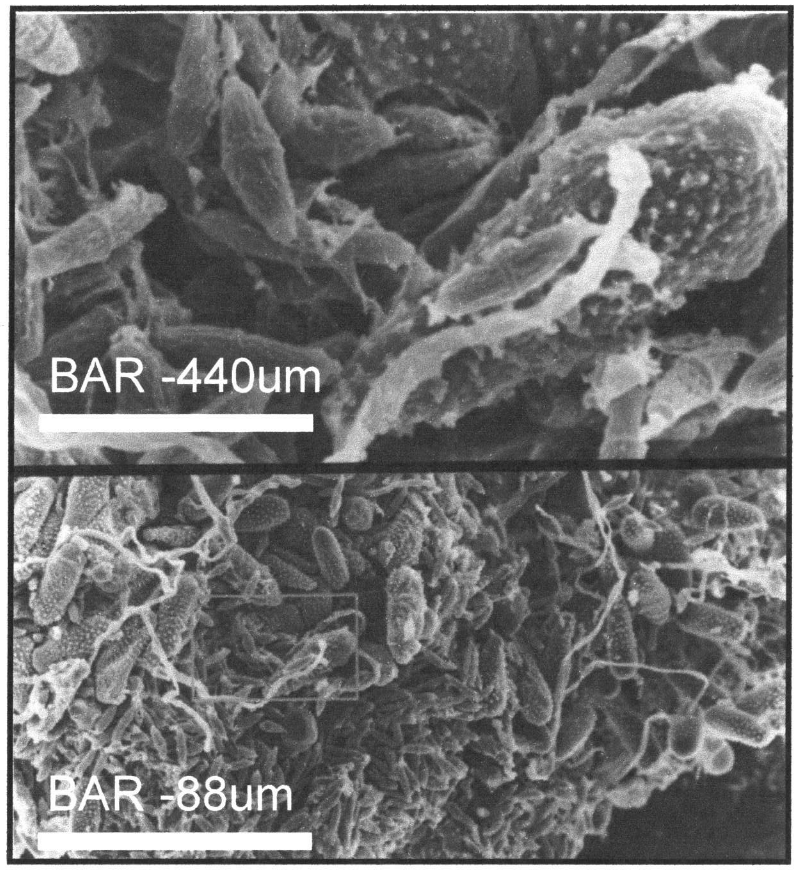

Fig. 7.3 Two celled conidia of *Sphaerellopsis filum* colonising a pustule of *Melampsora larici-populina* on a poplar leaf

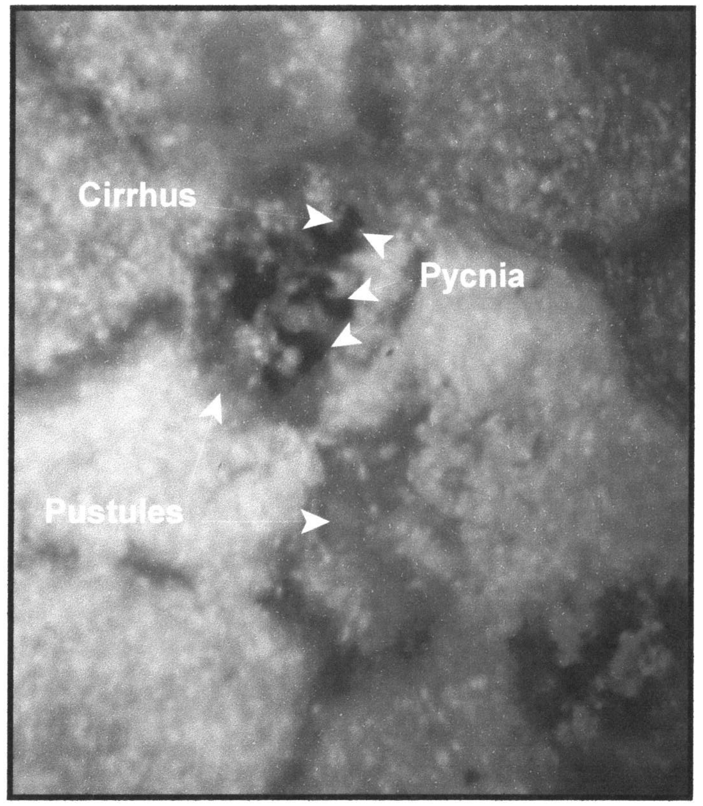

Fig. 7.4 Dark fruiting bodies (pycnia) of *Sphaerellopsis filum* seated deep within a pustule of a *Melampsora* spp. on a poplar leaf. The pycnia are exuding the conidia which are encased in a mucous-like matrix and emerge as a whitish cirrhus

7.6 PEST MANAGEMENT

Little mention has been made of the pests on poplars in South Africa. This topic will only be glanced at as a consideration in an integrated approach. At the Lion Match plantations, KwaZulu-Natal, one of the main pests are antelope. Trees under a metre are browsed, causing deformation of the form of the tree. Labour intensive pruning needs to take place to correct the form and restore the stand to a productive capacity. Trees up to a substantial girth (approximately 100mm) are prey to browsing bushbuck rams whose feeding method is very destructive. The upright tree is wedged between the horns which the ram twists, until the tree snaps, approximately 1.5m from the base. Electric fencing can be used to prevent antelope from entering the stand and regular hunting also takes place.

Other pests seen in large numbers on poplars in South Africa are aphids, spider mites and various caterpillar species. Chemical control is possible, but expensive.

7.7 HARVESTING AND TRANSPORT TO THE MILL

Most harvesting operations are out-sourced. This is more cost effective, and provides for a more specialist approach, where a contractor might only conduct harvesting operations, another, solely weeding operations, and so on. The down-side of out-sourcing is the inevitable reduction in quality of workmanship. Emphasis is placed on getting the job done as cheaply and as quickly as possible. Concerns of soil compaction are real as continuous exposure to heavy vehicles will decrease the oxygen content of the soil, and the hardness will prevent root penetration. Eventually the soil will be unable to sustain a poplar stand. Logs from the field are taken to the nearest railway siding where they are loaded onto a carriage and transported to the Durban Factory for processing. Rail haulage is at no expense to the farmer.

7.8 *QUO VADIS*

Poplar research in South Africa is in the beginning stages. Much information can be obtained from research conducted overseas. However, climate and soil types are conditions fundamental to South Africa. Further investigations into fertilizers (macro- and micro-nutrients) and their effects on growth and disease progress should take place. Ideally, the existing fertigation trial needs to be extended to include three replicates. In this way all interactions could be evaluated and greater significance could be obtained with smaller differences between the treatments.

As mentioned earlier, calcium should be considered as a factor within a fertilizer trial. Calcium is applied to soil mainly in the form of lime. Liming reduces acid saturation levels and many South African soils are typically acid. Soils become more acid as a result of leaching of calcium (Ca^{2+}), and magnesium (Mg^{2+}), and potassium (K^{+}) cations from the topsoil into the subsoil, through the removal of cations by growing crops, and by nitrification of ammonium (NH^{4+}) N. As cations are removed from soil particles, they are replaced with hydrogen and acid-forming aluminium ions. Quantities of lime that could be investigated include 0, 5, 10, 15, and 20t of lime per hectare, depending on the acid saturation of the soil on which the trial is to be conducted. Preferably,, a soil with high acid saturation should be used. At least four replications should be included in the trial, hence the experimental plot should be divided into 20 treatment blocks.

Drip irrigation allows greater control of water and nutrient application to plants. In a properly managed system, the roots are concentrated in the "onion" which is created by the dripper. Therefore, nutrients can be directly applied to the area where they are most needed and whenever they are needed. This "onion" becomes the sole nutrient source for the plant and the plant in turn becomes dependent on it. Through the systemic action of some fungicides the "onion" creates the perfect site for uptake of these systemic fungicides. A further trial should be conducted to investigate this. The use of Alto as a root application should be investigated. A granular formulation for the control of coffee rust has been developed by Novartis. These should be compared with granular Impact treatments. Due to the anticipation of uniform superior strength of these fungicide treatments, a min. of six replicates are suggested for this trial.

7.9 REFERENCES

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APPENDICES

APPENDIX 1 Tables of means for the effects of different fertilizer applications on soil nutrient levels, foliar nutrient levels, disease levels and growth.

App. 1A Table of means of soil nitrogen (N%), nitrate (NO_3^-) and ammonium (NH_4^+) levels as affected by fertilizer applications at Lion Match plantations, Seven Oaks, Kwazulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		N %	NO_3^-	NH_4^+	N %	NO_3^-	NH_4^+	N %	NO_3^-	NH_4^+
			0			15.5			31	
		0.3026	5.76	4.89	0.298	6.07	5.32	0.312	5.66	4.66
$LSD_{(0.05)}$						NS				
Main Effect:P(kg/ha)			0			5.3			10.6	
		0.3011	6.07	4.76	0.303	5.75	5.06	0.307	5.47	5.05
$LSD_{(0.05)}$						NS				
Main Effect:K(kg/ha)			0			16.7			33.3	
		0.2967	5.68	4.88	0.313	5.60	5.01	0.302	6.00	4.97
$LSD_{(0.05)}$						NS				
Interaction Effects										
N:P						**P (kg/ha)**				
			0			5.3			10.6	
	0	0.2910	6.34	4.54	0.309	5.71	5.35	0.308	4.62	4.77
N (kg/ha)	15.5	0.2903	6.45	4.95	0.286	5.71	5.29	0.317	6.04	5.72
	31	0.3220	5.40	4.79	0.315	5.83	4.54	0.298	5.74	4.65
$LSD_{(0.05)}$						NS				
N:K						**K (kg/ha)**				
			0			16.7			33.3	
	0	0.2927	5.10	4.81	0.305	6.08	4.63	0.310	5.49	5.22
N (kg/ha)	15.5	0.2910	6.48	4.94	0.313	5.03	5.52	0.288	6.69	5.50
	31	0.3036	5.46	4.90	0.320	5.69	4.89	0.309	5.82	4.20
$LSD_{(0.05)}$						NS				
P:K						**K (kg/ha)**				
			0			16.7			33.3	
	0	0.2933	5.29	4.41	0.310	6.00	5.01	0.300	6.91	4.86
P (kg/ha)	5.3	0.2977	6.37	4.77	0.324	6.25	4.99	0.288	4.63	5.42
	10.6	0.2990	5.39	5.47	0.304	4.55	5.03	0.320	6.46	4.64
$LSD_{(0.05)}$						NS				

App. 1B Table of means of soil phosphorus levels as affected by fertilizer applications at Lion Match plantations, Seven Oaks, Kwazulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		0	15.5	31
		12.3	9.1	10.4
$\text{LSD}_{(0.05)}$			NS	
Main Effect:P(kg/ha)		0	5.3	10.6
		3.8	11.9	16.2
$\text{LSD}_{(0.05)}$			NS	
Main Effect:K(kg/ha)		0	16.7	33.3
		9.9	10.2	11.8
$\text{LSD}_{(0.05)}$			NS	
Interaction Effects				
N:P			**P (kg/ha)**	
		0	5.3	10.6
	0	3.3	11.0	22.7
N (kg/ha)	15.5	4.3	11.7	11.3
	31	3.7	13.0	14.7
$\text{LSD}_{(0.05)}$			NS	
N:K			**K (kg/ha)**	
		0	16.7	33.3
	0	6.0	16.7	14.3
N (kg/ha)	15.5	10.0	9.0	8.3
	31	13.7	5.0	12.7
$\text{LSD}_{(0.05)}$			NS	
P:K			**K (kg/ha)**	
		0	16.7	33.3
	0	3.3	3.7	4.3
P (kg/ha)	5.3	5.3	15.3	15.0
	10.6	21.0	11.7	16.0
$\text{LSD}_{(0.05)}$			NS	

App. 1C Table of means of soil potassium levels as affected by fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		0	15.5	31
		184.1	157.2	169.6
LSD$_{(0.05)}$			NS	
Main Effect:P(kg/ha)		0	5.3	10.6
		163.4	161.0	186.4
LSD$_{(0.05)}$			NS	
Main Effect:K(kg/ha)		0	16.7	33.3
		150.4	168.1	192.3
LSD$_{(0.05)}$			NS	
Interaction Effects				
N:P			**P (kg/ha)**	
		0	5.3	10.6
N (kg/ha)	0	176.7	178.7	197.0
	15.5	159.7	147.0	165.0
	31	154.0	157.3	197.3
LSD$_{(0.05)}$			NS	
N:K			**K (kg/ha)**	
		0	16.7	33.3
	0	167.7	213.3	171.3
N (kg/ha)	15.5	134.0	127.3	210.3
	31	149.7	163.7	195.3
LSD$_{(0.05)}$			NS	
P:K			**K (kg/ha)**	
		0	16.7	33.3
	0	147.7	168.7	174.0
P (kg/ha)	5.3	155.3	143.0	184.7
	10.6	148.3	192.7	218.3
LSD$_{(0.05)}$			NS	

App. 1D Table of means of soil total carbon (T C%), total sulfur (T S%) and organic carbon (O C%) levels as affected by fertilizer applications at Lion Match plantations, Seven Oaks, Kwazulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		T C%	O C%	T S%	T C%	O C%	T S%	T C%	O C%	T S%
			0			15.5			31	
		4.78	3.26	0.04	4.66	3.28	0.04	4.92	3.44	0.04
LSD$_{(0.05)}$						NS				
Main Effect:P(kg/ha)			0			5.3			10.6	
		4.72	3.29	0.04	4.83	3.36	0.04	4.81	3.33	0.04
LSD$_{(0.05)}$						NS				
Main Effect:K(kg/ha)			0			16.7			33.3	
		4.72	3.44	0.04	4.88	3.28	0.04	4.75	3.26	0.04
LSD$_{(0.05)}$						NS				
Interaction Effects										
N:P						**P (kg/ha)**				
			0			5.3			10.6	
	0	4.59	3.13	0.04	4.92	3.37	0.04	4.82	3.27	0.04
N (kg/ha)	15.5	4.55	3.27	0.04	4.47	3.13	0.04	4.96	3.43	0.05
	31	5.01	3.47	0.05	5.09	3.57	0.04	4.65	3.30	0.04
LSD$_{(0.05)}$						NS				
N:K						**K (kg/ha)**				
			0			16.7			33.3	
	0	4.62	3.30	0.04	4.76	3.17	0.04	4.96	3.30	0.04
N (kg/ha)	15.5	4.60	3.57	0.04	4.90	3.13	0.04	4.48	3.13	0.04
	31	4.95	3.47	0.04	4.98	3.53	0.05	4.82	3.33	0.04
LSD$_{(0.05)}$						NS				
P:K						**K (kg/ha)**				
			0			16.7			33.3	
	0	4.68	3.57	0.04	4.76	3.07	0.04	4.71	3.23	0.04
P (kg/ha)	5.3	4.74	3.37	0.04	5.19	3.43	0.04	4.55	3.27	0.04
	10.6	4.74	3.40	0.04	4.69	3.33	0.04	5.00	3.27	0.04
LSD$_{(0.05)}$						NS				

App. 1E Table of means of soil exchangeable aluminum (Exch. Al), acid saturation (Acid Sat.) and pH (KCl) (pH) levels as affected by fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		Exch. Al	Acid Sat.	pH	Exch. Al	Acid Sat.	pH	Exch. Al	Acid Sat.	pH
			0			15.5			31	
		0.14	1.67	4.87	0.21	3.00	5.11	0.14	2.00	5.05
LSD$_{(0.05)}$						NS				
Main Effect:P(kg/ha)			0			5.3			10.6	
		0.17	2.44	4.87	0.16	2.22	5.07	0.16	2.00	5.09
LSD$_{(0.05)}$						NS				
Main Effect:K(kg/ha)			0			16.7			33.3	
		0.20	2.78	5.05	0.16	2.11	4.94	0.13	1.78	5.04
LSD$_{(0.05)}$						NS				
Interaction Effects										
N:P						**P (kg/ha)**				
			0			5.3			10.6	
	0	0.08	0.67	4.95	0.24	3.33	4.65	0.10	1.00	5.02
N (kg/ha)	15.5	0.14	2.00	4.97	0.19	2.67	5.29	0.30	4.33	5.07
	31	0.30	4.67	4.70	0.06	0.67	5.28	0.07	0.67	5.17
LSD$_{(0.05)}$						NS				
N:K						**K (kg/ha)**				
			0			16.7			33.3	
	0	0.27	3.67	4.64	0.07	0.67	4.96	0.08	0.67	5.02
N (kg/ha)	15.5	0.29	4.33	5.35	0.10	1.00	5.16	0.24	3.67	4.81
	31	0.04	0.33	5.15	0.30	4.67	4.70	0.09	1.00	5.30
LSD$_{(0.05)}$						NS				
P:K						**K (kg/ha)**				
			0			16.7			33.3	
	0	0.09	1.00	5.06	0.33	5.00	4.70	0.10	1.33	4.86
P (kg/ha)	5.3	0.17	2.33	5.29	0.09	1.00	4.94	0.22	3.33	4.99
	10.6	0.34	5.00	4.80	0.06	0.33	5.18	0.08	0.67	5.28
LSD$_{(0.05)}$						NS				

App. 1F Table of means of soil calcium and magnesium (mg/l) levels as affected by fertilizer applications at Lion Match plantations, Seven Oaks, Kwazulu-Natal in the 1999/2000 season

		Ca	Mg	Ca	Mg	Ca	Mg
Main Effect:N(kg/ha)		0		150		300	
		1451	140.6	1580	154.9	1582	160.8
LSD$_{(0.05)}$				NS			
Main Effect:P(kg/ha)		0		50		100	
		1408	151.0	1575	146.3	1630	158.9
LSD$_{(0.05)}$				NS			
Main Effect:K(kg/ha)		0		150		300	
		1570	153.7	1478	152.4	1564	150.1
LSD$_{(0.05)}$				NS			
Interaction Effects							
N:P				**P (kg/ha)**			
		0		50		100	
N (kg/ha)	0	1553	156.0	1235	125.0	1564	140.7
	150	1441	163.3	1709	153.7	1591	147.7
	300	1230	133.7	1780	160.3	1735	188.3
LSD$_{(0.05)}$				NS			
N:K				**K (kg/ha)**			
		0		150		300	
N (kg/ha)	0	1183	130.0	1487	151.3	1682	140.3
	150	1817	169.3	1664	155.3	1260	140.0
	300	1710	161.7	1284	150.7	1751	170.0
LSD$_{(0.05)}$				NS			
P:K				**K (kg/ha)**			
		0		150		300	
P (kg/ha)	0	1595	168.3	1180	142.7	1448	142.0
	50	1742	154.3	1545	142.0	1437	142.7
	100	1373	138.3	1710	172.7	1807	165.7
LSD$_{(0.05)}$				NS			

App. 1G Table of means of soil zinc and manganese (mg/l) levels as affected by fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		Zn	Mn	Zn	Mn	Zn	Mn
		0		150		300	
		0.44	3.33	0.44	3.11	0.47	3.44
LSD$_{(0.05)}$				NS			
Main Effect:P(kg/ha)		0		50		100	
		0.37	3.33	0.49	3.11	0.50	3.44
LSD$_{(0.05)}$				NS			
Main Effect:K(kg/ha)		0		150		300	
		0.41	3.11	0.49	3.44	0.46	3.33
LSD$_{(0.05)}$				NS			
Interaction Effects							
N:P				**P (kg/ha)**			
		0		50		100	
N (kg/ha)	0	0.30	3.00	0.50	3.67	0.53	3.33
	150	0.40	3.33	0.47	2.33	0.47	3.67
	300	0.40	3.67	0.50	3.33	0.50	3.33
LSD$_{(0.05)}$				NS			
N:K				**K (kg/ha)**			
		0		150		300	
N (kg/ha)	0	0.37	3.00	0.53	3.33	0.43	3.67
	150	0.40	3.00	0.47	3.00	0.47	3.33
	300	0.47	3.33	0.47	4.00	0.47	3.00
LSD$_{(0.05)}$				NS			
P:K				**K (kg/ha)**			
		0		150		300	
P (kg/ha)	0	0.33	3.00	0.43	3.33	0.33	3.67
	50	0.37	3.00	0.57	3.67	0.53	2.67
	100	0.53	3.33	0.47	3.33	0.50	3.67
LSD$_{(0.05)}$				NS			

App. 1H Table of means of foliar nitrogen levels as affected by fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		0	15.5	31
		3.318	3.536	3.387
LSD$_{(0.05)}$			NS	
Main Effect:P(kg/ha)		0	5.3	10.6
		3.393	3.402	3.444
LSD$_{(0.05)}$			NS	
Main Effect:K(kg/ha)		0	16.7	33.3
		3.484	3.346	3.41
LSD$_{(0.05)}$			NS	
Interaction Effects				
N:P			**P (kg/ha)**	
		0	5.3	10.6
N (kg/ha)	0	3.343	3.197	3.413
	15.5	3.437	3.653	3.517
	31	3.4	3.357	3.403
LSD$_{(0.05)}$			NS	
N:K			**K (kg/ha)**	
		0	16.7	33.3
N (kg/ha)	0	3.447	3.22	3.287
	15.5	3.73	3.497	3.38
	31	3.277	3.32	3.563
LSD$_{(0.05)}$			NS	
P:K			**K (kg/ha)**	
		0	16.7	33.3
P (kg/ha)	0	3.427	3.33	3.423
	5.3	3.573	3.297	3.337
	10.6	3.453	3.41	3.47
LSD$_{(0.05)}$			NS	

App. 1l Table of means of foliar phosphorus levels as affected by fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		0	15.5	31
		0.2878	0.31	0.3
LSD$_{(0.05)}$			NS	
Main Effect:P(kg/ha)		0	5.3	10.6
		0.2856	0.3033	0.3089
LSD$_{(0.05)}$			NS	
Main Effect:K(kg/ha)		0	16.7	33.3
		0.3056	0.2911	0.3011
LSD$_{(0.05)}$			NS	
Interaction Effects				
N:P			**P (kg/ha)**	
		0	5.3	10.6
	0	0.2833	0.2767	0.3033
N (kg/ha)	15.5	0.2967	0.33	0.3033
	31	0.2767	0.33	0.32
LSD$_{(0.05)}$			NS	
N:K			**K (kg/ha)**	
		0	16.7	33.3
	0	0.2933	0.2767	0.2933
N (kg/ha)	15.5	0.3433	0.2967	0.29
	31	0.28	0.3	0.32
LSD$_{(0.05)}$			NS	
P:K			**K (kg/ha)**	
		0	16.7	33.3
	0	0.2867	0.2933	0.2767
P (kg/ha)	5.3	0.3267	0.2767	0.3067
	10.6	0.3033	0.3033	0.32
LSD$_{(0.05)}$			NS	

App. 1J Table of means of foliar potassium levels as affected by fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		0	15.5	31
		2.241	2.179	2.322
$LSD_{(0.05)}$			NS	
Main Effect:P(kg/ha)		0	5.3	10.6
		2.168	2.258	2.317
$LSD_{(0.05)}$			NS	
Main Effect:K(kg/ha)		0	16.7	33.3
		2.143	2.22	2.379
$LSD_{(0.05)}$			NS	
Interaction Effects				
N:P			**P (kg/ha)**	
		0	5.3	10.6
	0	2.14	2.147	2.437
N (kg/ha)	15.5	4.3	2.23	2.203
	31	2.26	2.397	2.31
$LSD_{(0.05)}$			NS	
N:K			**K (kg/ha)**	
		0	16.7	33.3
	0	2.213	2.327	2.183
N (kg/ha)	15.5	2.177	2.05	2.31
	31	2.04	2.283	2.643
$LSD_{(0.05)}$			NS	
P:K			**K (kg/ha)**	
		0	16.7	33.3
	0	2.083	2.25	2.17
P (kg/ha)	5.3	2.263	2.15	2.36
	10.6	2.083	2.26	2.607
$LSD_{(0.05)}$			NS	

App. 1K Table of means of rust appearance 55, 68 and 83 days after planting (DAP) as affected by nitrogen, phosphorus and potassium fertilizer applications at Lion Match plantations, Seven Oaks, Kwazulu-Natal towards the end of the 1998 summer. Zero was assigned to trees with no disease, one to those with a few pustules and 10 to those with pustules prolific on the leaves

		55	68	83	55	68	83	55	68	83
Main Effect:N(kg/ha)						DAP				
			0			15.5			31	
		0.069	2.00	6.66	0.049	3.07	6.03	0.014	1.09	6.52
LSD$_{(0.05)}$						NS				
Main Effect:P(kg/ha)			0			5.3			10.6	
		0.054	1.51	6.79	0.039	2.39	6.40	0.040	2.26	6.01
LSD$_{(0.05)}$						NS				
Main Effect:K(kg/ha)			0			16.7			33.3	
		0.054	2.42	6.83	0.079	2.25	7.14	0.000	1.50	5.24
LSD$_{(0.05)}$						NS				
Interaction Effects										
N:P						**P (kg/ha)**				
			0			5.3			10.6	
	0	0.125	2.00	7.44	0.042	1.62	6.48	0.042	2.38	6.06
N (kg/ha)	15.5	0.037	1.75	5.64	0.074	4.15	6.04	0.037	3.31	6.42
	31	0.000	0.79	7.29	0.000	1.40	6.69	0.042	1.09	5.57
LSD$_{(0.05)}$						NS				
N:K						**K (kg/ha)**				
			0			16.7			33.3	
	0	0.125	2.57	7.22	0.083	1.76	7.98	0.000	1.67	4.78
N (kg/ha)	15.5	0.037	3.23	5.92	0.111	3.81	7.52	0.000	2.17	4.66
	31	0.000	1.47	7.34	0.042	1.16	5.94	0.000	0.65	6.27
LSD$_{(0.05)}$						NS				
P:K						**K (kg/ha)**				
			0			16.7			33.3	
	0	0.125	1.97	7.50	0.037	1.46	7.37	0.000	1.11	5.50
P (kg/ha)	5.3	0.037	2.72	7.10	0.079	3.51	7.11	0.000	0.94	5.00
	10.6	0.000	2.58	5.88	0.120	1.77	6.96	0.000	2.44	5.20
LSD$_{(0.05)}$						NS				

App. 1L Table of means of LAI (leaf area infected) as affected by fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		0	15.5	31
		32.45	32.13	33.98
LSD$_{(0.05)}$			NS	
Main Effect:P(kg/ha)		0	5.3	10.6
		32.66	33.01	32.89
LSD$_{(0.05)}$			NS	
Main Effect:K(kg/ha)		0	16.7	33.3
		33.13	32.03	33.39
LSD$_{(0.05)}$			NS	
Interaction Effects				
N:P			**P (kg/ha)**	
		0	5.3	10.6
	0	35.37	28.44	33.54
N (kg/ha)	15.5	27.98	33.85	34.55
	31	34.62	36.74	30.57
LSD$_{(0.05)}$			NS	
N:K			**K (kg/ha)**	
		0	16.7	33.3
	0	31.46	16.70	34.67
N (kg/ha)	15.5	32.37	33.67	30.35
	31	35.57	31.19	35.17
LSD$_{(0.05)}$			NS	
P:K			**K (kg/ha)**	
		0	16.7	33.3
	0	35.26	29.53	33.19
P (kg/ha)	5.3	32.63	33.28	33.13
	10.6	31.52	33.28	33.86
LSD$_{(0.05)}$			NS	

App. 1M Table of means of leaf drop weight as affected by fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		0	15.5	31
		5.29	5.45	3.80
LSD$_{(0.05)}$			NS	
Main Effect:P(kg/ha)		0	5.3	10.6
		5.01	11.90	4.10
LSD$_{(0.05)}$			NS	
Main Effect:K(kg/ha)		0	16.7	33.3
		4.74	4.94	4.87
LSD$_{(0.05)}$			NS	
Interaction Effects				
N:P			**P (kg/ha)**	
		0	5.3	10.6
	0	6.17	6.23	3.48
N (kg/ha)	15.5	6.46	6.22	3.67
	31	2.40	3.86	5.14
LSD$_{(0.05)}$			NS	
N:K			**K (kg/ha)**	
		0	16.7	33.3
	0	5.33	6.71	3.84
N (kg/ha)	15.5	5.86	4.52	5.97
	31	3.03	3.58	4.79
LSD$_{(0.05)}$			NS	
P:K			**K (kg/ha)**	
		0	16.7	33.3
	0	4.40	4.05	6.58
P (kg/ha)	5.3	6.20	7.01	3.10
	10.6	3.61	3.75	4.92
LSD$_{(0.05)}$			NS	

App. 1N Table of means of height growth as affected by fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		0	15.5	31
		205.2	214.3	226.1
$LSD_{(0.05)}$			NS	
Main Effect:P(kg/ha)		0	5.3	10.6
		214.7	222.1	208.9
$LSD_{(0.05)}$			NS	
Main Effect:K(kg/ha)		0	16.7	33.3
		216.8	214.3	214.5
$LSD_{(0.05)}$			NS	
Interaction Effects				
N:P			**P (kg/ha)**	
		0	5.3	10.6
	0	206.8	220.2	188.7
N (kg/ha)	15.5	223.7	210.2	208.9
	31	213.6	235.8	229.0
$LSD_{(0.05)}$			NS	
N:K			**K (kg/ha)**	
		0	16.7	33.3
	0	205.6	191.8	218.2
N (kg/ha)	15.5	213.5	234.7	194.5
	31	231.2	216.2	230.9
$LSD_{(0.05)}$			NS	
P:K			**K (kg/ha)**	
		0	16.7	33.3
	0	207.1	219.9	217.1
P (kg/ha)	5.3	230.8	237.1	198.2
	10.6	212.5	185.8	228.3
$LSD_{(0.05)}$			NS	

App. 1O Table of means of AUHGC (area under the height growth curve) as affected by fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		0	15.5	31
		32359	32501	33506
LSD$_{(0.05)}$			NS	
Main Effect:P(kg/ha)		0	5.3	10.6
		32911	33786	31668
LSD$_{(0.05)}$			NS	
Main Effect:K(kg/ha)		0	16.7	33.3
		33484	32283	32599
LSD$_{(0.05)}$			NS	
Interaction Effects				
N:P			**P (kg/ha)**	
		0	5.3	10.6
	0	32711	33423	30944
N (kg/ha)	15.5	34192	31985	31324
	31	31831	35951	32736
LSD$_{(0.05)}$			NS	
N:K			**K (kg/ha)**	
		0	16.7	33.3
	0	32446	30869	33763
N (kg/ha)	15.5	33788	34468	29247
	31	34218	31511	34789
LSD$_{(0.05)}$			NS	
P:K			**K (kg/ha)**	
		0	16.7	33.3
	0	32341	32919	33474
P (kg/ha)	5.3	36156	34184	31020
	10.6	31954	29745	33305
LSD$_{(0.05)}$			NS	

App. 1P Table of means of DBH (diameter at breast height) as affected by fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		0	15.5	31
		19.79	21.61	22.53
LSD$_{(0.05)}$			NS	
Main Effect:P(kg/ha)		0	5.3	10.6
		21.41	22.13	20.40
LSD$_{(0.05)}$			NS	
Main Effect:K(kg/ha)		0	16.7	33.3
		21.24	10.20	21.25
LSD$_{(0.05)}$			NS	
Interaction Effects				
N:P			**P (kg/ha)**	
		0	5.3	10.6
	0	19.59	21.47	18.32
N (kg/ha)	15.5	22.96	21.13	20.76
	31	21.68	23.79	22.12
LSD$_{(0.05)}$			NS	
N:K			**K (kg/ha)**	
		0	16.7	33.3
	0	19.73	18.84	20.81
N (kg/ha)	15.5	21.64	23.02	20.18
	31	22.35	22.49	22.75
LSD$_{(0.05)}$			NS	
P:K			**K (kg/ha)**	
		0	16.7	33.3
	0	21.12	21.51	21.59
P (kg/ha)	5.3	22.33	23.99	20.06
	10.6	20.26	18.85	22.08
LSD$_{(0.05)}$			NS	

App. 1Q Table of means of AUDBHGC (area under the DBH growth curve) as affected by fertilizer applications at Lion Match plantations, Seven Oaks, KwaZulu-Natal in the 1999/2000 season

Main Effect:N(kg/ha)		0	15.5	31
		3738	4036	4049
$LSD_{(0.05)}$			NS	
Main Effect:P(kg/ha)		0	5.3	10.6
		3973	4079	3771
$LSD_{(0.05)}$			NS	
Main Effect:K(kg/ha)		0	16.7	33.3
		3960	3913	3949
$LSD_{(0.05)}$			NS	
Interaction Effects				
N:P			**P (kg/ha)**	
		0	5.3	10.6
	0	3677	4040	3497
N (kg/ha)	15.5	4366	3829	3912
	31	3876	4367	3903
$LSD_{(0.05)}$			NS	
N:K			**K (kg/ha)**	
		0	16.7	33.3
	0	3835	3545	3835
N (kg/ha)	15.5	4128	4112	3867
	31	3917	4084	4145
$LSD_{(0.05)}$			NS	
P:K			**K (kg/ha)**	
		0	16.7	33.3
	0	3921	3957	4041
P (kg/ha)	5.3	4237	4296	3703
	10.6	3723	3487	4103
$LSD_{(0.05)}$			NS	

APPENDIX 2

App. 2 List of poplar growers in South Africa as of the year 2000

No.	Farm/Company/ Farmer	Contact person	Tel. no.	Location	No. of hectares (if known)
1	Hulley Brothers	James Murray	033 234 4699	Balgowan	
2	R & M Consultants	Ronald McClelland	033 234 4432/ 083 775 8549	Balgowan	
3	Baynesfield Estate	John Kennedy	033 251 0043/ 1	Baynesfield	0.1
4	Dougherty	Mark Dougherty	036 448 1334/ 082 806 0690	Bergville	8
5	Piccione	Vaughn Piccione	036 448 2337	Bergville	3
6	Scott	P.T. Scott	036 438 1331	Bergville	12
7	Southey	R.M. Southey	039 757 5453	Cadarville	5
8		Cascades	033 569 1742	Cascades	
9	Maritz	Piet Maritz	036 468 1672	Champagne Valley	
10	Klipp	F.O. Klipp	033 501 1724	Dalton	18
11	Meyer	L.A.	Unknown	Dalton	
12	Schroeder	J. Schroeder	Unknown	Dalton	
13	Union co-op	Peter Dyson	033 501 1101	Dalton	30
		Ray de Cray	033 501 1600	Dalton	
14	Brown	H. Brown	033 234 4653	Dargle	
15	Craig Darroch Farm		033 234 4488	Dargle	
16	Fowler	J. Fowler	033 234 4460/ 4583	Dargle	
17	Neal	P.A. Neal	033 234 4293	Dargle	1
18	Watling	Pam Watling	033 234 4546	Dargle	
19	Mingay	H.J. Mingay	033 631 8102	Donnybrook	
20	Mitchell-Innes	G.S. Mitchell-Innes	036 421 1860	Elandslaagte	18
21	Aberfoyle	D.F. Oldfield	033 413 3291	Greytown	
22	Angikitale	G.T. Oellerman	033 413 3326	Greytown	
23	Egner	Dr J. Egner	031 201 0214	Greytown	60
24	Hill	A.J. Hill	033 413 3342	Greytown	
25	Jarvie	I. Jarvie	033 413 1610	Greytown	5.5
26	Lee	Rob Lee	033 445 0766	Greytown	
27	Lion Match Co	Harold Churchill	033 413 2022/ 23	Greytown	
28	Odendaal	D.C. Odendaal	033 413 3351	Greytown	
29	Pidelta	Kevin	033 413 2074	Greytown	4
30	Gallagher	P. Gallagher	039 433 1820	Harding	

31	Harding Town Board		039 433 0110	Harding	
32	Payn	G. Payn	039 433 1494	Harding	
33	Kohne	R. Kohne	033 445 0721	Hermansburg	
34	Mondi Forests		033 445 0976	Hermansburg	
35	Hardingham	H.E. Hardingham	033 702 1286	Himeville	
36	Lund	R.J. Lund	033 722 1912	Himeville	
37	Shepscombe Farm	Rob Parker	033 330 2771	Howick	
38	Stockowners		033 330 7470	Howick	
39	Panbuilt Timbers	Hans Gherkin	017 820 607/ 812	Iswepe	
40	Benson M.	Tony Matchett	033 330 2525	Karkloof	5
41	Morphew	P.G. Morphew	033 330 2213	Karkloof	30
42	Shaw	Bundy Shaw	033 330 2337	Karkloof	
43	Shaw	W.V.C. Shaw	033 330 2692	Karkloof	6
44	Clarkstone	D. Lubbe	033 444 1924	Kranskop	
45	Green	H.R. Green	036 132 3041	Ladysmith	10
46	Hutchison	R.J. Hutchison	033 234 4368	Lidgetton	
47	Mann	Thomas Mann	033 234 4290	Lidgetton	0.75
48	Horn	D. Horn	033 234 4072/ 082 654 3832	Lions River	
49	Guillaume	G. Guillaume	015 517 7086	Louis Trichardt	3
50	North East Cape Forests		045 932 1662	Maclear	
51	Middleburg Municipality		013 225 331	Middleburg	
52	Sutherland	A.R. Sutherland	033 263 2511	Mooi River	
53	Cotcane Pty Ltd.	Patric Caetano	035 550 4404/ 083 433 6326	Mtubatuba	8
54	SAFCOL		Unknown	Natal	
55	SAPPI		033 347 6600	Natal	
56	Cambell A.M.A.	Peter Coetze	033 263 6234/ 6414	Nottingham Road	5
57	Haw	R.G. Haw	Unknown	Nottingham Road	
58	Hirst	W.S. Hirst	033 263 2131	Nottingham Road	
59	Stey Braes Holdings		033 263 6427	Nottingham Road	
60	Filter	H.P.E. Filter	038 995 0271	Paulpietersburg	18
61	Lammerding	V.H. Lammerding	038 995 2922	Paulpietersburg	
62	Pienaar	S.W. Pienaar	Unknown	Piet Retief	
63	Piet Retief Municipality		Unknown	Piet Retief	
64	Prigge	E. Prigge	013 43 2478	Piet Retief	
65	Prigge	G. Prigge	013 43 1665	Piet Retief	
66	Krause Boerdery		033 345 2336	Pietermaritzburg	
67	Kwagubeshe State Forest		033 505 0036	Pietermaritzburg	
68	Beavlieu	R.M. Nicholson	033 212 3378	Richmond	
69	Bruce	A.E. Bruce	033 212 3556	Richmond	

70	Earl	D. Earl	033 212 3519	Richmond	
71	Gemmel	T.D. Gemmel	033 212 3463	Richmond	
72	Morris	A.H. Morris	033 212 3270	Richmond	
73	Staley	J.	Unknown	Richmond	
74	Beaumont farms		033 263 7022	Rosetta	0.25
75	Sabie		013 154 1051	Sabie	
76	Sabie		013 764 2188	Sabie	7
77	Schagen		013 733 6092	Schagen	
78	Crowe	P.J.J. Crowe	033 552 1721	Seven Oaks	
79	Dixton Estates	Dave Reynolds	033 507 0036	Seven Oaks	
80	Lion Match Co	Harold Churchill	033 507 0022	Seven Oaks	
81	Redlands	W. Rommelspacher	033 507 0112	Seven Oaks	
82	Lion Match Co	Mr J. van Tonder	017 865 0002	Sheepmoor	1 500
83	Belmont	D.B. Cathcart	037 747 4682	Swartberg	
84	Du Plessis	D. Du Plessis	037 747 4582	Swartburg	
85	Mullen	K.	Unknown	Umlaas Road	
86	Fettercairn Farms		037 747 4382	Underburg	
87	James	R. James	033 712 2302	Underburg	
88	Palframan	A. Palframan	033 701 1947/ 082 821 4111	Underburg	2.5
89	Purchase	R.W. Purchase	033 712 1120	Underburg	
90	Bennent	W.O. Bennent	033 503 1622	Wartburg	
91	Eggers	E.W. Eggers	033 503 1442	Wartburg	
92	Gebers	W. Gebers	033 532 2311	Wartburg	
93	Hillerman Brothers		033 503 1047	Wartburg	
94	Holley Brothers		033 503 1245	Wartburg	
95	Safcol		039 553 0401	Weza	
96	Boetiger	Konrad Boetiger	036 488 1033/ 082 550 5262	Winterton	10
97	Cathkin Farm	Collette Hall	036 468 1630	Winterton	
98	Freese	L.E.L. Freese	036 488 3911	Winterton	
99	Green	G.R. Green	036 488 1264	Winterton	
100	Luffingham	L. Luffingham	036 488 1023	Winterton	4.5
101	Mann	Arthur Mann	036 635 3041	Winterton	
102	Robinson	M. Robinson	036 488 1528/ 1015	Winterton	11
103	Sclanders	D.B.A. Sclanders	036 488 1664	Winterton	9
104	Stockill & Sons	A.N. Stockill	036 488 1524	Winterton	5

APPENDIX 3

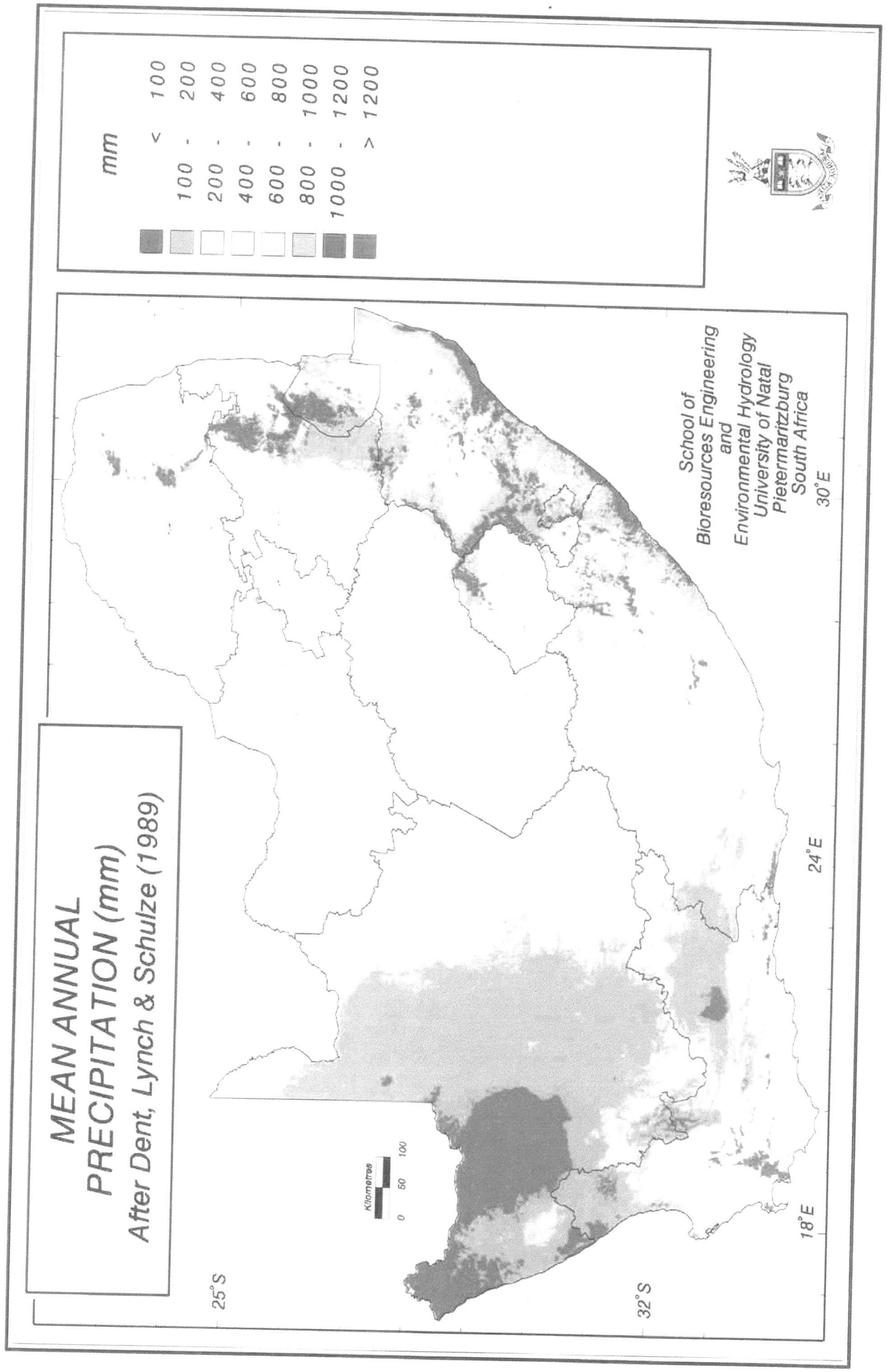
App. 3 Calculation of Nett Present Value of fungicidal applications using example of R 53.94 (cost of 0.1ml Alto/L of water)

Interest Rate : 12%

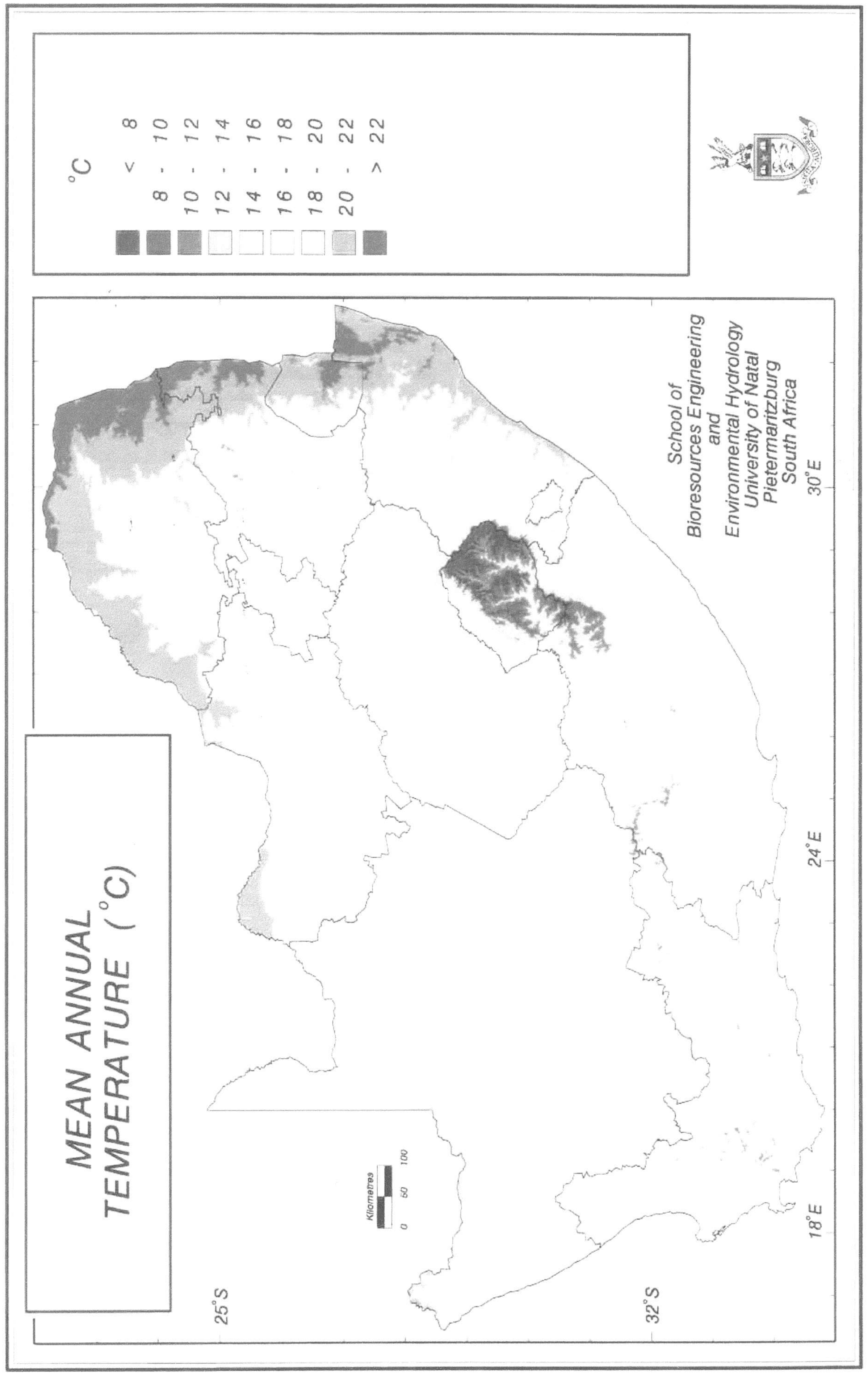
Inflation Rate : 6%

Year	Spend	PV	Spend Calculation	PV Calculation
2001	53.94	53.94	53.94	53.94
2002	57.17	51.05	53.94*1.06	57.17/(1.12)^(2-1)
2003	60.60	48.31	57.17*1.06	60.60/(1.12)^(3-1)
2004	64.24	45.73	60.60*1.06	64.24/(1.12)^(4-1)
2005	68.10	43.28	64.24*1.06	68.10/(1.12)^(5-1)
2006	72.18	40.96	68.10*1.06	72.18/(1.12)^(6-1)
2007	76.51	38.76	72.18*1.06	76.51/(1.12)^(7-1)
2008	81.11	36.69	76.51*1.06	81.11/(1.12)^(8-1)
2009	85.97	34.72	81.11*1.06	85.97/(1.12)^(9-1)
2010	91.13	32.86	85.97*1.06	91.13/(1.12)^(10-1)
2011	96.60	31.10	91.13*1.06	96.60/(1.12)^(11-1)
2012	102.39	29.43	96.60*1.06	102.39/(1.12)^(12-1)
	NPV	486.85		Sum of above column

APPENDIX 4



App. 4A Grid map of mean annual rainfall in South Africa based on 50 year means



App. 4B Grid map of mean annual temperature based on 50 year means of daily means