SEPARATION OF TWO ECOTYPES OF *PHYTOPHTHORA DRECHSLERI* TUCKER OCCURRING IN AUSTRALIAN NATIVE FORESTS

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Abstract

A total of 70 isolates of *P. drechsleri* from Australian native eucalypt forests were separated into two distinct ecotypes. The "northern" ecotype occurred from North Queensland to the south of New South Wales, while the "southern" ecotype occurred in South Australia, Victoria, Tasmania, and New South Wales.

The northern ecotype on average grew faster on a variety of media than did the southern one, but ecotypes could not be recognized unequivocally on this basis. The upper temperature limit for growth of the northern ecotype was in the range 36–37·5°C, while that for the southern was 33–36°C. Ecotypes could be separated on the bases of their growth rates in the presence of 33 μg/ml Cu^{2+} ions (23·54 and 55·66% of the growth in the absence of copper by the northern and southern ecotypes respectively), or in the presence of 1 p.p.m. pyronin G (63·34 and 33·87% of the growth in the absence of pyronin G by the northern and southern ecotypes respectively). Both ecotypes showed similar pH optima for growth, similar responses to the effects of exposure at 44°C on subsequent growth at 25°C, and similar degrees of growth inhibition by 0·05 p.p.m. malachite green.

It is tentatively suggested that the geographic distribution of the southern ecotype is related to the area enclosed by the 29·4°C (85°F) isotherm of average daily maximum temperature during the period November to March annually.

I. INTRODUCTION

Phytophthora drechsleri Tucker has been found in many countries and has been associated with diseases of safflower (*Carthamus tinctorius* L.) by Erwin (1950), velvet bean [*Mucuna deeringiana* (Bort.) Merr.] and sesame (*Sesamum indicum* L.) by Bates (1961), guayule (*Parthenium argentatum* A. Gray) by Hammond and Polhamus (1965), cucurbit (*Cucurbita pepo* L.) by Ershad and Mostowfipoor (1969), and tomato (*Lycopersicon esculentum* Mill.) by Tompkins and Tucker (1941). Distinct pathogenic races of the fungus have been described by Thomas and Klisiewicz (1963) and by Ershad (1971).

In Australia, *P. drechsleri* has been associated with diseases of velvet bean in Queensland (Anon. 1963) and safflower in New South Wales (Anon. 1970). Pratt

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and Heather (1973) provided the first record of an association of this fungus with disease of native vegetation and reported that *P. drechsleri* occurred widely in soils from native forest areas, where it was frequently associated with disease of a variety of tree and understorey species. The 55 isolates examined by them were morphologically similar, but could be separated into two groups according to the ability of isolates to produce oospores in the presence of other species of fungi. The "southern" group produced abundant oospores when isolates were mated with an A1 strain of *P. cinnamomi* Rands, or were exposed to a culture of *Trichoderma koningii* Oud. agg. for 3–5 days, whereas the "northern" group did not produce oospores when treated similarly. Isolates of the A1 mating strain of *P. drechsleri* were unavailable for study.

The aim of the present work was to examine the cultural characteristics of the two groups of isolates, to investigate the degree of variation occurring within them, and to develop rapid and unequivocal methods for their recognition, as a prerequisite to further studies on pathogenic behaviour. In addition, the detailed distribution of the two ecotypes has been determined.

II. Materials and Methods

(a) *Sample Collection and Isolation and Identification of Fungus*

During the period 1969–72, approximately 12,000 samples of soil and plant roots were taken from the root zones of native plants within natural eucalypt forest communities throughout Australia. *Phytophthora* species were recovered from these samples by lupin baiting (Pratt and Heather 1972). Following baiting, lupin radicles were surface-sterilized with 70% (v/v) ethanol, plated onto 2% water agar containing 50 μg/ml streptomycin sulphate, and incubated at 25°C. Fungi growing from the radicles were isolated on V-8 juice agar (Miller 1955). *Phytophthora* species were identified by the methods of Waterhouse (1963, 1970) and by comparison of their cultural, morphological, and reproductive characters with reliably named cultures of *Phytophthora* species.

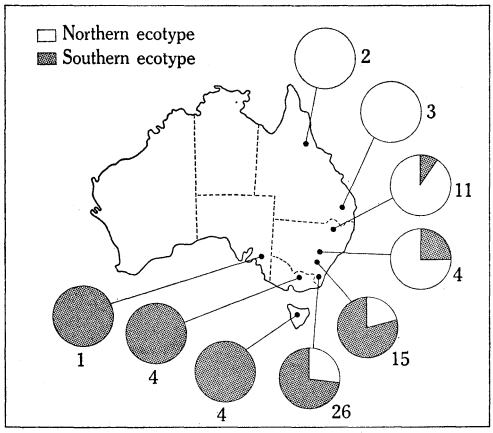

Fig. 1.—Distribution of ecotypes of *P. drechsleri* in Australia. The shaded and unshaded portions of the circles indicate the percentage of the southern and northern ecotypes respectively in the population from each region. The numbers beside the circles indicate the numbers of isolates studied from each region.

A total of 70 isolates of *P. drechsleri* were obtained and identified in this manner and examined for the presence of oospores following mating with A1 and A2 strains of *P. cinnamomi* and exposure to *Trichoderma koningii* for 3–5 days, using the procedure of Pratt *et al.* (1972). No isolate produced an oospore with the A2 mating strain of *P. cinnamomi*, but 42 isolates produced oospores when mated with the A1 strain, or after exposure to *T. koningii*, and were therefore considered to be A2 mating type of *P. drechsleri*. In view of their geographic area of origin, these 42 isolates were desig-

nated as "southern" in type. The remaining 28 isolates did not produce an oospore in either test, thus mating type remained undetermined; because of their places of origin, they were designated "northern" in type. The two types are referred to below as the northern and southern ecotypes of *P. drechsleri* and the numbers found in various areas of origin are shown in Figure 1.

In all, 26 isolates of each of the ecotypes were randomly selected for detailed study and the identities of four isolates were confirmed by Dr. Stamps of the Commonwealth Mycological Institute, Kew (IMI Code Nos. 129907, 133597, 157301, and 164185).

(*b*) *Determination of Growth Rates of Isolates*

Growth rates of isolates were determined on various media and at various temperatures in the range 6–37·5°C. Initially the growth tube method of Ryan *et al.* (1943) was used, but for the majority of the observations reported later radial growth rates were estimated on agar media in Petri dishes, this method being most convenient for handling large numbers of estimations simultaneously and being particularly suitable for studying the effects of inhibitors on fungal growth rates (Trinci and Gull 1970). Careful control of conditions was necessary in order to obtain the desired degree of reproducibility and, in particular, variation of agar depth was found to influence growth rate considerably, as previously reported by Leonian (1934). Agar (30 ml) was dispensed into each 85-mm-diam. Petri dish, giving an agar depth of approximately 5·5 mm. Each dish was inoculated centrally with a 7-mm-diam. agar plug cut from just behind the growing edge of a 4-day-old culture that had been grown at 25°C on corn meal agar, the culture surface of the agar plug being placed downwards in contact with the new agar. Electronically controlled incubators were used to maintain the desired incubation temperatures to $\pm 0 \cdot 1$°C.

Each culture and treatment was replicated four times. Colony diameters were measured at 24-hr intervals, starting 24 hr after inoculation, using a pair of dividers and a millimetre scale. Two measurements were made at right angles on each colony. Instances of colonial sectoring were rare throughout the course of the experiments, but when they did occur such cultures were discarded. At the termination of each experiment, the mean radial growth rates per 24 hr were calculated for each treatment, or isolate.

(*c*) *Growth Inhibition Experiments*

The basal medium used in growth inhibition studies had the following composition:

Sucrose	10·0 g	Thiamine hydrochloride	2 mg
Potassium dihydrogen phosphate	1·0 g	Oxoid casein hydrolysate (acid)	1·0 g
Magnesium sulphate	0·5 g	Trace element solution*	1·0 ml
Calcium chloride	0·1 g	Agar (Oxoid Ionagar No. 2)	18·0 g
		Water	1000 ml

* Trace element solution described by Hendrix *et al.* (1969).

The pH was adjusted to 4·8 with hydrochloric acid before autoclaving at 15 lb/in.^{2} for 15 min. A solution of β-sitosterol in ether was added to the medium immediately after autoclaving at the rate of 20 mg sterol per litre of medium.

When growth rate comparisons were made with either 10 g/l of sucrose or xylose as the carbon source in the absence of inhibitors, the pH of the medium was adjusted to 6·0 before autoclaving.

Growth responses of cultures in the presence of inhibitors were calculated as percentages of the growth rates measured in the absence of such compounds. The relationship of growth rate to inhibitor concentration was linearized by plotting $\log_{10}$(concentration of inhibitor) against the logit of the percentage response, the latter being calculated by use of the formula:

$$y = 0 \cdot 5 \log_{10}(0 \cdot 9R + 5)/(100 - 0 \cdot 9R + 5)$$

where y is the logit and R is the observed growth expressed as a percentage of the growth occurring in the absence of inhibitors.

Comparisons of intercepts and slopes were made and standard errors, variance ratios, and analysis of covariance calculated by the use of a specific programme in a CDC 3600 computer. Detailed results were plotted on computer-drawn graphs, or made manually on logistic scale paper (Gormack Graph Papers, E. and S. A. West Pty. Ltd., Sydney).

Discriminant function analysis was performed as an aid to ecotype differentiation, using a specific programme in the CDC 3600 computer.

III. Experimental Details and Results

(*a*) *Effects of Medium Composition and Temperature on Growth*

Four isolates of each of the northern and southern ecotypes were grown on five media at temperatures ranging from 15 to 37·5°C. As there appeared to be little difference between the growth rates of ecotypes, the results were pooled (Table 1). Growth rate was optimal at 25–27·5°C on corn meal agar and at 27·5°C on the other media tested. All isolates behaved similarly on all media except corn meal agar. On this medium the four southern isolates and two of the northern isolates showed maximum growth at 25°C, one northern isolate had a temperature optimum of 27·5°C, and one of 30°C.

TABLE 1

EFFECTS OF MEDIUM COMPOSITION AND INCUBATION TEMPERATURE ON RADIAL GROWTH RATE OF *P. DRECHSLERI* ISOLATES

Medium	Growth rate* (mm/24 hr) at incubation temperature (°C) of:							
	15	20	25	27·5	30	32·5	35	37·5
Oxoid corn meal agar	5·0	7·2	*9·1*	9·1	8·8	5·4	0·7	0
Oxoid malt agar	4·1	5·5	6·2	*6·5*	6·0	5·7	4·5	0
Campbell's V-8 agar	5·6	7·7	11·3	*11·5*	10·7	9·7	0·5	0
Difco lima bean agar	5·5	7·0	8·2	*9·0*	7·7	0·5	tr.†	0
Casein hydrolysate–sucrose agar	4·0	5·5	5·8	*6·5*	3·9	1·6	tr.†	0

* Radial growth rates are means of eight isolates (four northern and four southern types). Optimal growth rates on each medium are shown in *italics*.

† tr = trace (less than 0·5 mm/24 hr radial growth).

A more detailed examination of the growth–temperature relationships on corn meal agar was made using 26 isolates of each ecotype (Table 2). The greatest differences between the ecotypes are shown at the higher incubation temperatures. For example, at 36°C no southern isolates grew, while growth rates of northern isolates varied in the range 0·90–4·12 mm/24 hr. A temperature optimum of 25°C was shown by 18 of the southern isolates and the remaining eight exhibited an optimum at 30°C. Among the northern isolates, 10 showed an optimum at 25°C and 16 an optimum at 30°C. At most incubation temperatures used, the growth rates of northern isolates were greater than those of the southern ecotype.

Roncadori (1965) compared the nutrient requirements of a number of species of *Phytophthora* and found that several, including three isolates of *P. drechsleri*, made only a trace of growth when xylose was used as a carbon source. In our experiments, all 52 Australian isolates tested showed considerable growth with xylose as

the carbon source in a basal medium similar to that used by Roncadori (1965). The growths at 25°C of 26 northern and 26 southern isolates were compared on a medium containing xylose or sucrose as the carbon source, as well as on two more complex

TABLE 2

EFFECT OF TEMPERATURE ON RADIAL GROWTH RATE OF *P. DRECHSLERI* ISOLATES ON CORN MEAL AGAR

Ecotype	Growth rate (mm/24 hr)* at incubation temperature (°C) of:							
	6	15	20	25	30	33	36	37·5
Northern								
Mean	2·1	5·1	7·4	9·5	9·8	6·9	1·8	0
S.D.	0·2	0·4	0·4	0·5	1·1	0·5	1·0	0
Southern								
Mean	2·2	4·4	6·2	8·3	7·7	5·2	0	0
S.D.	0·3	0·3	0·4	0·6	1·0	0·9	0	0

* Each value represents the mean for 26 isolates.

media (Table 3). The relative mean growth rate on xylose, in comparison with sucrose, was 0·765 for the northern ecotype and 0·744 for the southern one. On all media, the northern ecotype tended to show a faster rate of growth than the southern one, but in no case was there a clear-cut separation of groups consisting of 26 isolates of each ecotype.

TABLE 3

RADIAL GROWTH RATES OF NORTHERN AND SOUTHERN ISOLATES OF *P. DRECHSLERI* ON VARIOUS MEDIA FOLLOWING INCUBATION AT 25°C

Medium	Radial growth rate (mm/24 hr)* of:			
	Northern type		Southern type	
	Mean	S.D.	Mean	S.D.
Corn meal agar	9·5	0·5	8·3	0·6
Lima bean agar	8·5	0·6	6·7	0·6
Casein hydrolysate–sucrose agar	8·6	0·9	6·1	1·0
Casein hydrolysate–xylose agar	6·5	0·6	4·6	0·6

* Each value represents the mean for 26 isolates.

It has been shown above that the northern ecotype is capable of growing at a higher temperature (36°C) than the southern. When tested at 37·5 and 40°C neither ecotype grew, but it was observed that if cultures were incubated at these higher temperatures for several hours and then returned to 25°C, growth would sometimes reoccur after a lag period. This phenomenon was further investigated and the results of one such experiment are shown in Table 4. Short exposures to high temperatures (2 hr at 44°C) had no effect on subsequent growth at 25°C, whereas long exposure (12 hr at 44°C) led to complete cessation of growth. Intermediate exposures to 44°C caused varying lengths of delay before growth occurred at 25°C. While reproducibility of results was poor for single isolates at the intermediate exposure, these general

relationships held in six replicate experiments using 59 separate isolates. Northern and southern ecotypes behaved similarly.

TABLE 4

EFFECT OF EXPOSURE AT 44°C ON SUBSEQUENT GROWTH OF *P. DRECHSLERI* AT 25°C ON CORN MEAL AGAR

Treatment	Isolate type	No. of isolates showing lag (days) of:					
		0	1	2	3	4	>7
2 hr at 44°C	Northern	26	0	0	0	0	0
	Southern	33	0	0	0	0	0
6 hr at 44°C	Northern	18	5	2	1	0	0
	Southern	20	10	2	1	0	0
8 hr at 44°C	Northern	0	5	11	3	1	6
	Southern	1	6	12	5	2	7
12 hr at 44°C	Northern	0	0	0	0	0	26
	Southern	0	0	0	0	0	33

(*b*) *Effect of Initial Medium pH on Growth*

The mean radial growth rates of 10 northern and 10 southern ecotype isolates were not altered by varying the initial pH values of the media from 3 to 9. The results (Fig. 2) indicate that both ecotypes behave similarly in response to pH of the medium, although the northern type exhibited a faster growth rate than the southern at all pH values tested.

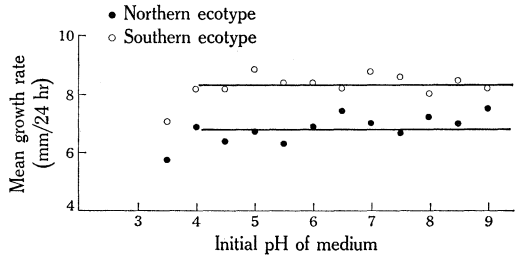

Fig. 2.—Effect of initial pH of basal casein hydrolysate–sucrose medium on growth of *P. drechsleri* ecotypes at 25°C.

(*c*) *Effects of Inhibitors on Growth*

Preliminary tests were made with 26 triarylmethane and other dyes and a range of heavy metal salts in order to examine their effects on the growth of *P. drechsleri* isolates. Compounds differed in their abilities to differentiate between the two ecotypes; pyronin G and copper sulphate gave the best differentiation, while the triarylmethane dyes, including malachite green, showed little or no capacity to differentiate between ecotypes. Further detailed experiments were limited to the use of malachite green, pyronin G, and copper sulphate.

It may be seen from the inhibition curves (Fig. 3) that the northern and southern ecotypes give lines of the same slope, but different intercepts, with a particular inhibitor but that both slopes and intercepts are different with different inhibitors.

When comparisons were made between different *Phytophthora* species in the presence of a single inhibitor, it was found that each species produced a curve with

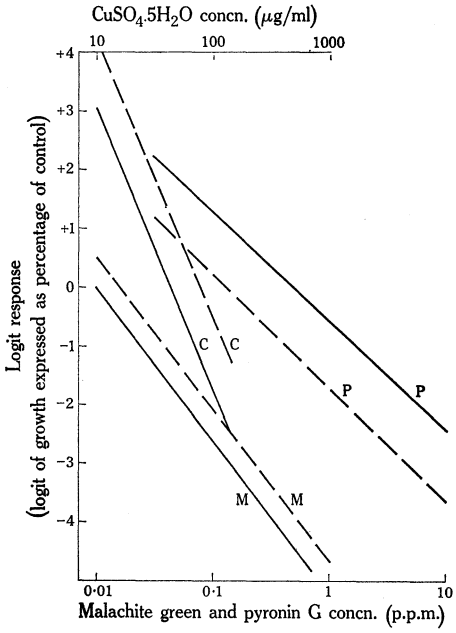

Fig. 3.—Growth responses of northern (——) and southern (– – –) ecotypes of *P. drechsleri* to pyronin G (*P*), copper sulphate (*C*), and malachite green (*M*).

characteristic features of slope and intercept. The responses of nine *Phytophthora* species to copper sulphate concentrations over the range 1·91–33·09 μg/ml Cu^{2+} are given in Table 5.

TABLE 5

GROWTH RESPONSES OF *PHYTOPHTHORA* SPECIES IN THE PRESENCE OF COPPER

Responses are expressed as the logit of the growth as a percentage of the control growth over the range 1·91–33·07 μg/ml Cu^{2+}

Species	Regression coefficient of logit curve	Standard error	Intercept value (logit)	Standard error
P. drechsleri	−0·387	±0·030	+0·359	±0·048
P. cinnamomi	−0·611	±0·043	+0·783	±0·061
P. cambivora	−0·395	±0·053	+0·386	±0·068
P. nicotianae var. *nicotianae*	−0·243	±0·023	+0·579	±0·034
P. megasperma var. *sojae*	−0·703	±0·101	+1·090	±0·154
P. cryptogea	−0·958	±0·182	+1·383	±0·267
P. vignae	−0·485	±0·072	+0·829	±0·113
P. cactorum	−0·851	±0·098	+1·027	±0·131
P. citricola	−0·742	±0·072	+1·122	±0·111

The responses of 10 isolates of each of the northern and southern ecotypes of *P. drechsleri* to various concentrations of copper sulphate are compared in Table 6. When plotted, these results gave curves showing different intercepts, but similar

slopes. The similarity of slope of the inhibition curves found here and given in Figure 3 and comparison with the data of Table 5 provides further evidence that the two ecotypes belong to a single species, *P. drechsleri*.

TABLE 6

INHIBITION OF GROWTH OF *P. DRECHSLERI* ECOTYPES BY COPPER IONS

Values represent mean growth rate expressed as a percentage of the control value in the absence of copper and are the means of 10 isolates of each type

Cu^{2+} concn. (μg/ml)	Northern ecotype		Southern ecotype		Cu^{2+} concn. (μg/ml)	Northern ecotype		Southern ecotype	
	Mean	S.D.	Mean	S.D.		Mean	S.D.	Mean	S.D.
0	100·00	—	100·00	—	14·001	62·88	11·277	83·14	5·498
1·909	99·67	0·869	99·31	1·123	17·820	49·14	12·196	77·84	4·309
3·818	97·81	3·404	97·83	3·243	21·639	36·32	8·889	72·18	6·633
6·364	91·34	5·847	94·39	3·517	25·457	32·27	8·419	62·76	9·925
10·183	78·87	7·348	88·28	5·511	33·094	23·95	5·125	47·05	5·960

Determinations of growth rates of all 70 isolates were made in the presence of 33 μg/ml Cu^{2+}, 1 p.p.m. pyronin G, or 0·05 p.p.m. malachite green. The results clearly indicate the existence of two discrete populations (Fig. 4), with no overlapping

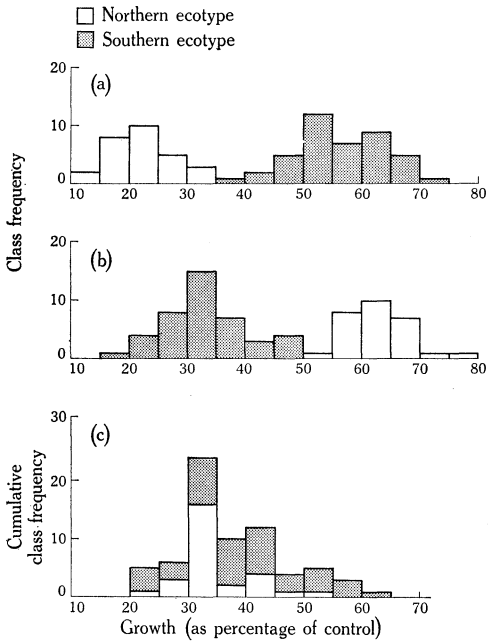

Fig. 4.—Growth responses of 70 isolates of *P. drechsleri* in the presence of 33 μg/ml Cu^{2+} (*a*), 1 p.p.m. pyronin G (*b*), or 0·05 p.p.m. malachite green (*c*).

of responses of the two ecotypes in the cases of copper and pyronin G. The differentiation into northern and southern ecotypes corresponded precisely with that obtained by oospore production following mating with an A1 strain of *P. cinnamomi*. In

contrast, growth responses in the presence of malachite green gave no separation of ecotypes. The application of discriminant analysis to the growth reponse data obtained in the presence of copper and pyronin G confirmed both the presence of two distinct types in the total population and the fact that there was no overlapping of response between ecotypes in the case of these two inhibitors.

In the final series of experiments, two groups of each of the northern and southern ecotypes from different areas in their distributional ranges were compared by five separate tests to investigate the possibility of subgroupings occurring within either ecotype. The results in Table 7 show no indication of such subgroupings.

TABLE 7

COMPARISON OF REGIONAL POPULATIONS OF BOTH ECOTYPES OF *P. DRECHSLERI*

Origin of isolates*	Growth† in the presence of			Growth rate (mm/24 hr) on	
	Cu^{2+} (33 μg/ml)	Pyronin G (1 p.p.m.)	Malachite green (0·05 p.p.m.)	Casein–sucrose	Casein–xylose
Northern ecotype					
Coff's Harbour (9)					
Mean	21·833	60·355	32·100	9·250	6·721
S.D.	3·471	3·874	5·311	0·353	0·367
Tallaganda Forest (9)					
Mean	19·102	65·196	37·089	8·811	6·359
S.D.	7·224	3·883	6·395	0·526	0·787
Southern ecotype					
Jindabyne (7)					
Mean	61·432	37·835	42·043	5·607	4·461
S.D.	4·571	7·605	8·812	0·453	0·382
Victoria–Tasmania (7)					
Mean	54·108	33·370	45·928	6·533	4·800
S.D.	11·513	6·106	9·280	0·653	0·585

* Numbers of isolates are given in parentheses.

† Values indicate growth as a percentage of that occurring in the absence of inhibitor.

IV. DISCUSSION

The only reports available that indicate the existence of separate groups of isolates within the species *P. drechsleri* are those concerning distinct pathogenic races of the fungus, by Thomas and Klisiewicz (1963) and Ershad (1971). This paper, together with that of Pratt and Heather (1973), presents the first demonstration of subspecific groups based on physiological rather than pathogenic criteria. The salient characteristics of the two ecotypes are summarized in Table 8.

Tucker (1931) reported that *P. drechsleri* grew optimally at 32·5°C on corn meal agar. Later authors (Leonian 1934; Tompkins *et al.* 1936; Katsura 1958; Waterhouse 1963, 1970; Roncadori 1965; El Helaly *et al.* 1968; Ershad 1971) have reported temperature optima in the range 25–35°C, with a modal value of approximately 30°C. In the present study, the temperature optima of Australian isolates of the fungus fell within the range of 25–30°C.

While Tompkins *et al.* (1936), Frezzi (1950), Schwinn (1959), and El Helaly *et al.* (1968) report the maximum temperature for growth to be approximately 35°C, Tucker (1931), Leonian (1934), Waterhouse (1963, 1970), and Ershad (1971) state that *P. drechsleri* showed slight growth in the range 37·0–37·5°C. In the present study, most but not all isolates of the northern ecotype grew at 36°C, while no isolate of the southern ecotype grew at a temperature above 33°C; no isolate of either ecotype grew at 37·5°C. Both Tucker (1931) and Roncadori (1965) noted that different isolates agreed closely in their temperature requirements, although relatively few isolates were examined. While the results of the present study give some indication of growth–temperature groupings of isolates (Tables 2 and 3), unequivocal separation of the two ecotypes cannot be made on this basis.

TABLE 8

CHARACTERISTICS OF *P. DRECHSLERI* ECOTYPES

Characteristics	Southern ecotype	Northern ecotype
Oospore production:*		
With A1 mating type of *P. cinnamomi*	+	0
With A2 mating type of *P. cinnamomi*	0	0
With other *P. drechsleri* isolates of either ecotype	0	0
In the presence of *Trichoderma koningii*	+	0
Homothallic oospore production	+	+
Presence of hyphal swellings*	+	+ → 0
Mean growth rates (mm/24 hr) at 25°C on:		
Corn meal agar	8·25±0·61	9·49±0·53
Lima bean agar	6·70±0·59	8·51±0·62
Casein hydrolysate–sucrose agar	6·13±1·00	8·62±0·92
Casein hydrolysate–xylose agar	4·56±0·57	6·52±0·58
Growth, as a percentage of control growth, in the presence of:		
Copper (33 μg/ml Cu^{2+})	56·66±8·55	23·54±4·86
Pyronin G (1 p.p.m.)	33·87±6·58	63·34±5·60
Malachite green (0·05 p.p.m.)	40·01±9·07	34·49±6·71
Upper temperature limit for growth on corn meal agar	33°C	36°C

* Data from Pratt and Heather (1973). + = present; 0 = absent.

Similarly, the two ecotypes are not differentiated by their abilities to withstand periods of high temperature (Table 4). Delay of growth following treatments at high temperature and return to a regime where growth is possible has not been reported previously. Further experiments by us (Shepherd and Pratt, unpublished data) indicate that all *Phytophthora* species tested showed similar behaviour, although considerable differences in their tolerances to high temperatures were observed.

The two ecotypes show similar effects of medium pH on growth rate (Fig. 2) indicating the tolerance of *P. drechsleri* to a wide range of pH values, as previously reported by Tompkins *et al.* (1936).

Three isolates of the fungus examined by Roncadori (1965) showed negligible growth after 14–21 days incubation when xylose was used as the carbon source in a synthetic medium similar to the one used in these studies. However, in our experi-

ments (Table 3) good growth with xylose as the carbon source was obtained with all Australian isolates tested. No explanation can be given to account for the difference in these findings.

Leonian (1930) first described the differential inhibition of growth of *Phytophthora* species by the triarylmethane dye, malachite green, and later (Leonian 1934) used this information to construct a key for the identification of those species. With the exception of Frezzi (1950), few authors have reported the use of Leonian's technique for species identification, perhaps because of the difficulty of obtaining reproducible results, as indicated by Grente (1961), who found that pH and nitrogen level of the medium could considerably influence the results obtained. Grente preferred to test the effects of malachite green on fungal growth in a medium devoid of added nitrogen source, but it is our experience that the greatest reproducibility of results is obtained when a nitrogen source is present. A low pH level was also found to be necessary in order to obtain close reproducibility of results (Shepherd and Pratt, unpublished data) and the probable reasons for this have been indicated by Goldacre and Phillips (1949).

Leonian (1934) and Frezzi (1950) reported that *P. drechsleri* did not grow in the presence of 0·25 p.p.m. malachite green, but did grow in 0·125 p.p.m. of the dye. Our results (Fig. 3) generally confirm these findings, as only 7 out of 70 isolates tested made a trace of growth in 1 p.p.m. of malachite green, while all 70 isolates grew in 0·1 p.p.m. At the intermediate concentration of 0·25 p.p.m. not all isolates grew and the mean growth rate of those that did was only 17·1% of the mean value found in the absence of the dye.

While the results of our experiments with dyes and heavy metals on the growth of *Phytophthora* species will be reported more fully elsewhere, it is apparent from the data (Table 5) that such methods provide a substantial aid for species identification, as suggested by the more limited results of Leonian (1934) and Frezzi (1950).

Morphologically the two ecotypes showed similar colonial appearance and identical branching patterns and hyphal morphology; both showed similar sporangial shape and size. Both also possessed hyphal swellings of the type described by previous authors for *P. drechsleri*, although these were more common in the southern than in the northern ecotype. Isolates of both ecotypes produced a few apparently homothallic oospores on inoculum plugs and oogonia, oospores, and amphigynous antheridia were similar in size, shape, and colour in each ecotype (Pratt and Heather 1973). In addition, the parallelism of the growth response curves in the presence of copper sulphate, pyronin G, and malachite green is considered to support strongly the claim that the two groups of organisms are ecotypes of a single species, *P. drechsleri*.

The area of distribution of the southern ecotype corresponds to the area of Australia enclosed by the average daily maximum temperature isotherm 29·4°C (85°F) (Watt 1941) during the period November to March. While it has been shown (Table 2) that both ecotypes will grow at this temperature, the average daily maximum is indicative of occasional periods of higher temperatures, and it is considered that these are the selective factor influencing ecotype distribution.

It is a safe assumption that growth characteristics in the soil nutrient environment are probably different from those on corn meal agar (Table 2) and thus it is

only tentatively suggested that occasional high temperature periods in soil may be exerting the selective effect that determines the distribution of the two ecotypes. There is no evidence of any selective effect in the lower part of the temperature range tested and no explanation can be given regarding the absence of the northern ecotype from the southern regions of Australia.

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