Progress Report to the Dieback Research Foundation

Research conducted at the Botany School, University of Melbourne

supervised by Gretna Weste

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Progress Report to the Dieback Research Foundation

by Gretna Weste
Botany School,
University of Melbourne

Summary

Research in 1980-81 demonstrated for the first time that Phytophthora cinnamomi can grow and spread through sand, soil or gravel even in the absence of hosts. Chlamydospores form and germinate to produce either zoospores or mycelium and new chlamydospores which may infect adjacent forests from infected roadside dirt or gravel. The fungus can therefore not only survive, but grow and multiply in host-free, forest soils.

Our research demonstrated that P. cinnamomi also forms chlamydospores in cells of infected roots and these survive in dead roots and root pieces. In rainy periods at temperatures between 22° and 35°C the fungus then produces zoospores on root surfaces and these infect other roots.

We have shown that root cells change in three ways during infection (1) cells become leaky, lose nutrients and die. (2) Root respiration increases. The diseased cells cannot function normally and "burn off" excess in respiration. (3) Resistant roots produce callose deposits which wall off infected hyphae and provide a visual expression of resistance which has not been previously reported and will be useful in screening forest eucalypts for resistance.

Infected susceptible eucalypts wilt and die from water stress whereas uninfected controls or resistant species show no change. We have just demonstrated for the first time that wilting occurs because infection immediately and dramatically reduces the transport of water through the whole root system, even though only a few roots are actually infected.

The implications for forest management are:

(1) The over-riding importance of not using infected soil or gravel in areas of healthy forest without treatment.

(2) The importance of removing dead roots when attempting to eliminate early disease.

(3) The use of a callose staining test as a rapid indication of host resistance before reafforestation programmes.

A brief summary of experimental results, their application and of work planned for 1982 are tabulated (Table 1).

TABLE 1. Research into dieback disease 1980-1982

Experimental Results 1980-81	Application of Results	Work planned 1982
Chlamydospore experiments		
Chlamydospores multiply in host-free soil, gravel, producing hyphae, chlamydospores, zoospores	Disease spread independent of hosts	Chlamydospores in disease spread and survival in different soils and moistures
Fungus forms chlamydospores and survives inside seedling roots	Dead roots a source of infection	Periods of survival in older roots at different soil moistures and temperatures
Root cell physiology		
3 changes with infection		
(1) Infected root cells become leaky (damaged membrane)	Nutrient loss causes chlorosis and death Screening for resistance	Are (1), (2), (3) hormonal? Hormone stopage results in chlorosis and root tip death. Hormone application reduces symptoms. Comparison of hormone production (a) with infection (b) with droughting.
(2) Root respiration increases	Diseased root cells can not absorb minerals or use nutrients	
(3) Resistant roots produce callose	Callose walls off infection. Screening test for resistance	
Plant Reaction to Water Stress		
Transpiration, leaf water potential, leaf relative water content & water transport in roots reduced in susceptible, not in resistant species.	Infection prevents water transport. Plants die from water stress under conditions which do not affect resistant species.	Application to jarrah & marri. Hourly monitoring of changes. Comparison between infected and droughted plants.

PLANS FOR 1982

The results of research conducted in 1981 lead to the following investigations:

1. Survival:- The role of chlamydospores - G. Weste

(a) Survival of inside roots

Recent research (1980-1981) investigated chlamydospore survival inside roots of 10-day old seedlings and the experiment only continued 30 days, at which time the fungus remained viable in 100% of the roots buried in soil. In 1982 survival will be tested inside older, larger roots with a suberised exodermis and for a longer period. The plants inoculated will be 3-month old seedlings of Eucalyptus sieberi or marginata (susceptible) and E. maculata or calophylla (tolerant). The effects of burial will be tested in sandy loam from Wilson's Promontory, conducive soil from the Brisbane Ranges, gravel, and suppressive soil from Olinda. Chlamydospore numbers and percentage survival will be examined at 10, 100, 200 and 300 days with replicate soils at three different matric water potentials, -100, -500 and -1000 KPa. Two isolates of P. cinnamomi will be tested, one from the Brisbane Ranges and one from Wilson's Promontory. All experiments will be maintained at 22°C.

(b) Chlamydospore activity in host-free soils

Investigations into chlamydospore activity (1980-1981) were confined to a period of 28 days. Additional experiments are planned to cover a longer period and to compare the effects of burial in conducive with suppressive soils for a range of matric water potentials.

Two isolates of P. cinnamomi will be prepared, one from the Brisbane Ranges and one from Wilson's Promontory. Nylon squares 1 cm^2 and of 35 µm mesh will be inoculated each with 40 fluorescent chlamydospores and buried in sandy loam from Wilson's Promontory, gravel, conducive soil from the Brisbane Ranges, suppressive soil from Olinda at three different matric water potentials, -100, -500 and -1000 KPa. After 10, 50, 100, 200 and 400 days, replicate squares will be examined to determine chlamydospore numbers, percentage germination, colonization and viability.

Progress Report - G. Weste

2. Changes in root physiology

Hormone levels must be measured on intact plants since excision or decapitation immediately alters hormone levels.

Materials	Susceptible	Tolerant
Seedlings of	Eucalyptus marginata	E. calophylla
	E. sieberi	E. maculata

Zoospores of P. cinnamomi

Inoculate root tips with 1-12 zoospores and compare hormone levels with uninoculated controls.

Measure cytokinins
abscisic acid
auxin (IAA)
gibberellic acid
ethylene

Cytokinin measurements

Seedlings to be grown in small rootbox, i.e. on a restricted root regime on scoria/sand mixture and watered with a nutrient solution. Root box to be transferred at 8 weeks to a large container of nutrient solution, resulting in rapid increase in root growth. Roots are inoculated with 10-12 zoospores at root tip. Plants are decapitated 2 days later, root xylem exudate collected and frozen for cytokinin determination by Amaranthus betacyanin bioassay. Cytokinin to be measured before and after infection and compared with similar uninfected controls and with controls with roots removed equivalent in number to those infected.

3. Changes in plant water relations:- - P. Dawson

The measurements - of transpiration, leaf conductance, leaf relative water content, leaf xylem water potential, root conductance, root growth and increase in number of roots - all need to be examined on a smaller scale, at least twice

daily, and hourly in periods of maximum change. Measurements need to be made for a number of species, including jarrah and marri to find whether the results obtained so far hold for other species. The decreased water flow through the roots may be due to factors such as damaged membranes, tyloses or root decay, but the increased resistance to water flow is evident for the whole root system even though only a portion is infected.

Measurements will be conducted to compare droughted with infected plants and also over a range of soil water potentials, since tree death is normally accelerated in zones of water stress or water saturation. We have already obtained basic experimental information on the relation between soil water potential and pathogen population density. We have acquired some irrometers to monitor changes in soil water potential, but will require more equipment from Irrometer Co., Riverside California.

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Dawson, P. and Weste, G. Changes in water relations associated with infection by Phytophthora cinnamomi. Aust. J. Bot.

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PROGRESS REPORT FOR ALCOA DIEBACK RESEARCH FOUNDATION

from G. Weste, School of Botany,
University of Melbourne.

Our application to the Dieback Research Foundation, Alcoa Ltd. cited the following objectives:

1) to define the requirements for survival of the cinnamon fungus P. cinnamomi, in the soil,

2) to investigate the responses of whole plants and plant tissues to invasion by the cinnamon fungus.

Our research has been concentrated in these two fields.

1. Survival

We studied the survival of 2 isolates of P. cinnamomi

a) inside the plant roots

b) in soils in the absence of any plant roots.

a) Inside plant roots

Seedlings of the susceptible blue lupin were inoculated with the cinnamon fungus, and excised roots were buried in non-sterile soil, sand, gravel or glassbeads at 22°C and at 3 different moisture levels. At burial roots contained mycelium, but no spores. After 10, 20, 30 days roots were removed, teased out, tested for viability of the pathogen, and the number of chlamydospores was counted. Data were subject to an analysis of variance. Results are presented in Tables 1 & 2 and demonstrate that larger numbers of resistant spores, chlamydospores, formed in the roots. Spore formation was rapid when roots were buried in glassbeads and gravel and slower with soil, but survival was greater when roots were buried in soil.

Initially no chlamydospores were present in the roots. Table 2 lists the numbers formed in the roots and pathogen viability. After 10-days many chlamydospores had formed in roots buried in coarse media, gravel and sand at all moisture levels. In glass beads large numbers of spores formed at high water

potentials but none in drier conditions. Counts were all low for roots buried in soil. Many competitors were present, particularly in the soil plates and presumably competition and antibiosis resulting from soil inhabiting micro-organisms were limiting pathogen activity in the non-sterile plates.

By 20 days chlamydospore numbers were reduced, particularly in gravel where few of either isolate survived, and in sand where none of isolate 2 survived. By contrast numbers increased in most roots buried in soil and, for the Brisbane Ranges isolate, in sand.

Survival was lower at the highest water potential for isolate 1 possibly because of increase microbial activity. At 30 days counts were further reduced and chlamydospores were confined to roots buried in soil where they survived at all moisture levels. P. cinnamomi remained viable only in soil and in roots buried in soil. Table 2 shows the progressive change over the period 10-30 days in (a) mean number and viability of chlamydospores and (b) in pathogen survival in the medium. Table 3 presents the percentage of inoculated roots in which the pathogen remained viable at 10, 20 and 30 days.

b) Survival in non-sterile soils in the absence of any plants

Suspensions were prepared containing fluorescent chlamydospores of 2 isolates of P. cinnamomi. A drop (0.1 ml) of suspension was placed on 1 cm^2 of nylon mesh (35 µm) and buried in soil, sand, gravel, or glassbeads at 22°C and at 3 different matric water potentials. After 10, 20 and 28 days the mesh squares were removed, the numbers of chlamydospores and their activities were assessed using fluorescence microscopy.

The initial number of chlamydospores deposited on 1 cm^2 of nylon mesh was 38. By 10 days the number had increased in most media and by a factor greater than 10 in some instances for example 430 and 472 were counted in gravel and soil respectively. Mean values are presented in Table 4. Most of the chlamydospores had germinated and produced a branched mycelium which covered 20-70% of each nylon mesh square. New chlamydospores were forming. At 10 days there was considerable variation in spore germination and growth (Table 5). At 20 days numbers of chlamydospores remained high, spore germination was nearly complete and 'ghost' or empty chlamydospores fluoresced on the nylon mesh. The fungus had colonized 25-100% of each mesh square and produced many new chlamydospores. By 28 days the number of 'ghosts' had increased and most retained

residual fluorescence. Numbers of chlamydospores with contents had declined on mesh buried in all media except soil (Table 4). In soil chlamydospore numbers continued to increase 28 days after burial. Colonization of the mesh was complete (Table 5) and the mycelium was in short lengths. All media at the highest moisture level were heavily contaminated with bacteria, yeasts, algae, protozoa and other fungi. In these experiments all inoculated chlamydospores germinated to produce mycelium and more chlamydospores, but in a test at a higher moisture level chlamydospores germinated to produce sporangia. Viability of the new chlamydospores at 10 and 20 days was demonstrated.

Mesh squares buried in each medium carried viable chlamydospores at 10 days, except for gravel at the lowest water potential. By 28 days viable chlamydospores were only found on mesh buried in glass beads at water potentials of -110 and -100 KPa and in soil at all water potentials. Three of the media contained viable fungus at various water potentials (Table 4) but P. cinnamomi did not survive 28 days in soil except when growing in the mesh.

Data were subject to analyses of variance using a square root transformation of chlamydospore counts. Chlamydospore numbers of nylon mesh varied with the isolate, with the media in which the mesh was buried, with its moisture level and over the period of time from 10 to 28 days. The highest mean count for the whole experiment was obtained from isolate 2 on mesh buried 10 days in soil at the medium moisture level. The highest mean count for 20 days was obtained from the same isolate under the same conditions. At 28 days the maximum mean was recorded for the same isolate in soil at the highest moisture level.

Factorial analysis indicated that isolate 1 from the drier Brisbane Ranges formed more chlamydospores under dry conditions but that chlamydospores of isolate 2 from Wilson's Promontroy have a greater capacity for survival.

Chlamydospores inoculated onto mesh in host-free, non-sterile media germinated, colonized the mesh and reproduced, forming new viable chlamydospores thus demonstrating some degree of competitive saprophytic ability. Chlamydospore germination varied with water potential from 50-90% at the minimum to 100% at the maximum (Table 5). Colonization ranged from 20-70% of the mesh at 10 days to 100% at 28 days and provides evidence of activities greater than those necessitated by survival in a hostile environment.

Conclusions

The results provide information on the behaviour and survival of P. cinnamomi either in root tissue buried in non-sterile media or as chlamydospores in host-free, non-sterile media at 22°C and at different water potentials.

The results obtained were unexpected and indicate the requirements for further experiments. These are planned using older roots, higher but equivalent matric water potentials and longer survival periods. Differences between the media are based on particle size and organic content, hence microbial populations and would repay examination. However the experiments have revealed chlamydospore survival and unexpected colonization of media in the absence of a host so that the concept of a chlamydospore of P. cinnamomi as a resistant spore which functions only as a static survival unit is insufficient and likely to lead to errors in disease management and control. In fact the role of the chlamydospore is dynamic with a saprobic life cycle. The spore can germinate and colonize media such as gravel, sand and soil independently of a host and in limited short term competition with other soil organisms. As a result of chlamydospore activity P. cinnamomi can increase in population density and in distribution in the absence of a host and this provides a competitive advantage unusual in a pathogen. For effective growth and reproduction chlamydospores require a medium with an organic content greater than 1.2% by weight and sufficient moisture. Chlamydospores can germinate to form either sporgania or mycelium and more chlamydospores depending on nutrient level or on moisture levels. The saprobic ability and the alternative germination procedure provide the pathogen with an adaptable mechanism for bridging space and time between hosts or for example between infested roadside gravel and adjacent forests.

Table 2. Chlamydospore formation and viability in inoculated roots.

+ = viable - = non-viable

Medium	ψ -KPa	Chlamydospore numbers & viability 10 days	viab.	20 days	viab.	30 days	viab.	Viability in medium (30 days)
Glass Beads	140	0	+	30 *	+	0	-	-
	110	161	+	35	-	3	-	-
	100	514	+	281	+	5	-	-
Gravel	90	360	+	0	-	0	-	-
	70	485	+	4	-	0	-	-
	60	393	+	0	-	3	-	-
Sand	70	55	+	0	-	0	-	-
	70	150	+	185	+	0	-	-
	70	87	+	264	+	0	-	-
Soil	3260	1	+	26	+	36	+	+
	1960	4	+	136	+	41	+	+
	690	10	+	30	-	45	+	+

* Each result is the mean of 6 replicates, 3 from each isolate.

Table 3. Survival of P. cinnamomi in seedling roots.

Medium (Non-sterile)	Percentage Survival after 10 days	20 days	30 days
Glass Beads	100	50	0
Gravel	100	0	0
Sand	100	33	0
Soil	100	100	100

Table 4. Chlamydospore numbers and pathogen viability when inoculated onto mesh and buried in non-sterile, host-free media.

Medium	ψ -KPa	Spore Number 10 days	20 days	28 days	Viability at 28 days In mesh	In medium
glass beads	140	218*	162	127	x	x
	110	93	162	104	✓	✓
	100	144	144	87	✓	✓
gravel	90	118	119	95	x	✓
	70	294	90	62	x	✓
	60	173	140	87	x	x
sand	70	46	57	69	x	✓
	70	137	79	63	x	✓
	70	75	55	78	x	x
soil	3260	102	173	207	✓	x
	1960	328	142	201	✓	x
	690	287	118	249	✓	x

* Each result is the mean of 6 counts comprising 3 replicates from each of 2 isolates.

Table 5. Chlamydospore behaviour on nylon mesh.

Media	ψ -KPa	Spore germination % 10 days	20 days	28 days	% mesh covered 10 days	20 days	28 days
glass	140	70	85	80	20	25-30	70
beads	110	70	95	80	20	25-30	70
	100	60-70	100	90	10	40	85
gravel	90	70	90	60-70	60	60-70	40
	70	80	100	80	70	80	50
	60	60	100	90	50	70	70
sand	70	50	80	80	30	80	20
	70	100	80	80	60	80	30
	70	50	100	100	30	100	50
soil	3260	75	50	70	40	70	90
	1960	100	100	70	50	70	100
	690	90	100	70	60	70	100

2. Responses of whole plants and plant tissues to invasion by *P. cinnamomi*

a) Changes in root tissue physiology and histology with infection.

Seedling roots of a number of native species were inoculated with 10-20 zoospores and the changes observed in association with increasing fungal penetration.

This work is partly funded by the grant from the Alcoa Dieback Fund and results are presented in the accompanying report by the recipient, David Cahill.

CHANGES IN ROOT PHYSIOLOGY ASSOCIATED WITH INFECTION BY *PHYTOPHTHORA CINNAMOMI*

by David Cahill, Botany School, University of Melbourne.

Studies of host-pathogen relations have demonstrated that the roots of all species examined both attract and are penetrated by zoospores, whether they are susceptible, tolerant or resistant.

Penetration and initial development of a pathogen in resistant and susceptible tissue appears nearly identical but both physiological and biochemical changes immediately following successful penetration may play a significant role in determining the plant's final reaction.

Little is known of the physiological responses of plant roots to the invasion of *Phytophthora cinnamomi*, particularly the mechanisms by which some roots can tolerate infection.

Three aspects of the post penetration host-pathogen interaction are currently under investigation. A range of host species including susceptible, tolerant and resistant species are being tested. Firstly root cell leakage associated with membrane damage and general root necrosis. Often the first detectable indication of damage to host tissues after infection is increased leakage of electrolytes from host cells Changes in the conductivity of a root bathing solution has been used as a sensitive assay for determining root cell damage, and hence is related to susceptibility and resistance.

Respiratory changes following infection are also being studied. Increased respiratory activity of root tissues after fungal infections is well known. Compatible and incompatible combinations of host and pathogen can often be characterized by their respiratory patterns.

Thirdly, a microscopical examination of the development of the pathogen within a range of host root tissues. Physiological changes must be related back to extent of root invasion, since this can differ markedly from species to species. Production of callose within root cells in response to infection also differs between species and may be related to a plant's resistance.

A time sequence of these physiological changes in host roots as a response to infection by P. cinnamomi have been recorded up to twenty-four hours.

Species examined:

Susceptible	Tolerant or Resistant
Xanthorrhoea australis	Acacia pulchella (hypersensitive)
X. resinosa	A. melanoxylon (possibly hypersensitive)
Themeda australis	Gahnia radula
Eucalyptus sieberi	Eucalyptus maculata
E. marginata	E. calophylla
	Juncus bufonius
	Zea mays (corn)
	Triticum sp. (wheat)

1. ROOT MEMBRANE DAMAGE/CELL LEAKAGE

Method: (a) Inoculate young roots each with 10-20 zoospores.

(b) Leave for 2, 8, 16 and 24 hours (plus uninoculated controls).

(c) Cut roots off and place in glass distilled water and measure the conductivity over 4 hours.

Cell contents may rapidly leak in some species - as early as 2 hours. Others may take up to 24 hours before significant leakage occurs.

Leakage responses:

Susceptible

	Time for significant leakage (hrs)
X. australis	16 hr
X. resinosa	16 hr
T. australis	8 hr
E. sieberi	16 hr
E. marginata	8 hr

Resistant and Tolerant

	Time for significant leakage (hrs)
Acacia pulchella	2 hr
A. melanoxylon	2 hr
G. radula	not tested
E. maculata	8 hr
E. calophylla	2 hr
J. bufonius	> 24 hr
Z. mays	> 24 hr
Triticum sp.	> 24 hr

Amount of leakage (μmhos/gram fresh weight) varies considerably between species, hypersensitive plants tend to release cellular electrolytes in greater quantity than susceptible species.

Degree of penetration by P. cinnamomi hyphae within the root can be correlated with amount of leakage.

Culture filtrates:

Preliminary work with a low molecular weight culture filtrate derived from P. cinnamomi hyphae has indicated that there may be a certain amount of leakage due to fungal products acting directly on root cells. The same conductivity assay has been used.

Leakage and a non-pathogen:

The presence of microorganisms around a growing root stimulates greater root exudation. To test if the observed leakage associated with P. cinnamomi was due to the presence of the fungus and not to its action directly on root cells a non-pathogen Mortierella ramaniana was inoculated onto host roots. Leakage was not recorded when compared to controls up to 48 hours after inoculation.

Conclusions:

1. All species roots became leaky after invasion by P. cinnamomi.
2. Time of appearance of significant leakage and amount of leakage differs between species and may be related to resistance.
3. Leakage response can be correlated with degree of pathogen penetration.

4. Basically three types of leakage response occur:
   (i) Susceptible plants - leakage after 8-16 hrs
   (ii) Tolerant/Resistant plants - leakage after 24 hrs
   (iii) Hypersensitive - massive leakage detected after 24 hrs.
5. The conductivity assay may be valuable in screening plants for resistance.

2. RESPIRATION

Method: (a) Inoculate young roots with 10-20 zoospores of each.
(b) Leave for 2, 8, 16 and 24 hours.
(c) Cut roots off and place in oxygen electrode chamber and run a set of controls.
(d) Rate of respiration can be determined.

Respiratory responses:

Two week old seedlings were used in these experiments and results are those taken at 24 hours after inoculation.

SPECIES	RESPIRATION AS A PERCENTAGE OF CONTROLS
E. sieberi	135
E. maculata	98
E. calophylla	179
A. melanoxylon	258
A. pulchella	131
X. australis	187
E. marginata	164

Although there is a general respiratory rise for most species there does not seem to be any relation between respiratory changes and susceptibility.

Respiratory changes along the root can be detected as infection spreads and is directly correlated with the proportion of root which is necrotic.

Conclusions:

1. There is apparently no relation between respiratory response i.e. increased respiration and a plant's susceptibility or resistance.
2. The pattern of respiratory change may be important.
3. The increase may be due to optimal supply of respiratory substrate (photosynthate) which the infected root is unable to use. Alternative respiratory pathways may be invoked.

3. MICROSCOPY

Any observed physiological changes must be related back to pathogen development within the host roots.

Roots of all species used both attract and are penetrated by *P. cinnamomi* zoospores. A time course study of post penetration development up till 24 hours after infection has been employed.

The hyphae appear to grow equally well in most species with only subtle differences between them. The vascular tissue

is invaded quite quickly and may occur as early as 8 hours after infection for some species.

Growth of hyphae may be considerably slowed down or even stopped in some resistant species for example Eucalyptus calophylla, E. maculata, Zea mays and Triticum sp. Acacia pulchella is known to be hypersensitive and rapid cellular necrosis and hyphal lysis has been observed, although the pathogen may still continue to invade the host.

Production of callose by invaded cells in response to hyphal penetration may impede growth as is the case in several other pathogen-host combinations.

Callose production: - rescorcinol blue or analine blue fluorescence detection.

	Callose detected		Callose detected
X. australis	-ve	A. pulchella	+ve
X. resinosa	-ve	A. melanoxylon	+ve
E. sieberi	-ve	E. maculata	-ve
E. marginata	-ve	E. calophylla	+ve
T. australis	-ve	G. radula	+ve
		Zea mays	+ve
		Triticum aestivum	+ve
		J. bufonius	+ve

It appears that callose production is in some way connected to resistance perhaps by stopping or restricting hyphal growth.

Conclusions:

1. Hyphae of P. cinnamomi initially develop well in all species except for A. pulchella.
2. Vascular tissue is rapidly invaded but there is no evidence of vessel blockage.
3. Hyphal growth may be drastically impeded in some species.
4. Callose production may be a resistance factor, and is not found in susceptible species but is found in most resistant species.

4. FUTURE AND CONTINUING LINES OF INVESTIGATION

(1) As most of this work has used young seedlings less then four weeks old it is important to determine whether older plants (6-12 months) will behave in a similar manner.

(2) Histochemical tests may provide a useful insight into the way in which cells are changed within roots after infection. A more detailed study of the timecourse of callose production is needed.

(3) Investigation of the nature of the respiratory rise observed with infected tissue.

(4) Hormonal changes after infection and relationships to the other physiological changes studies. Are there hormonal imbalances or excesses and which hormones are involved. Are hormones involved in leakage, respiratory rises and callose production etc.

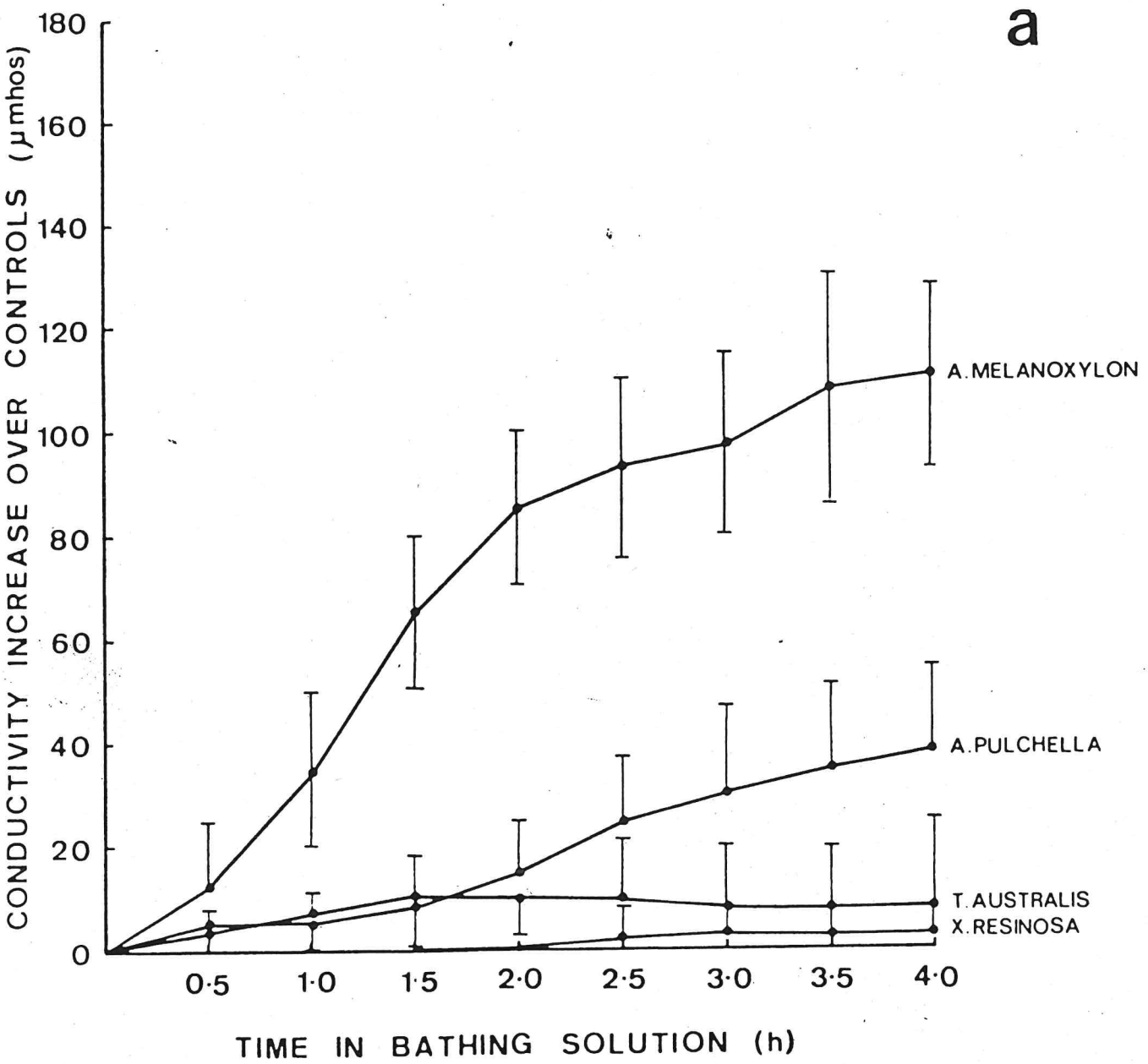
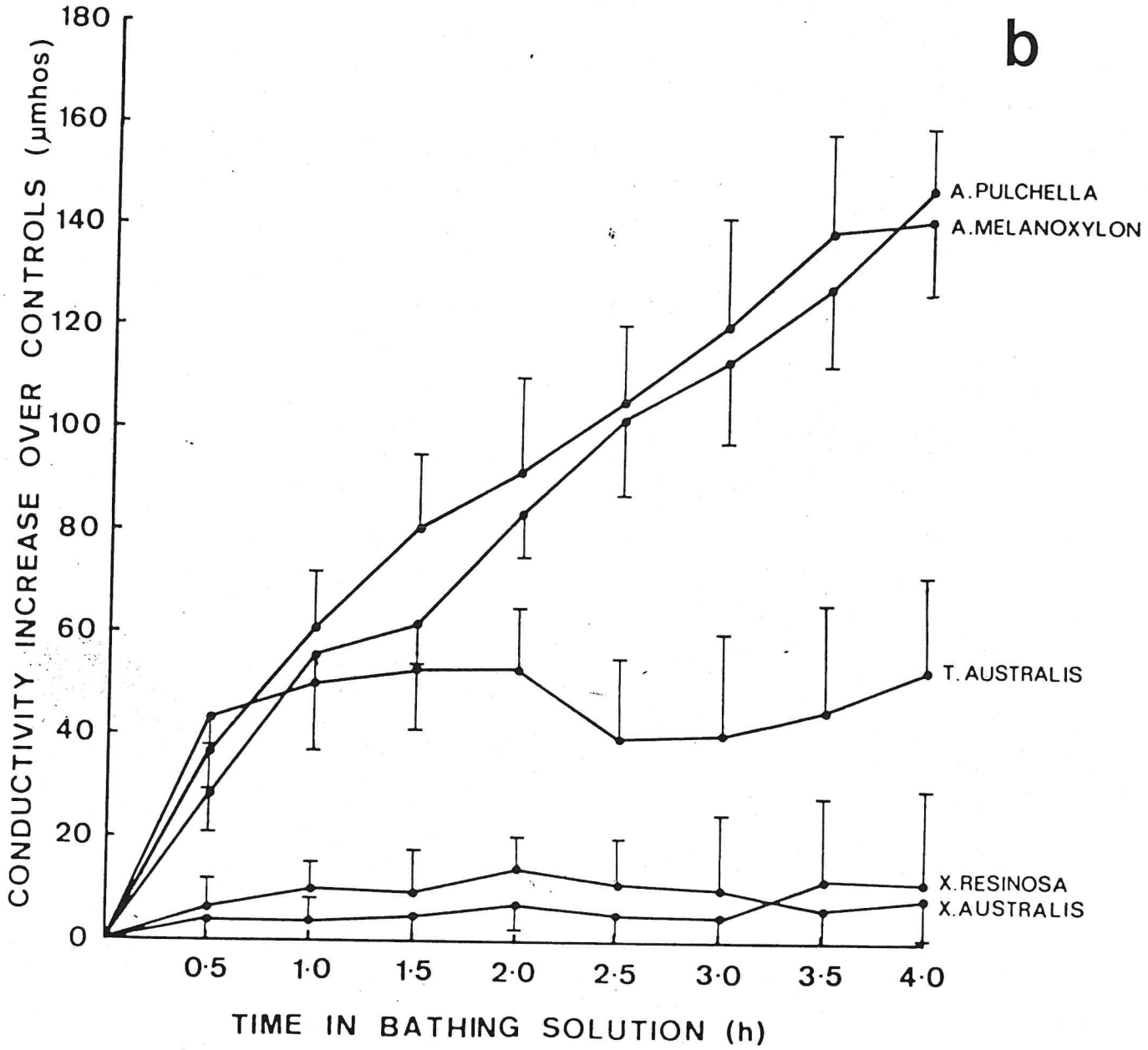


FIG. 1 (A) CHANGES IN THE CONDUCTIVITY OF THE ROOT BATHING SOLUTION TWO HOURS AFTER INOCULATION AND

(B) SIXTEEN HOURS AFTER INOCULATION.



(b) Sixteen hours after inoculation.

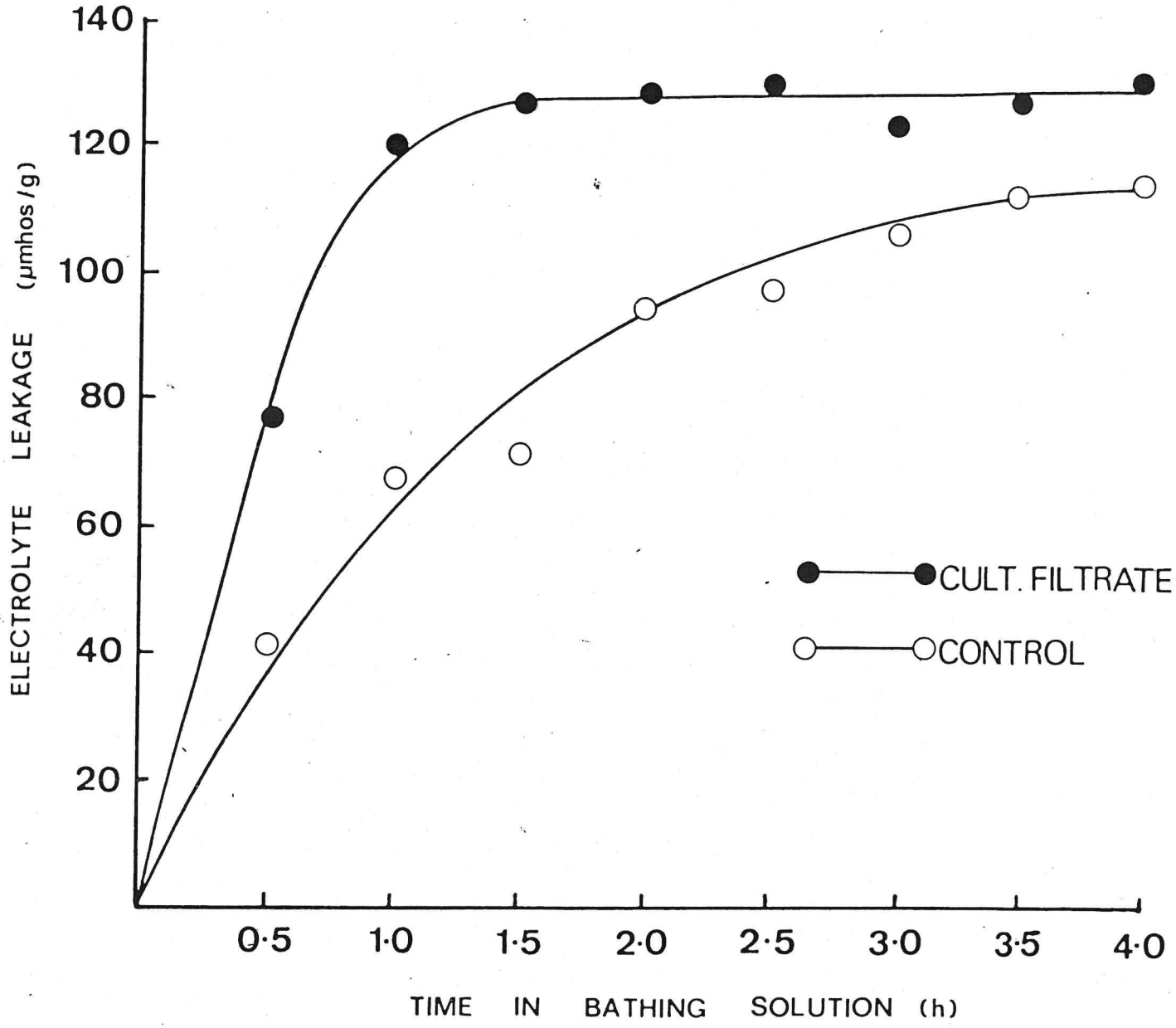
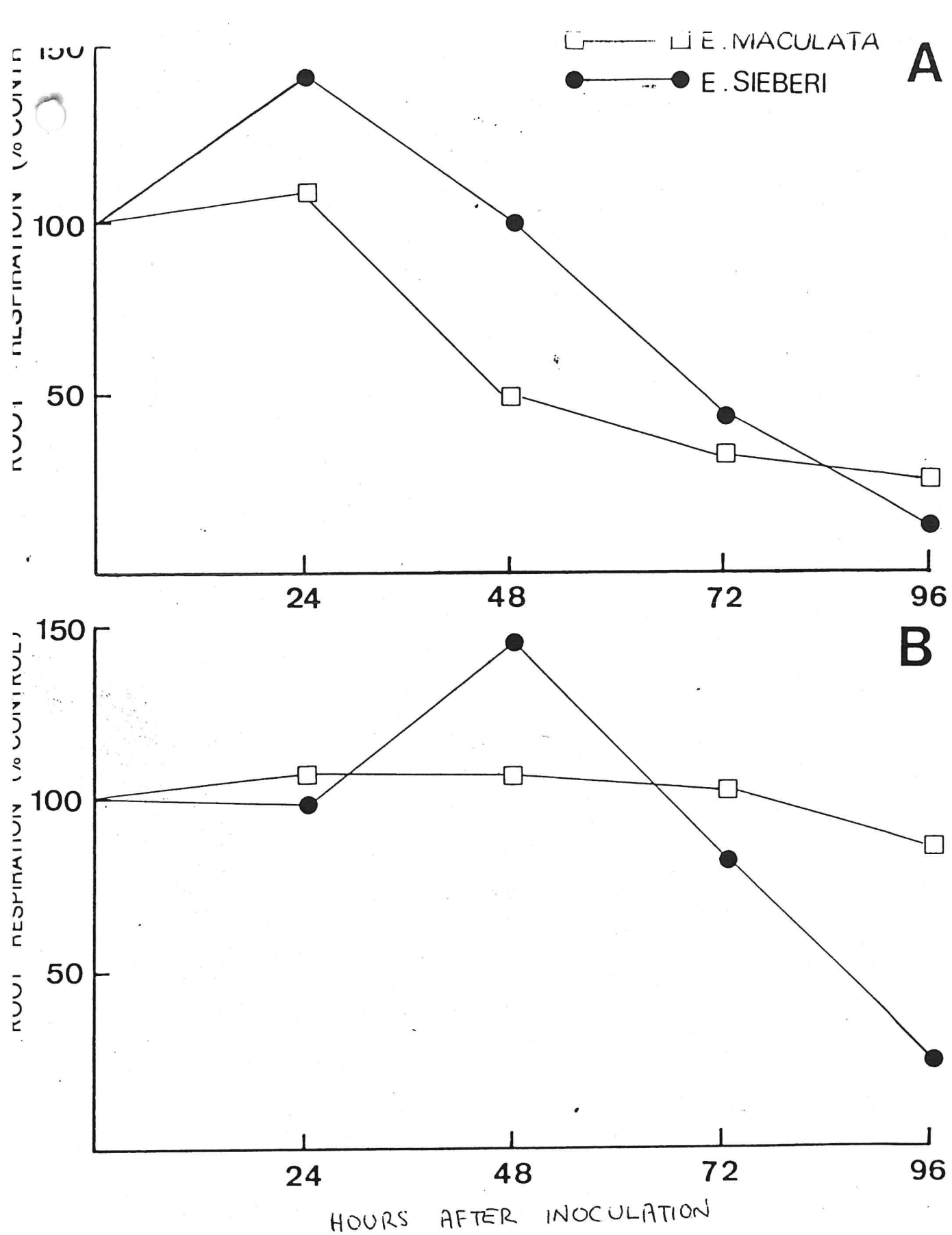


FIG. 2 CULTURE FILTRATES DERIVED FROM *P. CINNAMOMI* CAN ALSO CAUSE LEAKAGE OF ELECTROLYTES FROM HOST ROOTS. THE ABOVE GRAPH SHOWS THE EFFECTS OF CULTURE FILTRATES ON *XANTHORRHOEA AUSTRALIS* ROOTS.


E. MACULATA
E. SIEBERI
A
B
150
100
50
24
48
72
96
HOURS AFTER INOCULATION

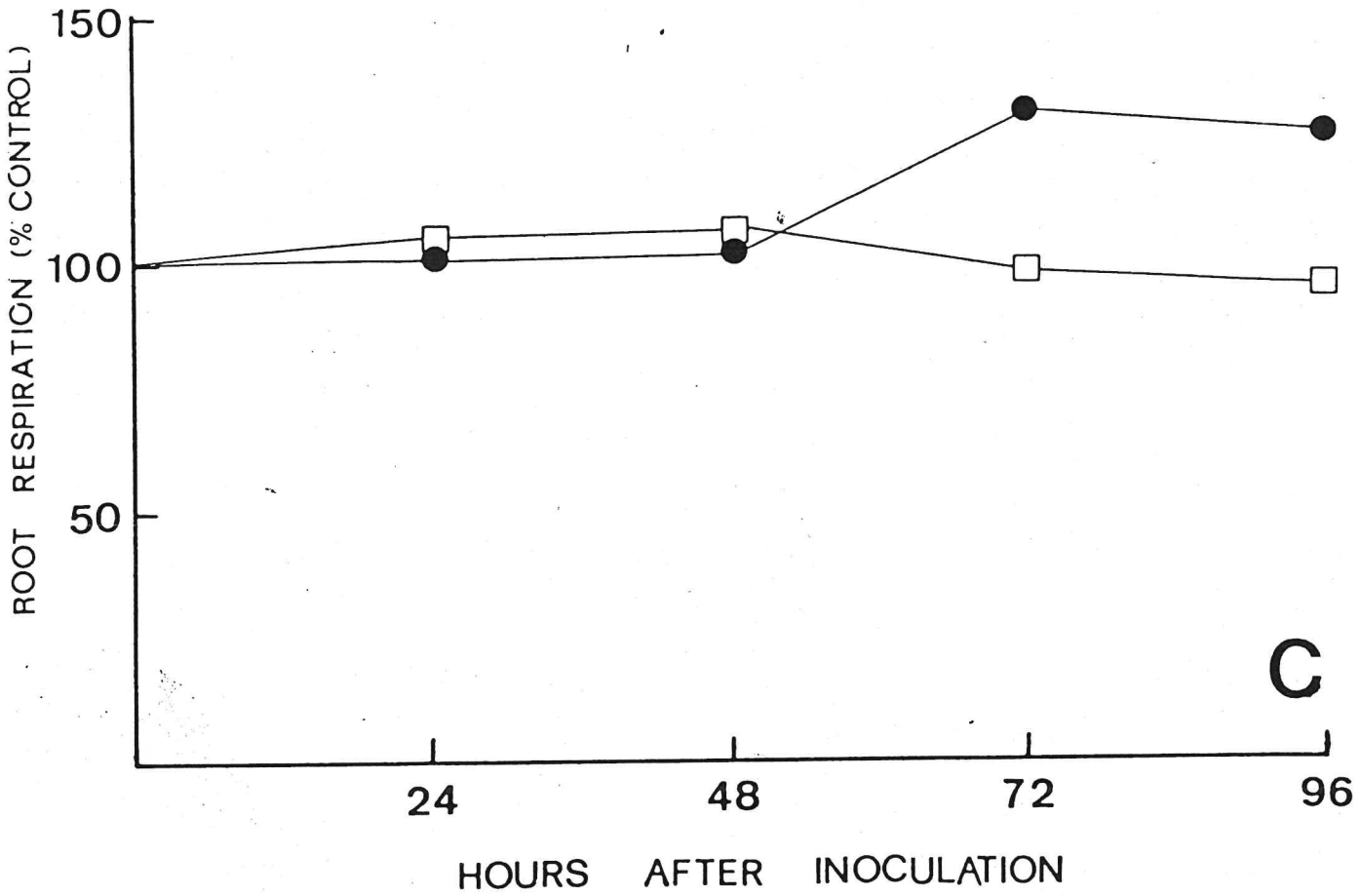


FIGURE 3. THE RESPIRATION OF ROOT SEGMENTS OF E. SIEBERI (SUSCEPTIBLE) AND E. MACULATA (TOLERANT) FROM 0 TO 96 HOURS AFTER INFECTION. RESULTS ARE EXPRESSED AS A PERCENTAGE OF THE RESPIRATION OF UNINFECTED CONTROL ROOT SEGMENTS.

THE GRAPHS SHOW THE RESPIRATION OF SEGMENTS FROM

A) THE LOWER 10MM OF ROOT.
B) 10-30MM ABOVE THE ROOT TIP.
C) 30-50MM ABOVE THE ROOT TIP.

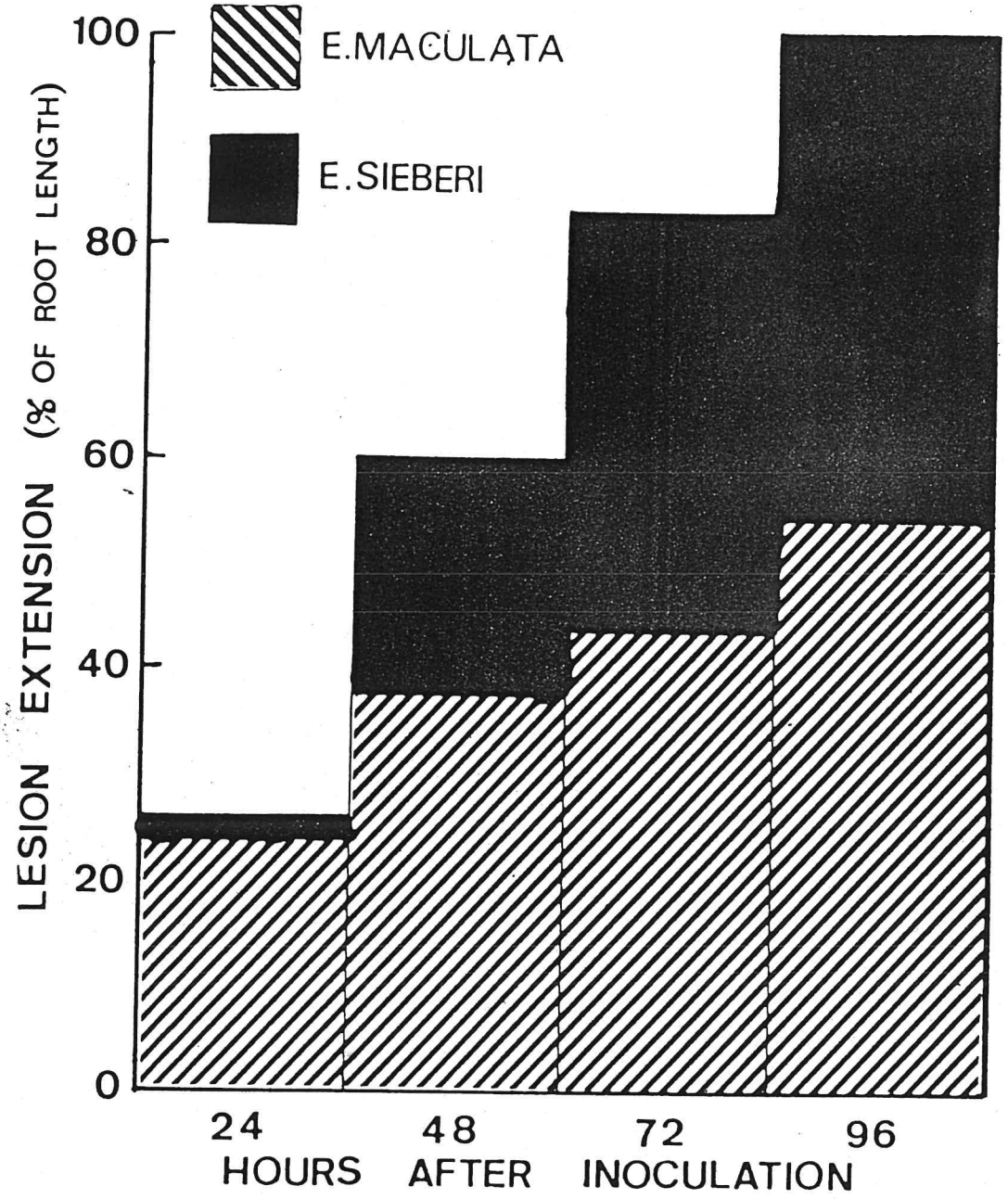


FIG. 4 LESION EXTENSION CAN BE CORRELATED WITH THE RESPIRATORY CHANGES OBSERVED IN THE TWO SPECIES.

Changes in water relations associated with infection by *Phytophthora cinnamomi*

by Peter Dawson and Gretna Weste, Botany School,
University of Melbourne

Introduction

Plants infected by *Phytophthora cinnamomi* show symptoms of water stress. Wilting has been observed in diseased forest trees (Weste and Taylor 1971; Marks *et al.* 1972) and several physiological studies have shown that the water relations of diseased plants are abnormal. De Roo (1969) observed that *Rhododendron catawbiense* infected with *P. cinnamomi* had low xylem water potentials similar to those of droughted plants. Infected, irrigated avocado trees behaved as if severely water stressed. Leaf conductance of water vapour, transpiration and leaf xylem water potential were altered by infection (Sterne *et al.* 1978). We have shown that infected *Isopogon ceratophyllus* (a proteaceous shrub) is severely water stressed too, even though well watered and grown in a glasshouse. Evapotranspiration was reduced and this was followed by a marked reduction of leaf water potential (Dawson and Weste) In this same study *Eucalyptus macrorhyncha* trees in infected forest appeared to suffer water stress in a dry autumn although these trees subsequently recovered from the stress.

The work reported here arose from the experience gained from this first study where we found:

(1) variability in measurements from forest trees was great and may hide effects due to the pathogen.

(2) the disease is hard to induce in plants grown under optimum conditions of the glasshouse

(3) the obvious importance of the root system as the site of host reaction and pathogenic action and hence the need to measure changes in the roots, not just the above ground parts of the plant.

We now have a system whereby plants are grown in a constant environment chamber, to reduce variability of measurements, and where susceptible plants will die after infection and where the plant's roots may be more easily studied.

Methods

(1) Plant material

a) Hosts. *Eucalyptus sieberi* (highly susceptible) and *E. maculata* (tolerant) were used. Both have broad leaves suitable for use in the porometer and petioles that allow use of the pressure bomb. The plants were grown in perspex-fronted pots to allow root growth to be seen. A section of the perspex is removable to allow inoculum to be placed directly on the underlying roots with little disturbance to the plant. The pots are water-tight so evapotranspiration can be measured. To allow easy access to the roots, the plants were grown in sand and fertilized with ¼ Hoagland's solution.

b) Pathogen. P. cinnamomi zoospores were prepared (Byrt and Grant, 1979) as inoculum and the plants were inoculated as per Byrt and Holland (1978).

The plants were grown in 14 hours of 2/3 sunlight at 23°C and night temperature was 19°C. Seedlings were initially raised in a glasshouse and transferred to the environment chamber two weeks before inoculation.

(2) Measurements

a) Evapotranspiration was determined by weighing the pots daily.

b) Leaf conductance was determined by a water vapour diffusion porometer.

c) Leaf water potential was determined using a pressure bomb.

d) Relative water content was determined by the method of Barrs and Weatherley (1962) which involved weighing fresh plant tissue and then placing the tissue in water to see how much water can be absorbed. This measure gives an idea of a plant's water deficit.

e) Root conductance was measured by using a pressure bomb modified in the School of Botany to allow water to be forced under pressure through the root system in a manner similar to that of Duniway (1977).

f) Root length was measured using a line intersection method.

Results

Results of evapotranspiration, leaf water potential, leaf conductance and leaf relative water content are shown in Figs. 1-2. Root conductance is shown in Fig. 3. In *E. sieberi* all these measurements were reduced in infected plants when compared with controls. Infected *E. maculata* plants behaved as their controls. Length of white root did not vary greatly between infected and control plants of either species. (Fig. 4).

Discussion

E. sieberi shows water stress after infection by *P. cinnamomi*. Root hydraulic conductance is most noticeably impaired after infection and all the root system appears to be affected although most of the roots seem to be uninfected. Because the roots have been measured when the host first shows signs of stomatal closure the pathogen's effect on the roots must be well advanced. Hence the pathogen must damage the root system when there is even less spread of infection than we have detected. The reduced flow of water through the roots may be due to a general loss of permeability or hydraulic conductance caused by a host response to invasion. Blockage of vascular tissue by tyloses or fungal products could occur but these results tend to discount the idea that the disease is due to the pathogen rotting the root system leaving insufficent roots to support the plant.

Future work

These results are extremely important as they show that infection prevents water transport within the root system. Obviously these findings merit further examination. Information is needed on a smaller time scale, for example twice daily measurements, and even hourly at times of maximum transport reduction. This would allow us to determine how soon after infection the root system begins to be impaired and how much infection is required to affect the water balance of the entire plant. More information about the rate of infection would also allow us to plan gas analysis experiments to more accurately monitor changes in host physiology upon infection. It is proposed to continue the work in this fashion as our apparatus and procedures have proved so successful. It is also proposed to extend this work by testing other species, e.g. jarrah and marri, to see how general the response is in hosts. A comparison of droughted hosts and infection water-stressed plants is also planned to see whether the plants undergo similar physiological changes. A proposed study program in given in Table 1.

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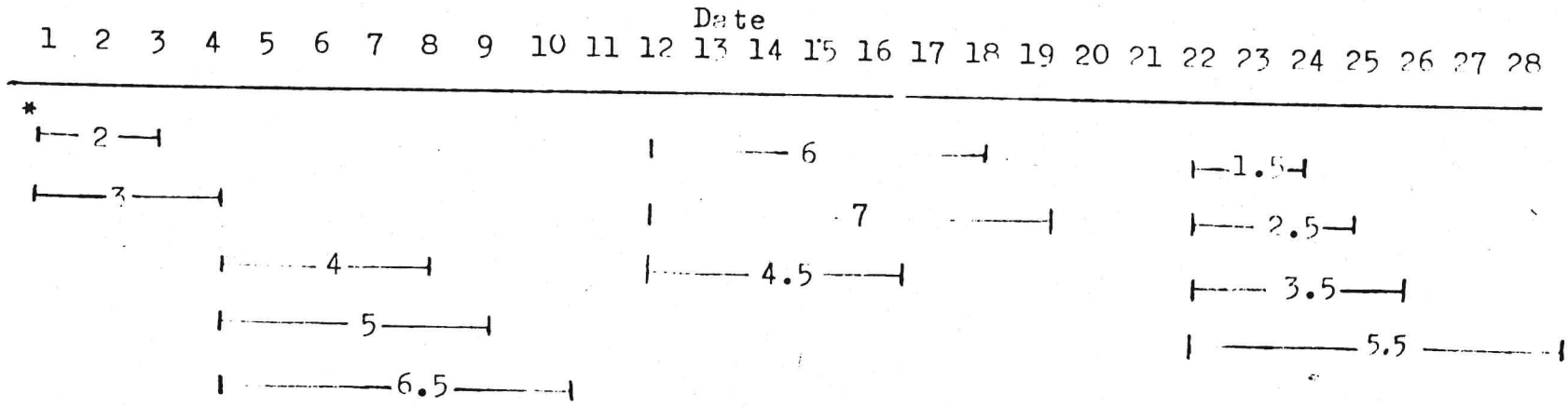
Table 1. Proposed program of study in 1982 on the effects of *Phytophtora cinnamomi* on the water relations of hosts.

Month	Species	Measurements	Aim and methods
Feb.	*E. sieberi*	Evapotranspiration Leaf conductance Leaf water potential Leaf R.W.C. Root conductance Root length Root number	To measure changes in the hosts' water relations at ½ daily intervals. - known provenance seed to be used to reduce variation. - plant growth and infection to be staggered to allow time to complete measurements - see plan.
	E. marginata *E. cal ophylla*	Evapotranspiration	Pilot experiment to determine length of time needed for symptoms to show in our system.
March	*E. maculata*	------- as for *E. sieberi* in February ----------	
April	*E. sieberi* *E. maculata*)	Anatomical study	To determine the extent and manner of tissue damage caused by the fungus.

Table 1. continued.

Month	Species	Measurements	Aim and methods
May	*E. marginata*	---------- as for *E. sieberi* in February ----------	
June	*E. cal ophylla*	---------- as for *E. sieberi* in February ----------	
July	*E. marginata* *E. cal ophylla*	) Anatomical study	To determine the extent and manner of tissue dammage caused by the fungus.
August	*E. sieberi*	-- as in February --	To compare drought stress (water witheld from plants) with stress due to infection to see whether the same physiological changes occur.
September		Gas analysis of water relations parameters.	To obtain data on physiolog ical changes on a continuous basis at times of greatest pathogen effect as determined from experi- ments completed from Feb. to June.

Plan showing staggered infection times of plants to enable ½ daily measurements to be taken.



* bars represent length of infection of plant . Half daily measurements from 1½ to 7 days can thus be achieved without having too many plants to measure at once.

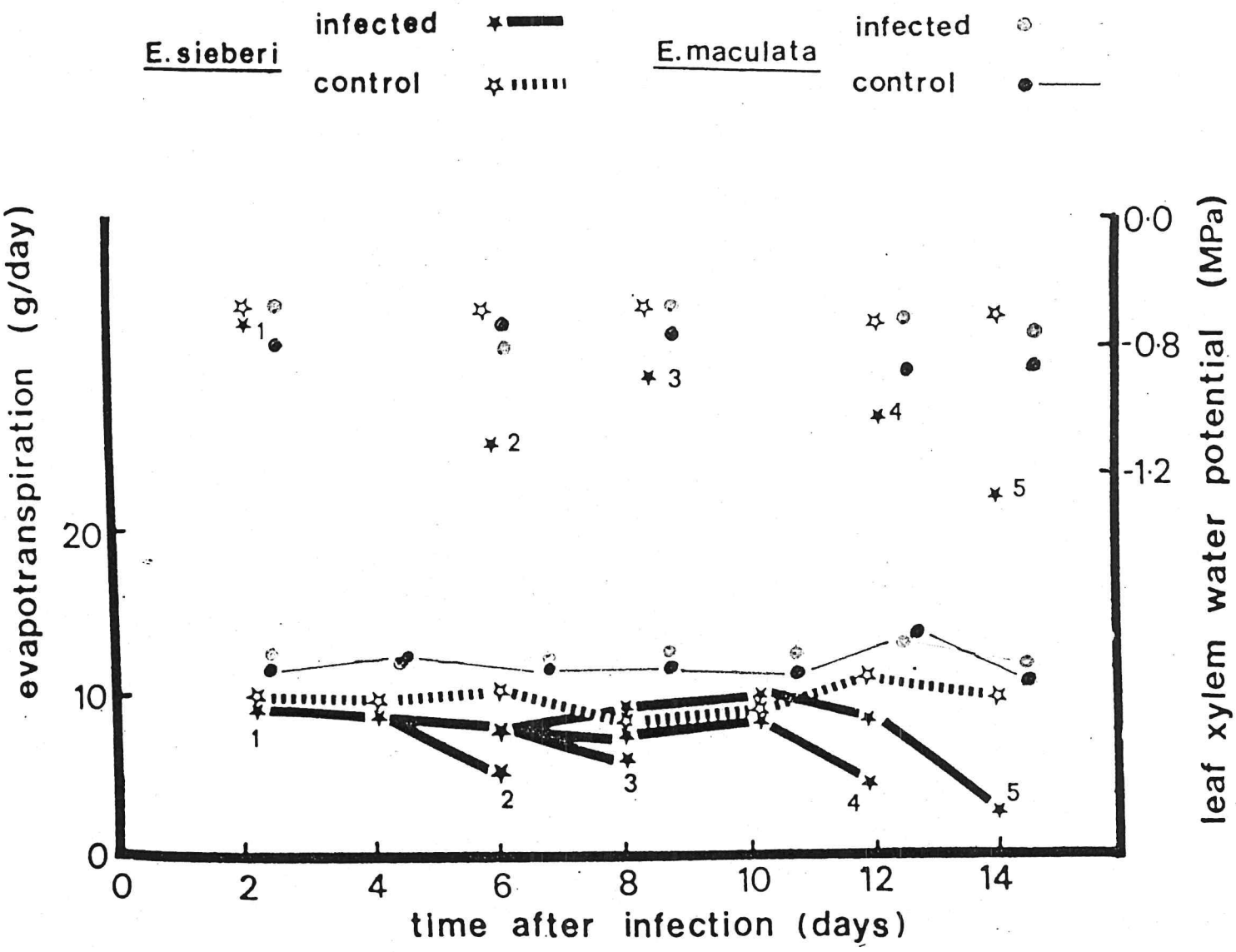


Figure 1. Evapotranspiration and leaf xylem water potential of Eucalyptus sieberi and E. maculata infected with Phytophthora cinnamomi. Numbers 1-5 represent single plants of E. sieberi.

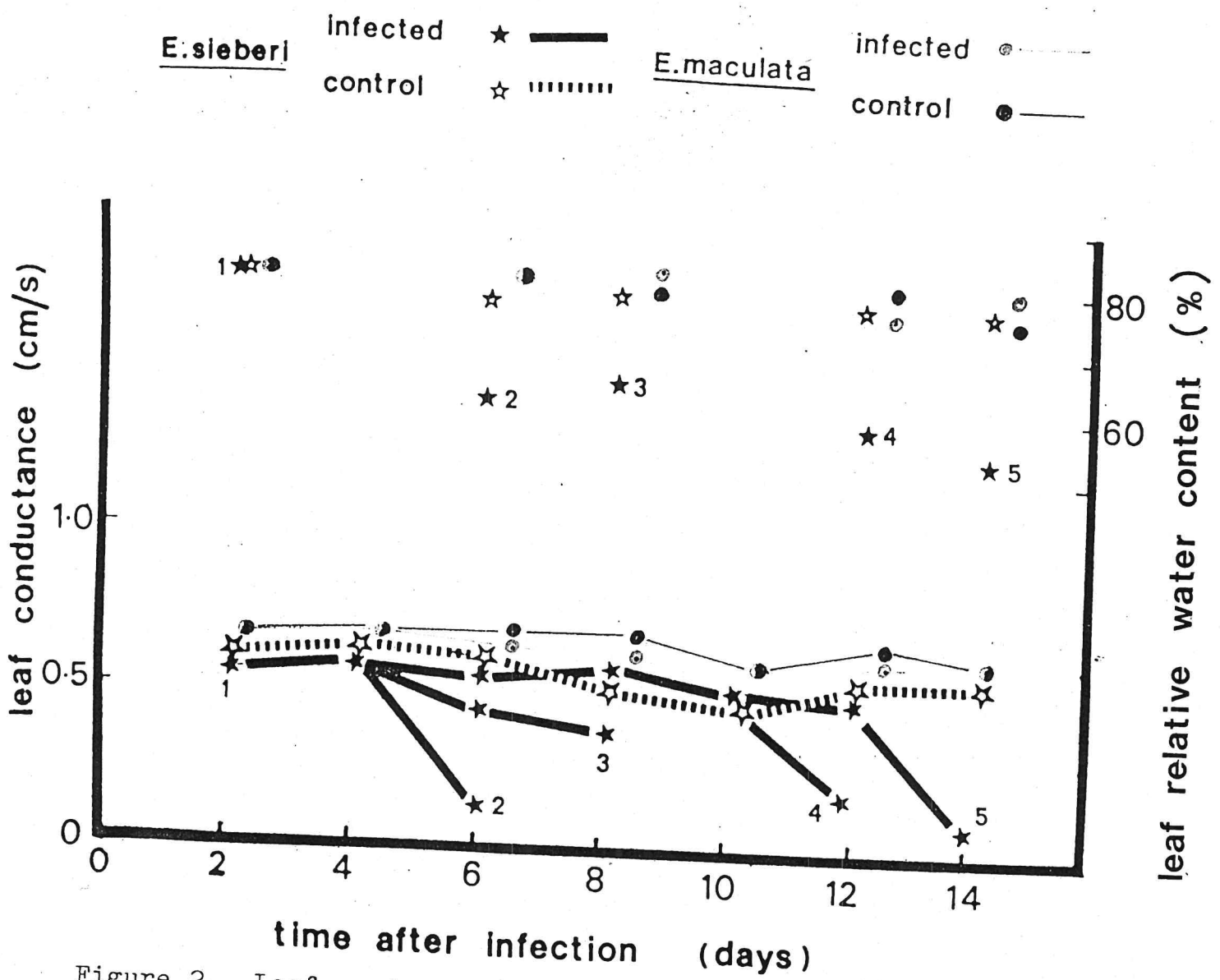


Figure 2. Leaf conductance and leaf relative water content of Eucalyptus sieberi and E. maculata infected with Phytophthora cinnamomi. Numbers 1-5 represent individual plants of E. sieberi.

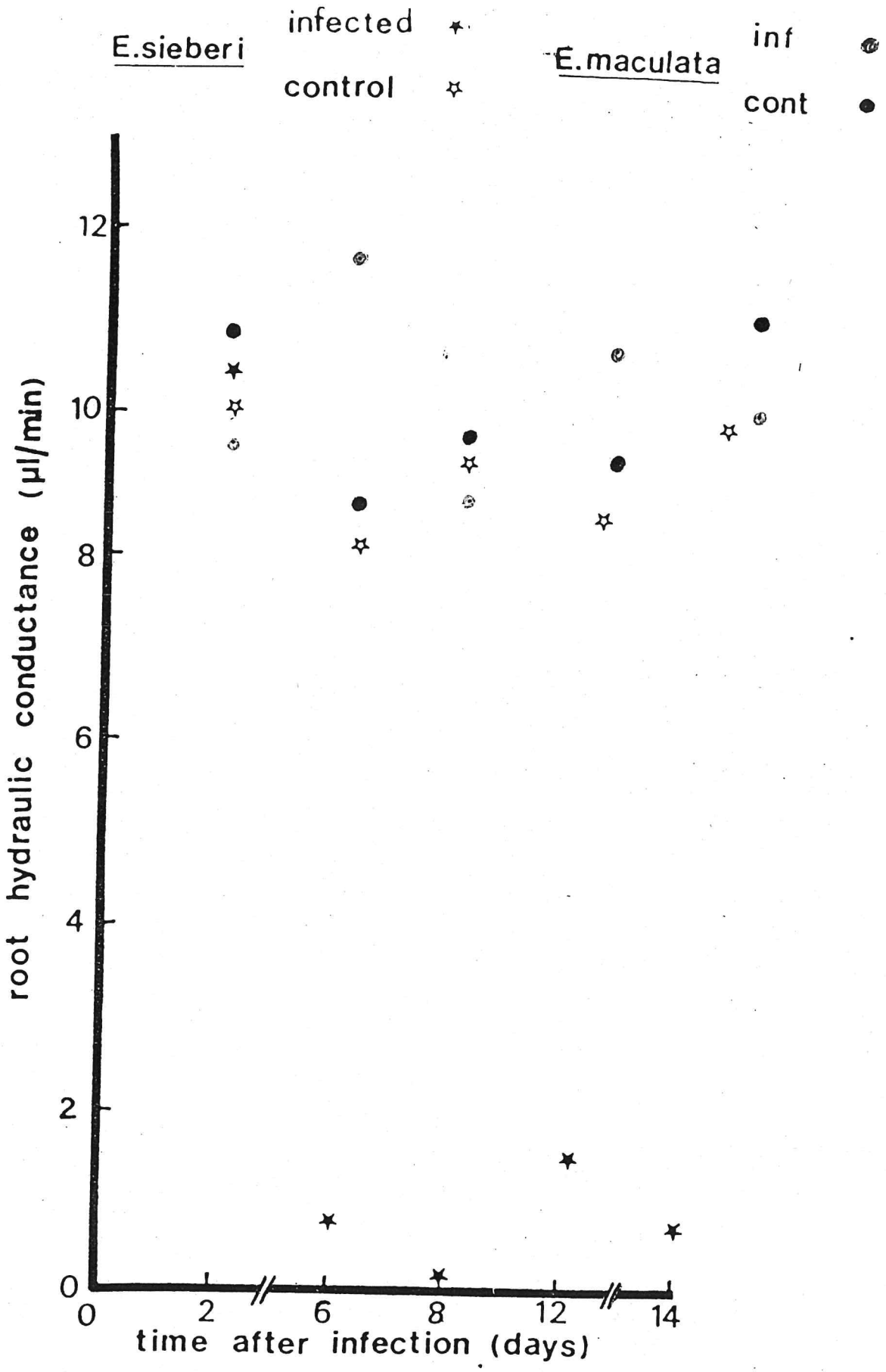


Figure 3. Root hydraulic conductance of Eucalyptus sieberi and E. maculata infected with Phytophthora cinnamomi.

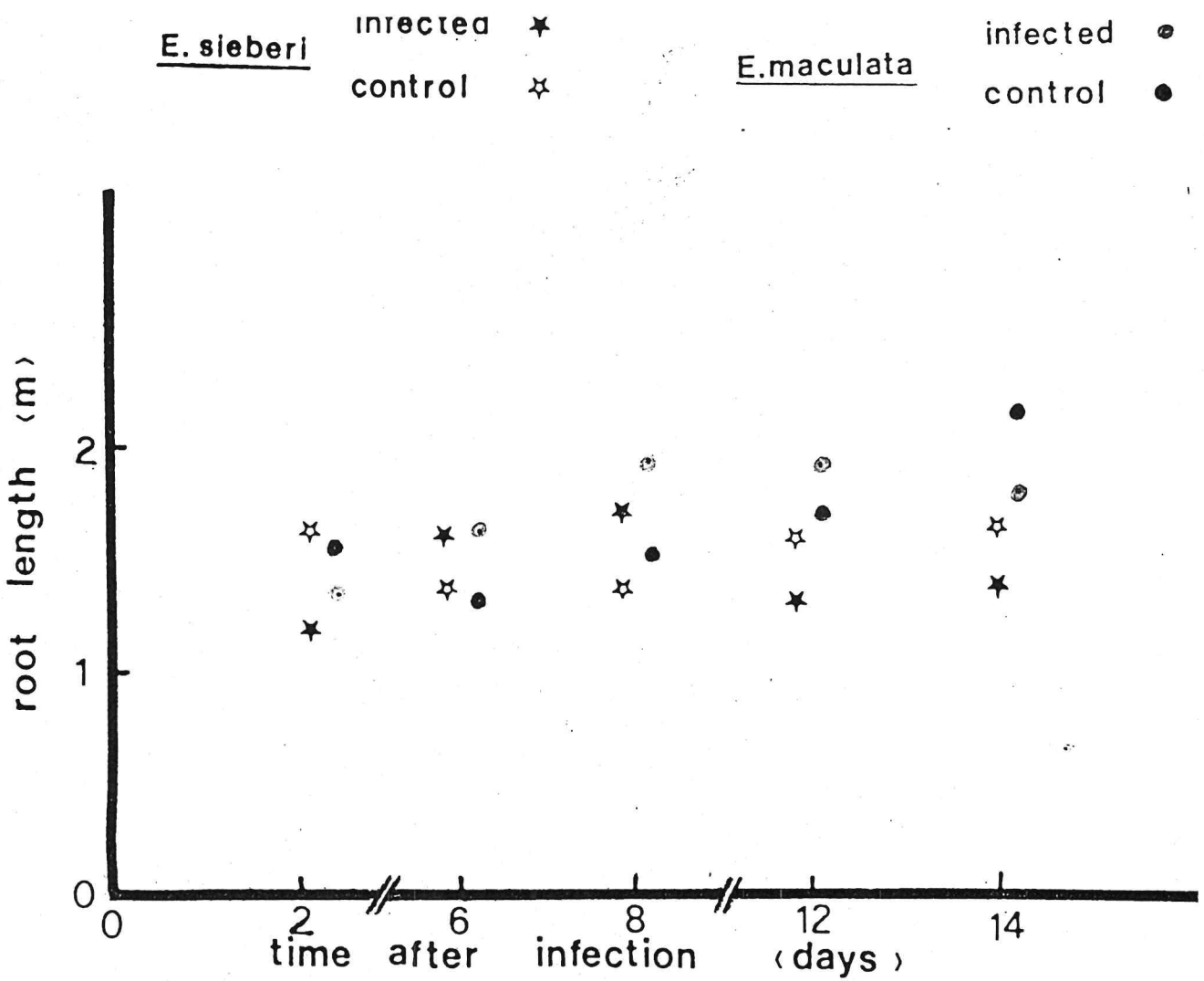


Figure 4. Root length of Eucalyptus sieberi and E. maculata infected with Phytophthora cinnamomi.

Figures

1. Chlamydospore germination and subsequent fungal growth on nylon mesh after 3 weeks burial in non-sterile, host-free soil. Note formation of new species and general spread of fungus.

2. Chlamydospores in host-free, non-sterile soil sometimes form a sporangium with zoospores.

3. Callose fluorescing in section of mani root infected with *P. cinnamomi*.

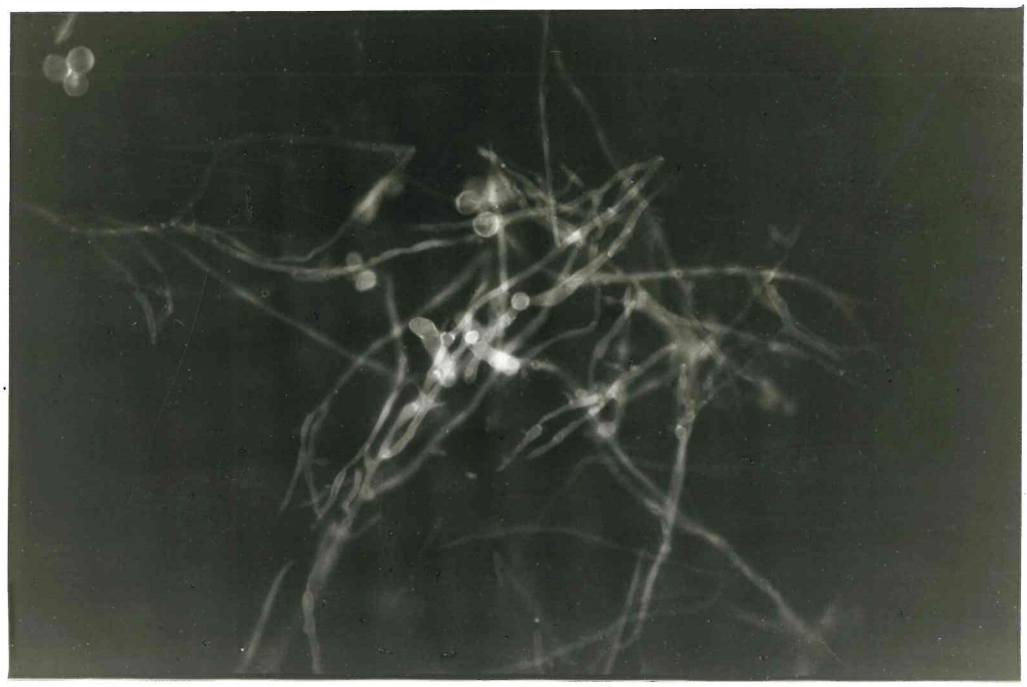


FIGURE 1.

FIGURE 2.

FIGURE 3.