BIOLOGY OF *CRYPTOSTROMA CORTICALE* AND THE SOOTY BARK DISEASE OF SYCAMORE

by

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ABSTRACT

Isolates of *Cryptostroma corticale* (Ell. & Everh.) Gregory and Waller varied considerably in their ability to grow in sycamore (*Acer pseudoplatanus* L.) but there was no evidence of distinct pathogenic strains. The extent of colonisation of inoculated trees varied, and growth of the fungus appeared reduced after c. 6 weeks suggesting a host response. Thirteen other *Acer* spp. were susceptible to *C. corticale* infection.

C. corticale infected sycamores via wounds at 4 different times of year. The fungus gained entry via wounds but not through intact bark or autumn leaf scars. The cell wall around the penetration point was dissolved and *C. corticale* colonised the sapwood.

Conidial germination, germ-tube growth and mycelial extension on 3% MEA were optimal at 25°C. Dry conidia at 25° or 30° lost viability more rapidly than those stored at lower temperatures. *C. corticale* grew more extensively in young sycamores kept at 25° than in those at 15°. Also, at 25°, its growth was greatest in trees subjected to water stress. In all inoculation experiments there was a significant correlation between the extent of staining and recovery of *C. corticale* from the tissues. The results are discussed in relation to the hypothesis that sooty bark disease is associated with high summer temperatures.

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TABLE OF CONTENTS

	Page
ABSTRACT	2
ACKNOWLEDGEMENTS	3
INTRODUCTION	6
MATERIALS AND METHODS	17
Source of Isolates	17
Standard Germination Test	18
Maintenance of Isolates	18
Trees	18
Inoculation of Trees with *C. corticale*	19
Assessment of Fungal Colonisation	19
Fixation of Sycamore Tissue	21
Dehydration for Scanning Electron Microscopy (SEM)	21
Sputter Coating for SEM	21
SEM	23
Sectioning	23
EXPERIMENTAL	24
(1) Variability of *C. corticale* Isolates	24
In Vitro Studies	24
Growth on MEA	24
Growth on other agars	26
In Vivo Studies	30
Growth in sycamore at 25^{o}	30
Growth in sycamore under field conditions	32
(2) Susceptibility of Other Tree Species	39
Growth in a Range of *Acer* spp. at 25^{o}	39
Growth in Other Genera at 25^{o}	46

Page

Sporulation of *C. corticale* on Sterile Wood from a Range of Species 49

(3) Time of Inoculation 50

(4) Mode of Entry 62

Infection of young sycamore 62

Infection of sycamore under field conditions 65

Penetration of sycamore wood at 25° 66

(5) Colonisation of Sycamore Tissues 70

Light microscopy 70

SEM 76

Investigation of the apparent limitation of growth in sycamore 82

Investigation of changes resulting from colonisation by *C. corticale* 91

(6) Temperature and Water Stress 93

Effects of Temperature *in vitro* 93

Longevity of conidia 93

Growth on agar 93

Effects of Temperature *in vivo* 95

Experiment 1 95

Experiment 2 97

Effects of Water Stress 103

Experiment 1 103

Experiment 2 106

DISCUSSION 111

REFERENCES 123

APPENDIX 127

INTRODUCTION

Sycamore (*Acer pseudoplatanus* L.) is native to the mountains of Central Europe and was introduced to Britain in the 15th or 16th Century as an ornamental. It has now become thoroughly established. Although its wood has limited uses commercially, sycamore is very resistant to wind and is thus a useful shelter tree on exposed hill farms and on the coast. Also it is a valuable amenity tree in urban areas because it tolerates high levels of atmospheric pollutants.

The disease known as sooty bark is caused by the fungus *Cryptostroma corticale* (Ell. Everh.) Gregory and Waller. The first symptom is usually wilting of leaves over part or the whole of the crown (Fig. 1) and sometimes yellowing of the leaves (Fig. 2). Later some shoots die and in spring the tree partially or completely fails to leaf (Fig. 3). Sections of recently infected wood reveal a dark greenish-brown to yellow stain, extending vertically for long distances. When the fungus has girdled and killed the tree the stain fades and the wood is uniformly pale. A sporing lesion is usually found where the stain meets the bark (Gregory & Waller, 1951). Areas of the bark become raised in blisters, usually elongated vertically. The affected bark slowly peels off to expose a characteristic, 1-2 mm thick, dry sooty layer of spores (Fig. 4). Sometimes spores are formed over the entire surface of a dead tree but on live trees they are frequently limited to small areas. Large lesions may result in loss of bark for many feet up the trunk. The spore layer (often of c. 30-170 million conidia/cm^2) is produced between stromatic sheets of hyphae in any part of the bark, outside the cambium, and occasionally several spore layers occur at different levels in the bark. The floor and roof stromata are separated by black columns of fungal tissue up to 1 mm long and 0.33

Fig. 1 : Sycamore showing wilting over part of the crown.

Fig. 2 : Sycamore with yellowed leaves.

Fig. 3 : Sycamore which has partly failed to leaf.

Fig. 4 : Blistered bark exposing the characteristic sooty layer of conidia.

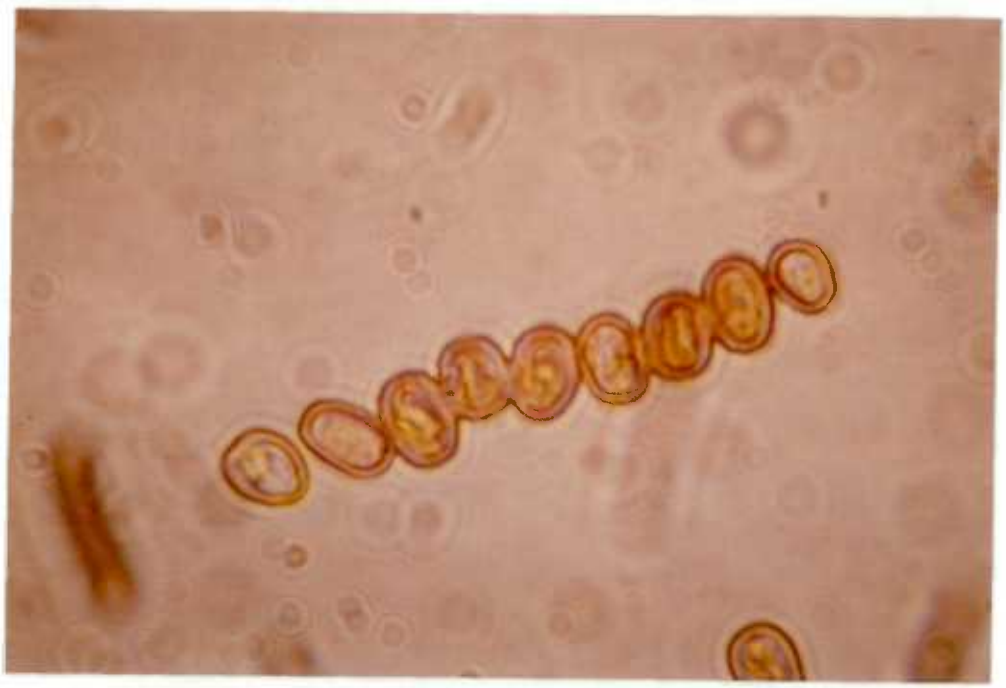
mm diameter. These columns (c. 50/cm^2) break across when the bark is shed and usually persist as spikes on the exposed surface (Fig. 5) and on the shed bark. The conidia (Fig. 6) are dry phialospores which arise endogenously from short, erect, unbranched conidiophores. They are typically ovoid, 4-6.5 x 3.5-4 µm, although their shape may be distorted by pressure from adjacent conidia, and they occur in chains of up to 30 conidia (Gregory & Waller, 1951). The young conidium is hyaline but soon becomes brown to black. The conidial mass is anchored by a system of threads, as many as 10^5 threads/cm^2, which arise from the floor stroma. These threads are unbranched, 1 mm long, and sticky so the exposed spores are only slowly released by wind. Once the conidia are dispersed a bluish-grey layer of conidiophores is exposed; this erodes to reveal the hard black surface of the floor stroma. A black cambial stroma sometimes forms between the bark and wood and this then remains adhering to the wood when the bark is shed.

The fungus was first recorded, as *Coniosporium corticale*, on the bark of maple logs (probably *Acer saccharinum*) from London, Ontario by Ellis and Everhart (1889). There are further records of the fungus on dying maples, hickories (*Carya* spp.) and basswoods (*Tilia* spp.) in Wisconsin (see Gregory & Waller, 1951), and Towey *et al* (1932) reported that in Michigan some workers inhaling spores from maple logs developed severe bronchial asthma. However, the relatively few records suggest that the fungus is not common in N. America.

The fungus was first reported in Britain on sycamores in Wanstead Park, London in 1945 and within 2 years it had apparently killed about 40 trees there. Other isolated outbreaks occurred near Wanstead and those at Plumstead and Plaistow, near docks, suggested that the fungus had been

Fig. 5 : Columns of fungal tissue persisting as characteristic spikes after bark shedding.

Fig. 6 : Chain of *C. corticale* conidia. X 5300.



imported on timber (Waller, 1949).

Gregory and Waller (1951) were the first to describe the fungus in the UK. They confirmed its identity with the fungus originally described by Ellis and Everhart but because of the special characters of its stroma, conidiophores and conidia they transferred it to a new genus *Cryptostroma* as *C. corticale*. There were reports of *C. corticale* from France in 1951, apparently killing sycamore at Vincennes. It was also found on field maple (*Acer campestre*) and in 1952 on *Acer negundo*. However, records indicated that sycamores in Paris had been affected by sooty bark since 1947 (Moreau & Moreau, 1951). These authors suggested that *Eutypa acharii*, a common saprophyte on sycamore, was the perfect state of *C. corticale* (Moreau & Moreau, 1951, 1954) but there is no good evidence to support this and in Britain *E. acharii* appears to be more widely distributed than *C. corticale* (Peace, 1962).

From 1948-51 sooty bark caused considerable damage to sycamores in and around Wanstead Park and appeared a threat to the sycamore population of the entire country. During the winter of 1949-50 over 200 diseased trees were felled. By July 1952 *C. corticale* had apparently extended its distribution around London, south to Croydon, south-west to Guildford, west to Henley and north-east to Mundford in Norfolk. However, in the east and south-east it was recorded only in the London suburbs. Within the London area it was generally distributed in parks and cemeteries, though extensively only at Wanstead, and appeared to be acting as a virulent parasite. Elsewhere, with one exception (Waterford, nr Hertford) it was growing on trees probably weakened or killed by other agencies (Peace, 1955).

By 1953 even in the London area the disease had subsided, few trees

were killed and it became increasingly difficult to find actively sporulating lesions. During the next two decades sooty bark caused little damage; there were only a few minor outbreaks where trees were killed. During this period the disease was generally restricted within a 30 mile radius of London though it was reported in Surrey and Northamptonshire in 1962 and 1963 (Pawsey, 1973) and Peace (1962) recorded *C. corticale* as a saprophyte in Somerset. Then in 1976 there was a major epidemic of sooty bark with reports of the disease as far as 160 miles from London. It became well-established in S.E. England (Kent, Sussex, Surrey) and in Hampshire, Wiltshire and neighbouring countries. It has now spread to Norfolk and the Midlands to a line stretching from Hull to the Mersey. Wales remains relatively free of the disease except for the south coast between Newport and Swansea (S.M. Dennis, personal communication). Outbreaks have also been recorded in Devon (Abbott *et al.*, 1977).

The means by which *C. corticale* infects sycamore and the factors which influence the severity of sooty bark are not fully understood. The wind blown spores are small and in this they resemble the spores of other fungi which infect through broken branches, entering exposed vessels by capillarity when wetted by rain. As a result of their inoculation experiments and observation that most infections begin high in the tree on small branches, Gregory and Waller (1951) suggested this was the usual mode of infection. However, Townrow (1954) concluded that the spores are not drawn into vessels. He observed germination on decapitated shoots followed by growth of germ tubes into the twig. Dry wounds did not become infected, but if the wound was moist some penetration occurred. The number of infections in the field is very low in proportion to the enormous spore production, suggesting that special conditions are required for infection and/or subsequent spread.

Grey squirrels have been implicated in a few disease outbreaks. They often strip the bark from healthy sycamore, thus allowing access of spores and also sometimes feed on the layers of exposed *C. corticale* spores in infected trees (Abbott *et al.*, 1977). However, evidence is lacking for their involvement in most disease outbreaks.

The beetles, *Enimus brevicornis* and *Diplocoelus fagi*, shelter and feed on spore layers which after several months still remain protected by bark, but there is no evidence that they have any role in spreading *C. corticale*.

The period from initial infection to death can take several years. Young (1978) reports that on one wilted tree the fungus progressed slowly down one limb for six or seven years before disease became acute. His inoculation experiments suggested that it takes a year or longer for severe injury to develop, although small trees only a few years old may wilt within 10 months. However, the fungus varies considerably in the extent to which it colonises trees. On some it behaves as an aggressive parasite and rapidly kills the tree; on others its growth is restricted to damaged or dead branches. Also, after the conidia have been shed lesions on some trees heal, thus attack even of the main stem is not necessarily fatal.

Disease outbreaks in Britain appear to be related to the occurrence of hot, dry summers. In reviewing the available data, Young (1978) suggested that disease outbreaks followed months with a mean daily maximum of 23^{o}C or more. There were three such months during the summer of 1976 when there was a major epidemic, and the summer of 1975 was also hot. The original 1948-51 disease outbreaks followed the hot summers of 1947 and 1949 and there were scattered minor outbreaks associated with warm summers

in 1955, 1959, 1967, 1969, 1970 and 1973. The greater incidence of the disease in and around London may similarly be associated with the higher summer temperatures in this area compared with the rest of Britain.

The initial aim of the present investigation was to examine the effects of temperature and water stress in sycamore on the growth of *C. corticale*. The ready availability of material following the 1976-77 epidemic also enabled a study of variation in *C. corticale* and the ability of different isolates to infect sycamore both in the glasshouse and in the field. The susceptibility of other trees was examined.

MATERIALS AND METHODS

Source of Isolates

Conidia of *C. corticale* were collected from typical lesions on sycamore at Laleham Park, Staines (Grid Ref: TQ 053 685) on 12 October 1977 and were stored at 5°. These conidia or a mycelial isolate derived from them were used in most experiments. Viability of the original conidial collection decreased with time and conidia produced in culture on sterile wood were used for later experiments.

Conidia were also collected from Sunninghill Park near Ascot (Grid Ref: SU 933 701) on 19 October 1977 (Isolate SP) and on 3 April 1978 (SU 934 712; Cr), from Wanstead Park in London (TQ 417 875; WP) on 13 June 1978, from Datchet (SU 996 772; D) and Remenham near Henley (SU 783 828; H) on 20 June 1978 and from Thamesmead in London on 9 May 1979. These or corresponding mycelial isolates were used in some experiments.

Mycelial isolates were obtained from a sporulating log collected from Holland Park, London by the Forestry Commission in 1975 and from a culture under oil originating from Whip's Cross, London 1962-3 collected by Dr. B.E.J. Wheeler.

The Forestry Commission Research Station at Alice Holt Lodge kindly provided mycelial isolates from conidia collected in the London area. Isolate 57 from Wanstead Park was probably obtained during the period 1948-50; isolates 108, 112, 113 and 114 were obtained in 1975 and isolate 3 in 1976, all from Holland Park. Isolates 250, L10, L13 and L16 were obtained from Lambeth Palace and 251 from Holland Park in 1976. Isolate Ch was obtained from conidia collected from Chester in 1978.

Standard Germination Test

Viability of the conidial collections was tested at intervals. Conidia were streaked onto a thin layer of 3% malt extract agar on glass slides, which were then incubated in a damp chamber at 25° for 18 h when germination was arrested by adding drops of lactophenol. Percentage germination was assessed from counts of 100 conidia on each of 4 replicate slides.

Maintenance of Isolates

Mycelial isolates were subcultured regularly on plates of V8-juice agar (V8; Appendix Table 1). Stock cultures were made by covering V8 slope cultures with sterile paraffin oil. Stock cultures of conidia were prepared by inserting sterile sycamore stem pieces (4-6 cm long) into V8 slope cultures and incubating at 25°.

Trees

Sycamores, 1-2.5 m tall, were transplanted from Silwood Park or nearby Crown Estate woodlands. Container-grown sycamores, 1.5-2 m tall, from a single seed stock were provided by the Forestry Commission. Other *Acer* spp. were purchased from Hilliers Nurseries, Winchester, or Slococks Nurseries, Knaphill, Surrey. These were not container-grown. Trees and shrubs of other genera (which were container-grown) were obtained from various sources.

The trees were grown in John Innes Compost No. 3 in 30 cm pots for several weeks or months before use. They were watered every 2-3 days, except in the water stress experiments. The trees in the first temperature experiment (p. 95-97) were dormant; all others were in full leaf.

Sycamore trees, c. 8-10 m tall, growing on a plot at Silwood Park were used for field experiments. These were planted as seedlings in 1963.

Inoculation of Trees with *C. corticale*

A disc (6 mm) of bark was removed with a cork borer half way up the stem or branch and a millipore (Type SM, 5 µm pore size) disc of similar size charged with conidia (c. 2.3×10^3) was inserted. The bark was replaced and the wound sealed with parafilm on stems or with "Scotch" pressure sensitive tape on branches. Spore-free discs were used for controls.

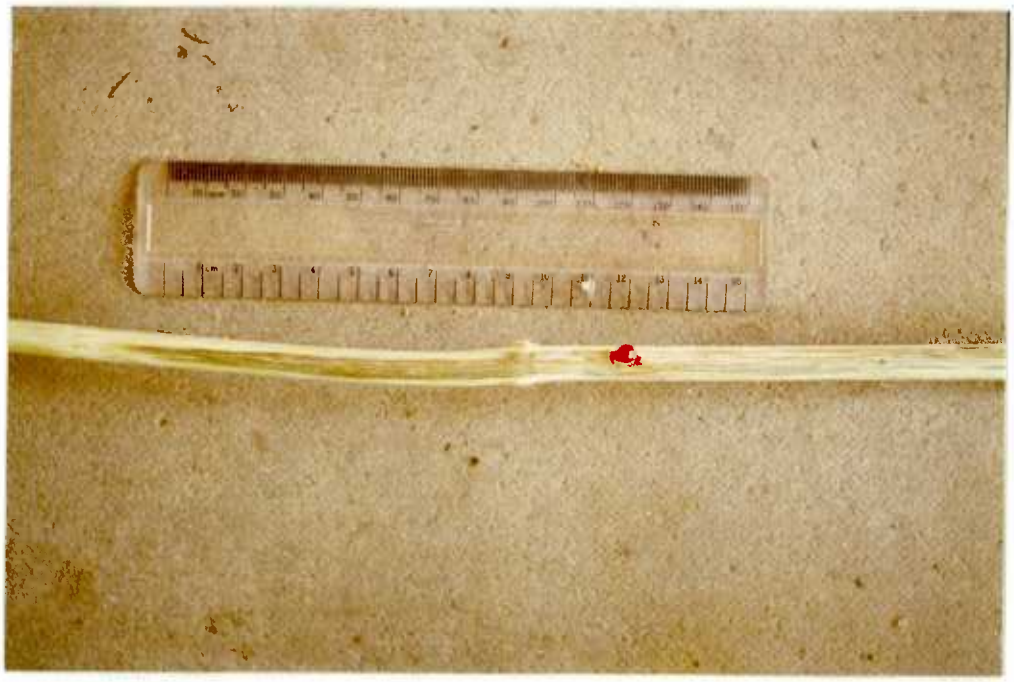
In experiments involving comparisons between isolates, where relatively few conidia were available, a disc of mycelium cut from the margin of a colony on 3% malt extract agar (MEA) was used as inoculum. Agar discs were used in the controls. The cork-borer was surface-sterilised by flaming in alcohol between isolates. In inoculations of trees in the field the replaced bark was fixed by two staples and the wound was not otherwise sealed.

Assessment of Fungal Colonisation

Bark was removed from the stem or branch to expose the dark stain (Fig. 7) which appeared to be associated with the fungus in inoculated trees and the extent of this was recorded. In long-established infections it was sometimes necessary to remove later sapwood to expose the stain. Samples, each c. 5 mm^3, removed at intervals of one to 10 cm along the stained area and beyond its limit were surface-sterilised in 10% Chloros (1% available chlorine) for 10 min and rinsed in sterile distilled water. Three slivers of the tissue were then plated aseptically onto V8-juice agar and were incubated for c. 7 days at 25°. The mycelial isolates obtained (Fig. 8) were checked against typical colonies of the *C. corticale* isolate used. They were confirmed as *C. corticale* by introducing into slope cultures on V8-juice agar sterile sycamore stem pieces (4-6 cm long)

Fig. 7 : Staining of sapwood typically induced following inoculation with *C. corticale*. The red pin indicates the point of inoculation.

Fig. 8 : Typical mycelium of *C. corticale*, reisolated onto V8-juice agar from infected wood.



on which, after some weeks, the fungus usually produces its typical conidial fructifications (Fig. 9) (Townrow, 1954). It was found that sporulation was accelerated if the stem pieces were transferred to sterile containers (without agar) when mycelium of the fungus had developed on them.

Fixation of Sycamore Tissue

Infected sycamore tissue and controls were cut into pieces 4-5 cm long and fixed immediately in Formalin acetic acid (FAA : 130 ml formalin, 50 ml glacial acetic acid, 1 ℓ of 70% ethanol).

Dehydration for Scanning Electron Microscopy (SEM)

Initially pieces (1 cm^3) of FAA-fixed wood were put through three 15 min changes of acetone. These pieces were then trimmed to blocks, 1 mm thick or less, with a new single-edge razor blade taking care not to allow the specimen surface to dry out. In subsequent preparations blocks were cut prior to acetone treatment since this dehydration rendered the surfaces brittle and difficult to cut. The samples were critical point dried using a Polaron E3000 unit. The acetone was replaced with liquid carbon dioxide (800 lb/in^2 i.e. 56248 g/cm^2), flushed three times and the chamber was left sealed for at least $1\frac{1}{2}$ h. The chamber was flushed again with CO_2 before slowly running water around the chamber until the critical point was reached at about $34-35^{o}$ (c. 1200 lb/in^2 i.e. 84372 g/cm^2). The pressure was let down to that of air as quickly as possible without allowing condensation to occur (1-2 min).

Sputter Coating for SEM

The dehydrated specimens were mounted onto stubs using Silverdag high conductivity paint. The stubs were fixed into the vacuum chamber of a Polaron SEM coating unit E5000 and were coated with gold for 3 min at 1.2 kV. As the coating deteriorates stubs were re-coated for $1\frac{1}{2}$ min if

Fig. 9 : Blistered bark exposing typical conidial fructifications of *C. corticale*.


15
16
17
18
19
20
2

left for more than a few weeks.

SEM

The stubs were examined using a Phillips PSEM 500 at electron gun potentials of 12 or 25 kV and a spot size of 320 Å. Potentials of 25 kV give better resolution but were less satisfactory since the specimens tended to break up under the electron beam.

Sectioning

FAA-fixed or fresh wood was sectioned at 20 or 40 µm using a Reichert sledge microtome with the knife set at c. 10^{o} angle to the specimen, or occasionally by hand using a single-edged razor blade. Sections were collected and stored in a 1 : 1 mixture of glycerol and ethanol, which helps to prevent curling, except where required for certain microchemical tests when they were collected in water or ethanol as appropriate.

EXPERIMENTAL

(1) VARIABILITY OF *C. CORTICALE* ISOLATES

The growth characteristics of a range of *C. corticale* isolates were examined to establish particularly whether more than one strain of the fungus exists.

In Vitro Studies

Growth on MEA. A preliminary experiment compared radial growth rate of isolate 113 on agars known to favour rapid growth of *C. corticale*. Growth was faster on V8-juice agar (6.1 mm/day) than on 3% Malt Extract Agar (4.1 mm/day) over a 5 day incubation period at 25°. However, for further experiments 3% MEA was chosen because on this the edge of the colony was more distinct.

The growth rates of 20 *C. corticale* isolates were measured at a range of temperatures. Petri dishes containing 15 ml of 3% MEA were inoculated centrally with a disc (6 mm) taken from the perimeter of a 7-9 day old culture. They were incubated at 25°C for 2 days to establish growth and then three replicate plates of each isolate were transferred to 5, 10, 15, 20, 25 and 30°. Daily measurements of typical isolates (LP and SP) over the same temperature range had previously shown that at 25° the plate was covered after 6-7 days; hence in this experiment growth was measured on Day 2 and on Day 5 or 6 along two marked diameters at right angles. Some isolates grew faster than others but the growth rates of all isolates increased from 5° to 25° and then declined at 30° (Table 1). Isolate 57 grew exceptionally slowly and had a waxy appearance unlike that of other isolates.

Cultures of some isolates often blackened after c. 7 days growth at

TABLE 1 : Growth of *C. corticale* isolates on 3% MEA.

Isolate[b]	Mean growth rate mm/day[a] at 5°	10°	15°	20°	25°	30°C
57	0.0	0.3	0.4	1.0	1.1	0.2
L10	0.6	1.3	2.1	3.4	4.1	1.6
113	0.8	1.0	1.9	3.1	4.1	1.7
WP	0.1	1.3	2.1	3.4	4.7	3.7
3	0.9	2.1	3.2	4.5	5.0	2.3
SP	0.8	1.3	3.0	4.4	5.8	4.7
112	0.8	1.9	3.1	4.6	6.2	3.7
H	0.2	1.2	2.4	4.4	6.4	2.8
HP	0.2	1.1	3.4	5.4	6.6	3.5
D	0.4	1.7	3.3	5.1	6.8	3.4
108	0.4	1.7	2.8	5.1	6.9	4.0
L16	0.5	1.2	2.9	4.7	7.0	1.5
251	1.4	1.3	2.0	5.1	7.0	4.4
LP	1.0	1.9	2.9	4.8	7.1	5.0
WC	0.7	1.5	3.1	4.5	7.2	5.1
Ch	0.9	1.9	3.5	4.9	7.5	5.4
L13	0.7	1.4	3.8	5.1	7.5	2.1
Cr	0.7	2.0	3.3	5.6	7.8	1.3
250	0.9	1.5	3.6	6.1	8.3	4.6

Key: a. Each figure is the mean of 3 replicates.

b. Ranked on growth at 25°.

25° (e.g. LP) and after a longer period at lower temperatures. This blackening was sometimes preceded by a yellowing of the colony. Other isolates (e.g. 113) remained uniformly white under similar conditions. Examination of discoloured areas showed no evidence of changes in hyphal organisation or sporulation, but the change in colour appeared first near the centre of the colony suggesting that it is related to ageing.

Growth on other agars. (a) Growth of isolates LP and 113 at 25° was examined on a range of agars, including 3% MEA, viz:- Czapek-Dox agar (CZD), Sabouraud Dextrose agar (SDA), Nutrient agar (NA), Corn Meal agar (CMA), Water agar (WA), 5% MEA, V8-juice agar (V8), Rose Bengal agar (RBA) and Potato Dextrose agar (PDA). Details of the constituents and preparation of these agars are given in Appendix Table 1. The original purpose of this experiment was to find a medium on which *C. corticale* could be readily identified on mycelial characteristics. Isolate LP grew well on SDA and after 11 days was more intensely blackened than on 3% MEA; isolate 113 remained white on both. The cultures on SDA smelled of celery, unlike uninoculated plates. *C. corticale* grew less well on CZD, covering only half the agar surface; isolate LP produced a fluffy colony whilst isolate 113 was relatively adpressed. Both isolates had a yeast-like odour on 5% MEA and had covered the agar. On PDA the fungus grew well, isolate 113 was fluffy with larger aggregations of hyphae than LP. Growth on CMA and WA was sparse, and on RBA and WA only half-covered the medium.

(b) The growth of 19 *C. corticale* isolates was compared on SDA and 3% MEA to examine particularly the blackening of colonies. Two replicate plates of each medium were inoculated centrally with a 6 mm disc of mycelium. They were incubated at 25° in the dark for 7 days and then transferred to a laboratory bench for 5 days before assessment. None of

the cultures blackened when incubated in the dark, but many did so subsequently (Fig. 10). Blackening did not appear to be related to growth rate on 3% MEA (cf columns 5 and 6 with column 4 of Table 2).

(c) The time taken for isolates LP and 113 to sporulate was examined by inserting pieces of sterile sycamore wood (4-6 cm) into twelve V8 slope cultures of each isolate and incubating them at 25°. After c. 8 weeks most of the LP cultures showed typical conidial fructifications on the wood, but none of the cultures of 113 had sporulated. This result reflects the faster growth of isolate LP on 3% MEA and suggests that such isolates may be more successful colonizers of sycamore than ones with a slower growth rate.

(d) *C. corticale* isolates were incubated at 25° under different light regimes in an attempt to induce sporulation in culture. Isolate HP and single spore isolates LP2, SP2 and SP3 were each grown on 2 plates of 3% MEA in darkness, black light or fluorescent light. After several weeks cultures in fluorescent light had blackened most, but no sporulation occurred under any regime.

(e) Antagonism between genetically distinct fungal mycelia of the same species may be observed as "barrages" or "demarcation zones" between basidiomycete colonies placed opposite each other in culture (Rayner & Todd, 1977). The sexual stage of *C. corticale* has not been observed and the fungus may not be a basidiomycete. However, this is not important since antagonism between individual mycelia of a single fungus may occur amongst the fungi as a whole. Alternatively, if there is more than one mating type, sexual spores may be produced where hyphae of two strains meet. To examine these possibilities all combinations of two isolates from the available 19 *C. corticale* isolates were examined by inoculating

Fig. 10 : Differential blackening of *C. corticale* isolates on Sabouraud dextrose agar.

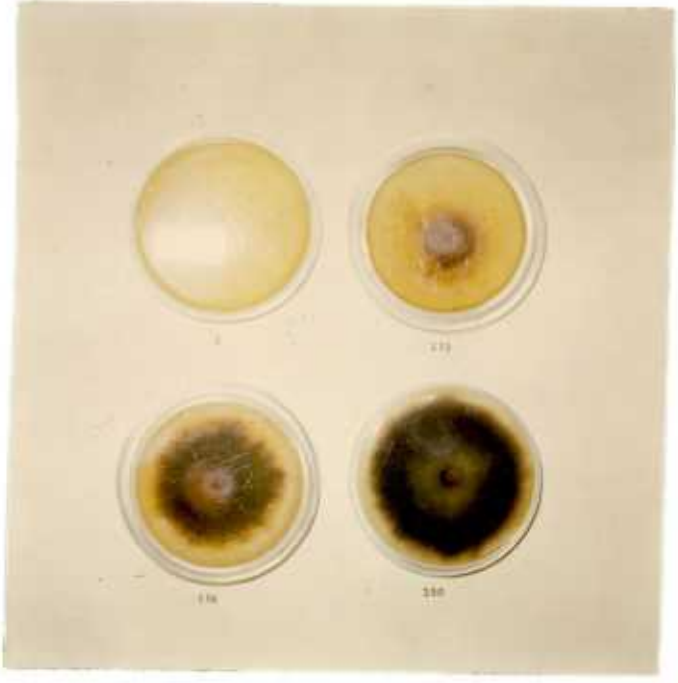


TABLE 2 : A comparison of various isolates of *Cryptostroma corticale*.

Isolate* No.	Source	Growth		Blackening on	
		in sycamore[a]	on agar[b]	SDA[c]	MEA[d]
57	Wanstead Park 1948-50	1.8	1.1	1	0
Cr	Windsor Great Park 1978	7.3	7.8	1.5	1
L10	Lambeth Palace 1976	8.5	4.1	0.5	0
3	Holland Park 1976	16.3	5.0	0	0
L13	Lambeth Palace 1976	20.8	7.5	1.5	0
113	Holland Park 1975	21	4.1	0	0.5
251	Holland Park 1976	33	7.0	1	1
114	Holland Park 1975	39.5	8.1	0	0
250	Lambeth Palace 1976	49.5	8.3	3.5	3
WP	Wanstead Park 1978	62	4.7	2	1
112	Holland Park 1975	> 64.5	6.2	2	2
HP	Holland Park 1976	67.5	6.6	3.5	2
SP	Sunninghill Park 1977	68	5.8	4	2.5
L16	Lambeth Palace 1976	68.3	7.0	3	2.5
H	Henley 1978	68.8	6.4	2	1.5
D	Datchet 1978	> 69	6.8	2	1.5
LP	Laleham Park 1977	84.5	7.1	2	3
108	Holland Park 1975	104.5	6.9	3	3
WC	Whips Cross 1962	>130.5	7.2	3	1.5

Key: a. Mean growth (cm) in 4 trees after 4 wk at c. 25°.

b. Mean growth (mm/day) on 3% MEA at 25°C.

c. Blackening on SDA and

d. on 3% MEA assessed on scale 0 = no blackening, to 4 = marked blackening

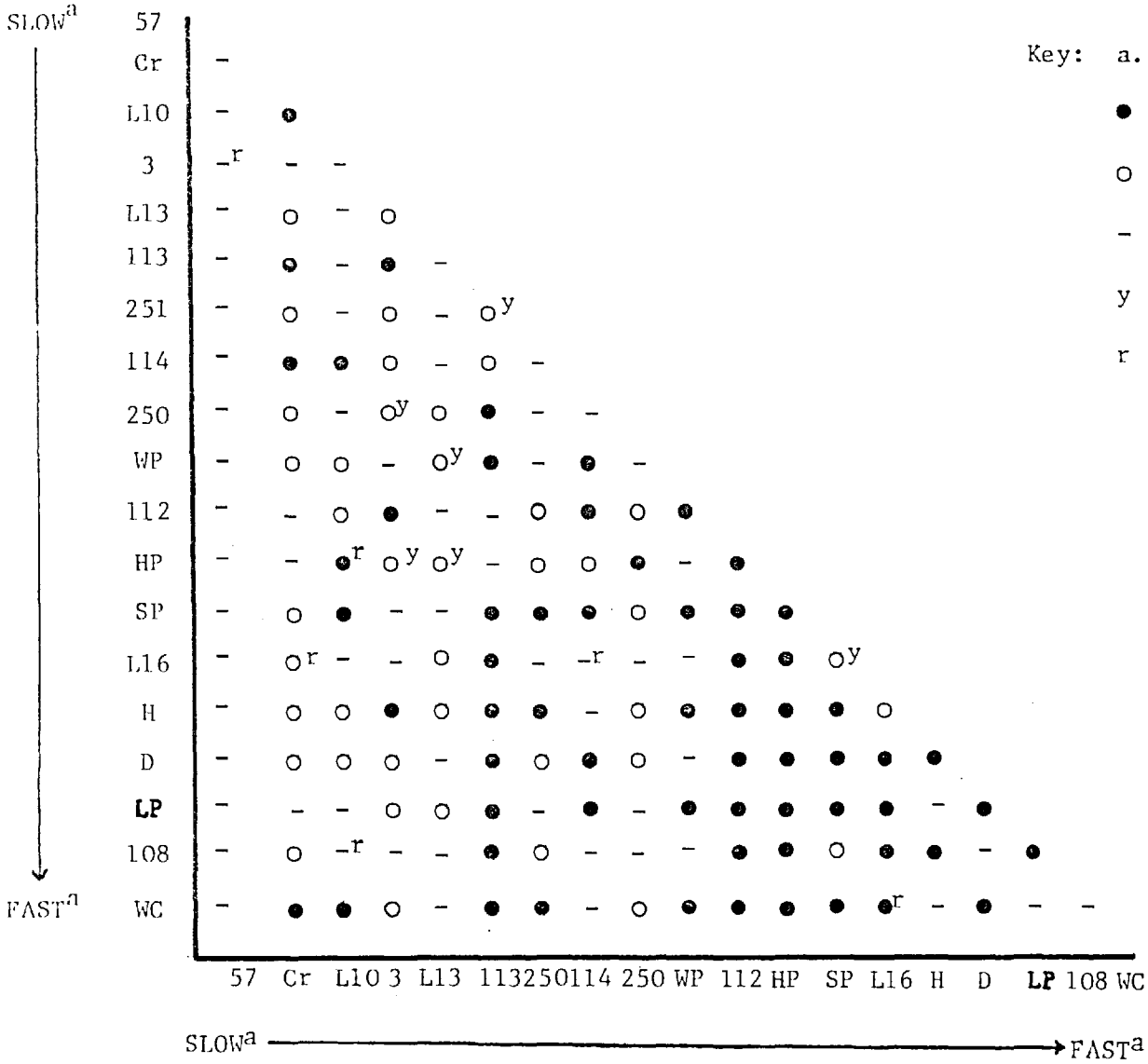
* Ranked on growth in sycamore.

plates of 3% MEA with two, widely-spaced 6 mm discs of mycelium. Plates were incubated at 25° and growth of *C. corticale* was assessed after 12 and 28 days. Demarcation zones of less dense growth were visible on most plates after 12 days; sometimes the zone appeared yellow or the growth was raised and fluffy. No sexual structures were observed. The demarcation was most obvious in combinations of two fast-growing isolates. In combinations involving a slower growing isolate the demarcation was less distinct, there was sometimes yellowing or no zone at all (Fig. 11). Most isolates showed antagonism towards other *C. corticale* isolates even when they were from the same geographic source.

In Vivo Studies

Growth in sycamore at 25°. The infection of young sycamore by 19 *C. corticale* isolates was investigated. Four trees (1.5-2 m) from a single seed stock were inoculated per isolate on 2 August 1978, using a mycelial plug inserted under the bark and all trees were transferred immediately to a glasshouse maintained as near as possible at 25°, a thermograph indicated a range for the daily means of 21°-24° with an overall mean of 21.9° (Appendix Table 5). After 4 weeks the trees were cut at soil level, the leaves were trimmed off, and the cut ends were sealed with "Vaseline". These excised shoots were stored at 5° and later examined for stain and presence of *C. corticale*. At harvesting, sporulating lesions were observed on one tree inoculated with isolate HP (lesion size c. 2.2 x 0.8 cm) and another inoculated with isolate H (lesion size c. 2.7 x 0.9 cm). One tree was apparently killed by isolate WC; most of the aerial parts were dead and the wood was uniformly pale brown, while the base of the stem was heavily stained but there was no sporulation of *C. corticale*.

The identification of cultures re-isolated from infected wood was



Key:
- a. Relative growth rate on 3% MEA.
- ● Distinct demarcation zone of less dense growth.
- o Less distinct demarcation zone or yellowing.
- – No demarcation zone.
- y Yellowing at interaction zone.
- r Raised fluffy growth c. 1 cm wide at zone of interaction.

Fig. 11 : Antagonism between *C. corticale* isolates on 3% MA, after 28 days at 25°.

based mainly on mycelial characteristics since not all could be induced to sporulate on sterile wood. There were several possible reasons for this: (i) the time allowed was insufficient for the slow growing isolates, (ii) some wood pieces dried out too quickly to permit sporulation and (iii) some wood cultures became contaminated with mites and had to be discarded.

The results (Table 2, column 3 and Appendix Table 2) showed that the isolates varied considerably in their ability to grow in sycamore but an analysis of the ranked means for growth using Duncan's new multiple range test provided no evidence of distinct pathogenic strains (Fig. 12; Appendix Table 3). This experiment also provided data for determining statistically the relationship between the stain commonly seen in the wood and colonisation by the fungus as determined by reisolations of characteristic mycelium. The correlation was highly significant ($P \leq 0.001$), the coefficient being 0.811.

The virulence of the isolates did not appear to be related either to geographic source or year of collection nor did growth rate on 3% MEA relate well with growth in the tree at 25°. However, on SDA and 3% MEA, isolates of greater virulence produced more blackening than those of low virulence (Table 2, columns 5 and 6). The relationship was most marked with SDA.

Growth in sycamore under field conditions. The ability of *C. corticale* to grow in 16-year-old sycamores in the field was investigated. Four trees per isolate were inoculated on 21 June 1978 at two points on opposite sides of the trunk c. 140 cm above ground level, using plugs of mycelium as inoculum. In one part of the experimental plot the trees were slightly smaller than elsewhere and tended to have multiple stems, so the plot was divided approximately into 4 blocks and a tree was selected at random

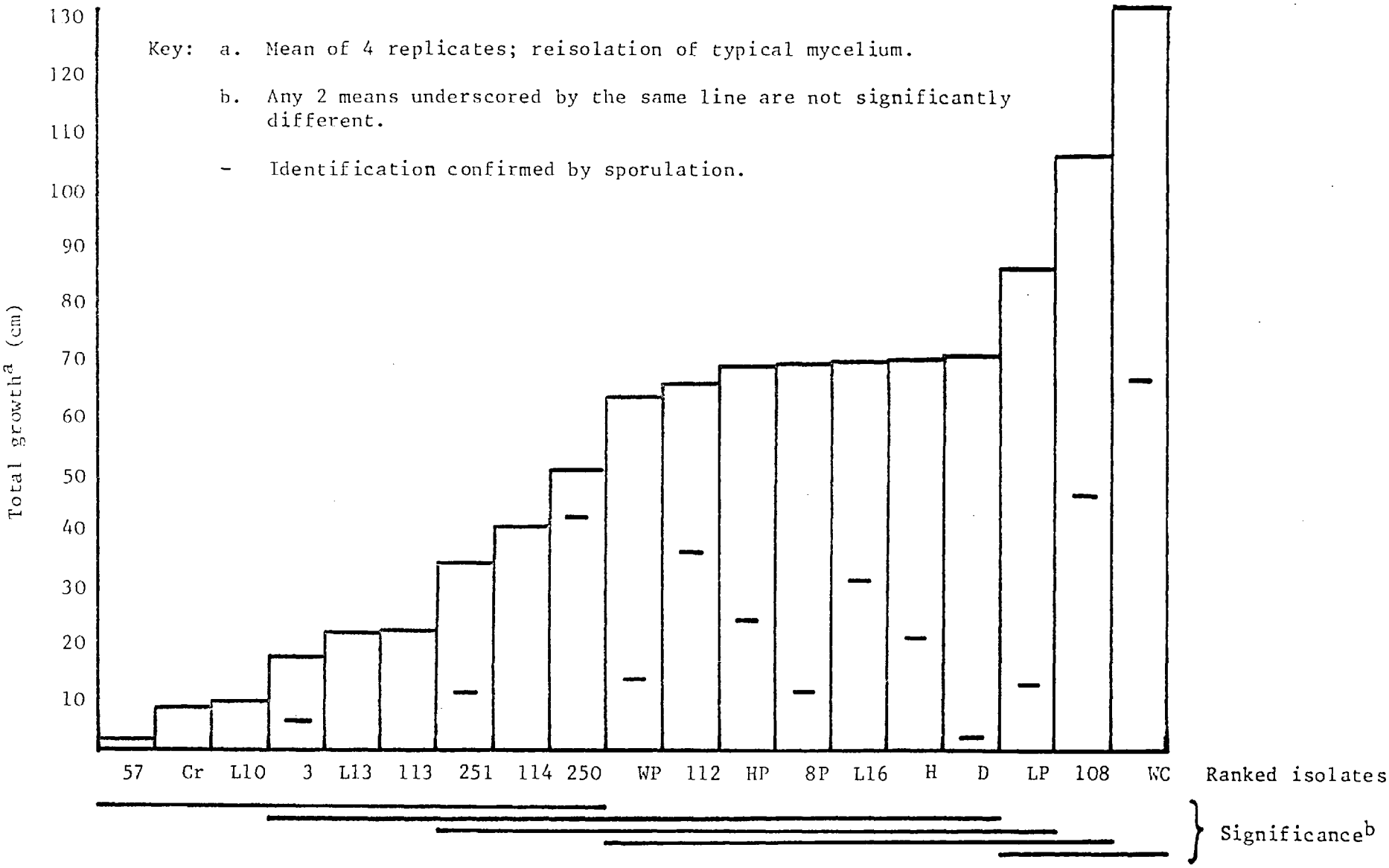


Fig. 12 : Growth of *C. corticale* isolates in sycamore trees at 25°.

within each block for inoculation with one isolate. A plan of the site is shown in Fig. 13 and the girth measurements of the trees are given in Appendix Table 4. A thermohydrograph at canopy height (4 m) within the plot recorded temperature during the experiment (Appendix Table 6), the mean, calculated from average monthly temperatures, was 10.3^{o}.

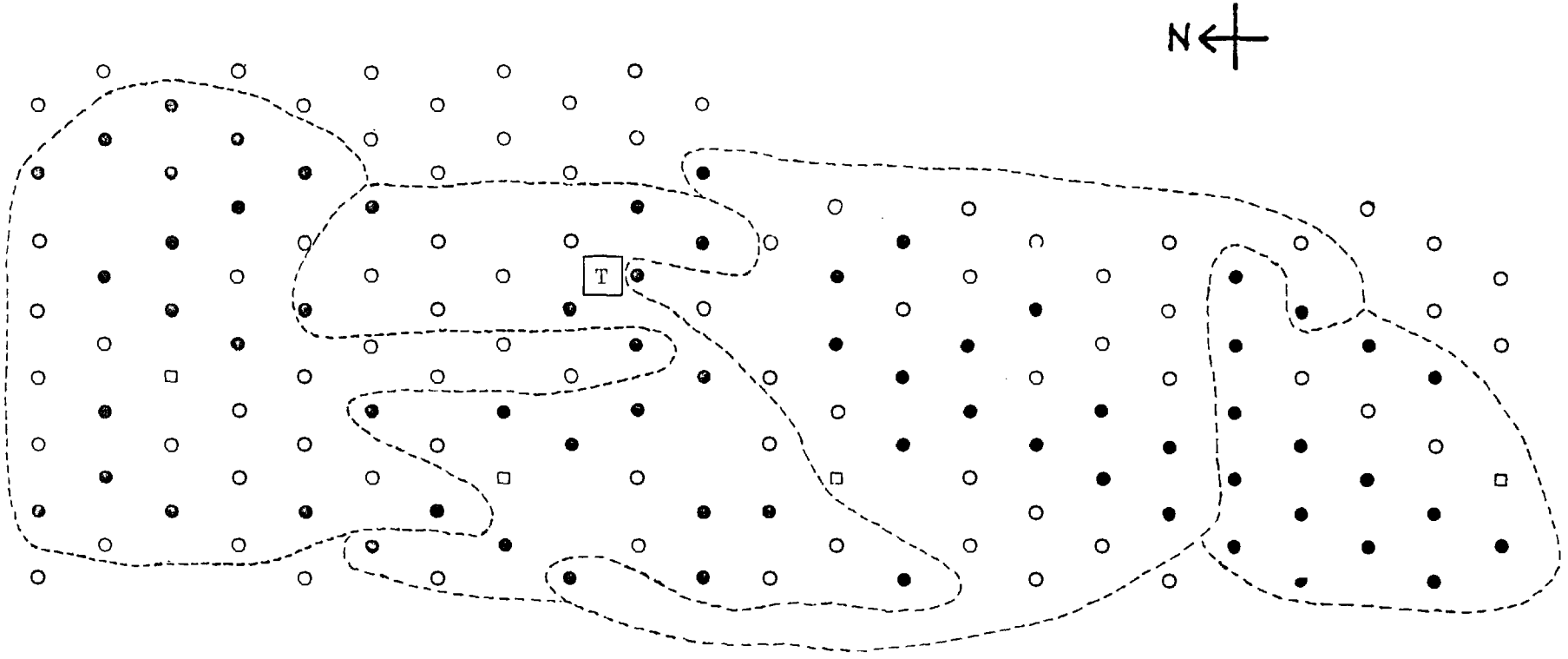
The trees were sampled 16 months after inoculation by removing pieces (c. 1 cm^3) of wood tissue along the length of any stain exposed by removing bark above and below the points of inoculation, and beyond. The extent of staining was recorded and slivers of the excised wood tissue were plated on V8-juice agar to recover *C. corticale*. The stain extended vertically from the inoculation point and was generally less than 1 cm in width.

The results, based on the extent of staining, are shown in Fig. 14 and Appendix Table 7. These indicate considerable variation between isolates of *C. corticale* in respect of their growth in sycamore as did the glasshouse experiment previously described. Again there was no evidence of clearly-defined pathogenic strains. However, the order in which isolates were placed differed from that obtained in the glasshouse experiment and from that shown by growth on 3% MEA at 15^{o} (Table 1).

Further analysis indicated that variation between two inoculation points on a single tree was generally less than variation between different trees inoculated with the same isolate (Table 3) since variance between blocks was greater than within blocks ($F = 3.714$; $P \leq 0.05$). The significance of the F value obtained for isolates indicates that at least one isolate is significantly different, as shown in the Duncan's new-multiple range analysis (isolates 57 and 108 are significantly different from 251).

Reisolation from stained tissue proved difficult although the

Fig. 13 : Growth of *C. corticale* in sycamore under field conditions: plot plan.



Key: T. Thermograph.

□ Control.

o Inoculated trees.

O Other trees.

--- Blocks A, B, C and D.

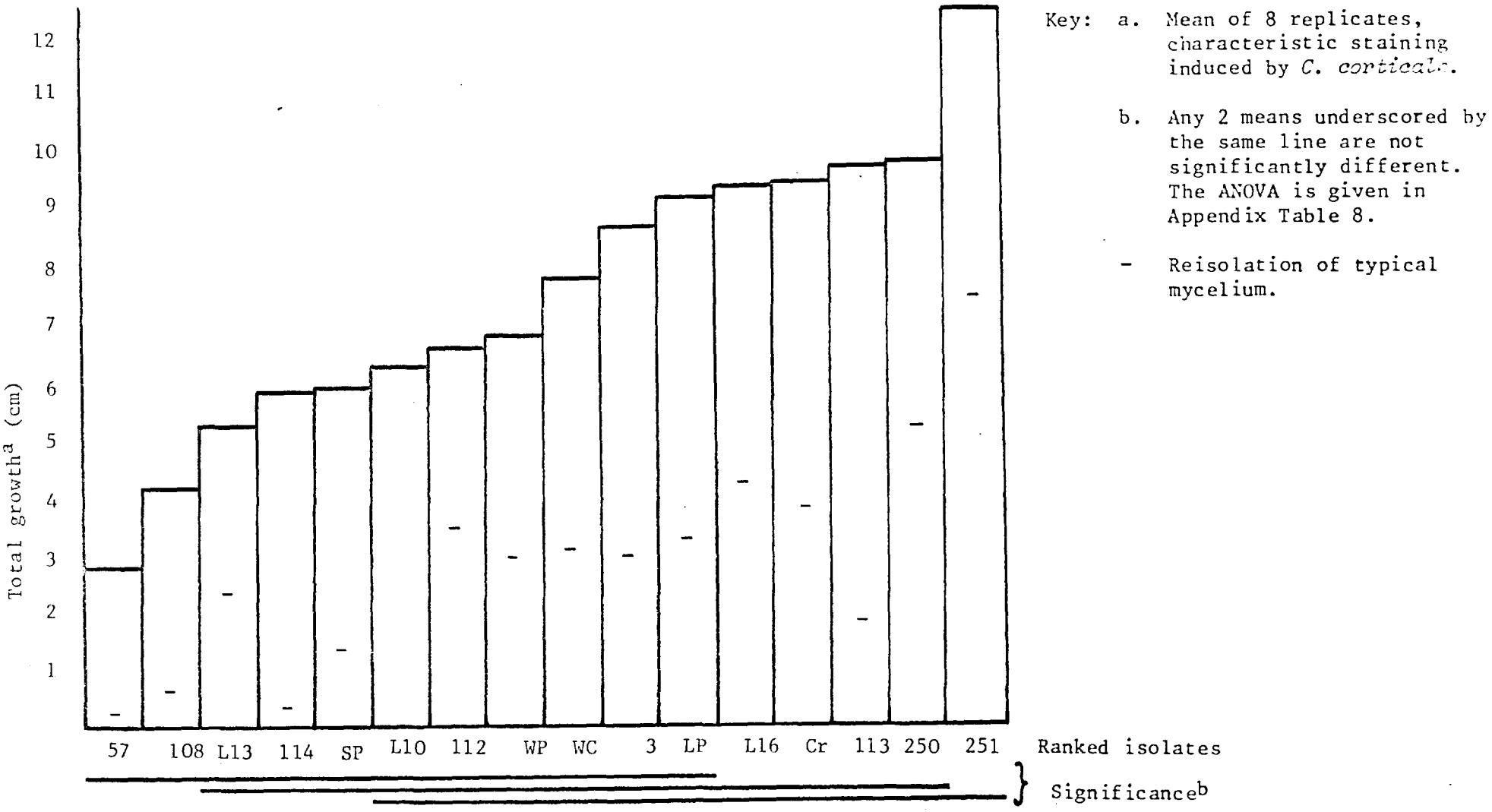


Fig. 14 : Growth of *C. corticale* in sycamore under field conditions.

TABLE 3 : ANOVA: stain induced by *C. corticalc* isolates in sycamore trees under field conditions.

	DF	SS	MS	F
Isolates	15	734.5	49.0	2.000 *
Blocks of trees	3	272.9	91.0	3.714 *
Interaction	45	1373.3	30.5	1.245 n.s.
Error	64	1569.0	24.5	

Key: *. Significant at $P \leq 0.05$.

correlation between reisolations and stain was high ($P \leq 0.001$, correlation coefficient calculated from mean values for each isolate = 0.834). Neither did success in reisolating seem related to position along the stain: treating the stained areas above and below points of inoculation separately, the fungus was reisolated along the length of the stain in 27, only near the point of inoculation in 18, only from the middle of the stained length in 9 and only from the limit of the stain in 14. *C. corticale* was not reisolated from 188 of 256 stained areas sampled.

The possibility that micro-organisms antagonistic to *C. corticale* had developed within stained areas was investigated. Slivers of tissue from the wood samples derived from block C were plated aseptically on V8 and NA. The fungi and bacteria reisolated were divided into groups which could be distinguished by their cultural characteristics. The isolates in each group were counted and a typical sample was tested for antagonism to *C. corticale*. Potential bacterial antagonists were streaked across freshly prepared PDA plates and fungi were applied as mycelial discs. Plates were incubated at 25^{o} and assessed after 9 and 14 days. Of the 37 groups of bacteria 9 proved antagonistic, these groups comprised 23 from a total of 161 colonies. Seven of the 23 groups of fungi inhibited growth of *C. corticale*, comprising 38 colonies from a total of 141. None of the fungi and bacteria which proved antagonistic to *C. corticale* were isolated frequently and thus they are unlikely to be important in displacing it from the wood.

(2) SUSCEPTIBILITY OF OTHER TREE SPECIES

The susceptibility to infection by *C. corticale* of a range of *Acer* spp. and other genera was investigated under conditions favourable for colonisation of sycamore tissue.

Growth in a Range of *Acer* spp. at 25°

The susceptibility of 12 *Acer* spp. was investigated in June 1979: *A. platanoides* L. (Norway maple), *A. campestre* L. (field maple), *A. saccharum* Marsh (rock or sugar maple), *A. nigrum* Michx. f. (black maple), *A. saccharinum* L. (silver or soft maple), *A. rubrum* L. (red maple), *A. negundo* L. (box elder), *A. cappadoicum* Gleditsch., *A. hersii* Rehd., *A. japonicum* Thunb., *A. palmatum* Thunb. (Japanese maple) and *A. palmatum dissectum atropurpureum*. The first six of these species are grown for their timber and attractive foliage; the remainder are grown primarily as ornamentals.

One tree of each type was inoculated on 1 June 1979 half-way up the stem by inserting under the bark a millipore disc charged with conidia collected from Laleham Park (p. 17). The germination of these conidia was checked by a standard test (p. 18) and found to be 70%. Inoculated trees and controls were placed in a glasshouse maintained as nearly as possible at 25°. Temperature records (Appendix Table 5) indicated that the daily mean over the experimental period varied between 20° and 26° with an overall mean of 23.1°. The heights and girths of the inoculated trees are given in Appendix Table 9.

The inoculated *A. nigrum* wilted after 19 days (Fig. 15), and was cut 2 days later for investigation. A lesion with young, thin-walled conidia of *C. corticale* extended 7 cm above and 2 cm below the point of inoculation. Here it was 5 mm wide but narrowed towards its limits. Externally the

Fig. 15 : *Acer nigrum* wilting 19 days after inoculation with *C. corticale*.

roots appeared healthy but when washed and cut many were found to be stained by *C. corticale*.

None of the other inoculated trees wilted during the experiment which was terminated after 5 weeks. The stems were cut at soil level and sampled within 2 days.

In a small, supplementary experiment, young trees of *A. griseum* Pax. (paperbark maple) and *A. nigrum* (kept from the collection purchased in 1979) were inoculated on 3 April 1980 with conidia (79% viability) of the Lalcham Park isolate produced on sterile sycamore wood. These trees were kept in the 25° glasshouse (daily means between 21° and 27° with an overall mean of 24°; see Appendix Table 5) for 6 weeks and then were cut and examined immediately.

C. corticale grew in all the *Acer* spp. tested but to different extents (Table 4; Appendix Table 9). *A. nigrum*, *A. saccharum*, *A. hersii* and *A. griseum* appeared relatively more susceptible than other spp. The fungus grew far more extensively in *A. nigrum* (1) than in other trees, this was not obvious from measurement of stain length since *C. corticale* had killed the upper part of the tree and the wood was uniformly pale brown in colour. The unexpectedly low values obtained for growth of the fungus in sycamore and the difference between colonisation of the two *A. nigrum* trees shows the importance of replication in comparisons of relative susceptibilities.

Growth of *C. corticale* in selected *Acer* spp. was compared to that in sycamore in replicated experiments:

a) *A. platanoides* is common in parts of Britain and its susceptibility (relative to sycamore) was investigated. Six trees of *A. platanoides* and 5 sycamores were inoculated on 1 June 1979 using millipore discs charged

TABLE 4 : Susceptibility of *Acer* spp. to infection by *C. corticale*.

Species	Stain	Reisolation	Confirmation[c]
EXPERIMENT 1[a]			
A. nigrum (1)	> 26.0	59	59
A. saccharum	19.0	21	20
A hersii	20.0	17	17
A. rubrum	8.5	8	3
A. negundo	15.0	7	7
A. palmatum	3.0	6	6
A. cappadoicum	8.0	6	4
A. saccharinum	6.5	5	5
A. japonicum	5.5	5	5
A. psuedoplatanus (1)	5.0	5	4
A. campestre	5.0	5	3
A. platanoides	3.0	3	1
A. palmatum dissectum atropurpureum	1.0	2	2
EXPERIMENT 2[b]			
A. griseum	12.0	12	8
A. nigrum (2)	7.0	6	-
A. psuedoplatanus (2)	2.0	2	-

Key: a. Growth in cm after 5 weeks at c. 25°.

b. Growth in cm after 6 weeks at c. 25°.

c. Sporulation on sterile wood.

with conidia from Laleham Park (70% viability), and kept at c. 25° (daily mean ranging from 20° to 26°, overall mean of 23.1°; Appendix Table 5). Three trees of each species were harvested after 2 weeks, the remainder after 5 weeks. All excised shoots were examined for stain. Those of sycamore were retained for other microscopic work (p. 70); samples from the stained areas in maple (*A. platanoides*) were plated on V8 juice agar to reisolate *C. corticale*. Analysis (Appendix Table 10) of the results (Table 5) showed there was no difference in the extent of staining in sycamore and Norway maple.

b) Results from inoculation of various *Acer* spp. indicated that susceptibility of *A. nigrum* to *C. corticale* may be greater than that of sycamore. The synonym of *A. nigrum* is *A. saccharum nigrum*, and it is closely related to *A. saccharum* which also appeared highly susceptaible to *C. corticale*. *A. saccharum* was selected for further investigation since it has greater economic importance than *A. nigrum*. Dormant trees (6 of *A. saccharum* and 7 of sycamore) were put into the 25° glasshouse on 10 January 1980. Day-length was extended to 15 h by a series of 100 W tungsten bulbs and under these conditions the trees produced leaves in c. 4-6 weeks. On 3 March 1980 they were inoculated with conidia produced on sterile wood (Laleham Park isolate; 79% viability) using millipore discs; a thermograph recorded daily means between 23° and 27.5° with an overall mean of 24.9° over the experimental period (Appendix Table 5). The trees were examined after 4 weeks for staining typical of *C. corticale* and for presence of the fungus. Results are given in Table 6; analysis (Appendix Table 11) showed the extent of colonisation by *C. corticale* to be similar in *A. saccharum* and in sycamore.

In 1978 ornamental *Acer* trees at Laleham Park were affected by sooty

TABLE 5 : Growth of *C. corticale* in *A. platanoides* and in *A. psuedoplatanus*[a].

Tree		Time (weeks)	Stain		Reisolation		Confirmation[b]	
			-	+	-	+	-	+
Norway maple (*A. platanoides*)	A	2	3.5	12	4	11	-	11
	B	2	7	6	5	7	-	7
	C	2	1	6	1	4	4	4
	D	3	2	4	2	4	-	4
	E	3	6	9	8	9	-	6
	F	3	10	12	10	9	10	9
Sycamore (*A. psuedoplatanus*)	A	2	9	15	not tested			
	B	2	12	5				
	C	2	3	10				
	D	3	8	6				
	E	3	9	8				

Key: a. Growth in cm at c. 25° below (-) and above (+) the inoculation point.

b. Sporulation on sterile sycamore wood.

TABLE 6 : Growth[a] of *C. corticale* in *A. saccharum* and in *A. psuedoplatanus*[a].

Tree		Stain		Reisolation		Confirmation[b]	
		-	+	-	+	-	+
Rock maple (*A. saccharum*)	A	3.5	1.0	4	1	4	-
	B	23.5	17.0	23	17	23	17
	C	12.5	24.0	12	23	12	23
	D	1.0	9.0	1	9	-	9
	E	0.5	2.0	1	2	-	2
	F	5.5	12.0	6	12	-	12
Sycamore (*A. psuedoplatanus*)	A	3.0	8.5	1	7	-	-
	B	3.6	3.6	4	4	4	4
	C	8.5	8.0	9	9	9	9
	D	1.7	8.5	2	8	2	8
	E	2.5	8.0	3	8	3	-
	F	9.0	6.0	9	7	9	7
	G	6.0	3.0	7	2	7	2

Key: a. Growth in cm at c. 25^{o} below (-) and above (+) the inoculation point.

b. Sporulation on sterile wood.

bark (Fig. 16 & 17). This observation of naturally infected ornamentals and the proven susceptibility of young trees of various *Acer* spp. at 25° suggests that sooty bark can affect any *Acer* when field conditions are suitable for disease development.

Growth in Other Genera at 25°

C. corticale has been reported on species of *Carya* and *Tilia*. This experiment was set up to examine the susceptibility of a wide range of trees and shrubs, viz:- *Cornus sanguinea* L. (common dogwood), *Corylus avellana* L. (hazel), *Euonymus europeaus* L. (spindle), *Fraxinus excelsior* L. (common ash), *Hippophae rhamnoides* L. (sea buckthorn), *Ligustrum vulgare* L. (common privet), *Platanus* x *hispanica* Muenchh. (London plane), *Populus nigra* L. (black poplar), *Populus tacamahaca* x *trichocarpa* (a hybrid balsam poplar), *Prunus spinosa* L. (sloe or blackthorn), *Quercus robur* L. (English oak), *Salix caprea* L. (goat willow or great sallow), *Salix viminalis* L. (common osier), *Ulmus carpinifolia* Gleditsch. (smooth-leaved elm), *Viburnum lantana* L. (wayfaring tree) and *Viburnum opulus* L. (guelder rose).

The young trees and shrubs and 3 sycamore controls were inoculated on 30 July 1979 using conidia from Laleham Park (82% viability) and transferred to a glasshouse at c. 25° (daily mean ranged from 19°-26° with a mean of 22.4°; Appendix Table 5). In most instances, two trees of each species were inoculated, but only one specimen of *C. avellana*, *Q. robur* and *U. carpinifolia* was available. After 5 weeks the trees were cut and examined for staining and presence of *C. corticale*. Staining was observed only in the sycamore controls. *C. corticale* was obtained from samples taken 1 cm from the inoculation point in both specimens of *E. europeaus* and one of *P. tacamahaca* x *trichocarpa*, *S. viminalis* and *V. opulus*, but could not be reisolated from the remaining species. In contrast *C.*

Fig. 16 : Die-back of ornamental *Acer* infected by *C. corticale*.

Fig. 17 : Sporulation of *C. corticale* beneath the bark of an ornamental *Acer*.

corticale grew a mean of 28 cm in the sycamore controls.

Sporulation of *C. corticale* on Sterile Wood from a Range of Species

C. corticale produced little or no growth in a wide range of genera in the previous experiment. However, the fungus has been reported on *Carya* and *Tilia* spp., and it may be able to sporulate on wood killed by other agencies to provide a reservoir of inoculum for infection of *Acer* spp. growing nearby. Sterile wood from *Acer* spp. was also used since the fungus had only been observed to sporulate on *A. nigrum* and sycamore in the short time allowed for inoculation experiments. The ability of *C. corticale* to sporulate on sterile wood was examined using *A. campestre*, *A. cappadocicum*, *A. hersii*, *A. japonicum*, *A. palmatum*, *A. rubrum*, *A. saccharinum*, *A. saccharum*, *Aesculus hippocastanum* L. (horse chestnut), *E. europeaus*, *Fagus sylvatica* L. (common beech), *L. vulgare*, *Platanus* x *hispanica*, *Platanus orientalis* L. (oriental plane), *P. nigra*, *P. spinosa*, *Q. robur*, *S. caprea*, *S. viminalis*, *Taxus baccata* L. (common yew) and *Tilia* x *europaea* L. (common lime).

Three replicate stem pieces (c. 5 cm) from each species were autoclaved at 125° for 90 min. The surface of the wood was allowed to dry under sterile conditions and each stem piece was inserted into a V8 slope covered by mycelium of isolate LP. After 2 months at 25° the stem pieces were examined for the characteristic conidial fructifications of *C. corticale*. Those which had not sporulated were transferred to sterile universal containers and re-examined after 5 months. The fungus sporulated on most wood species within 2 months and did so on the remainder within 5 months.

(3) TIME OF INOCULATION

The relationship between time of inoculation of sycamore and infection and colonisation by *C. corticale* was investigated.

Thirty trees each with at least 4 branches suitable for inoculation were selected within the experimental plot at Silwood Park (Fig. 18). One branch of each tree was inoculated with conidia collected from Laleham Park, using the millipore disc technique, at the following times during 1978:

(1) 24 February, when the trees were dormant,

(2) 16 May, at bud burst,

(3) 10 August, when the trees were in full leaf,

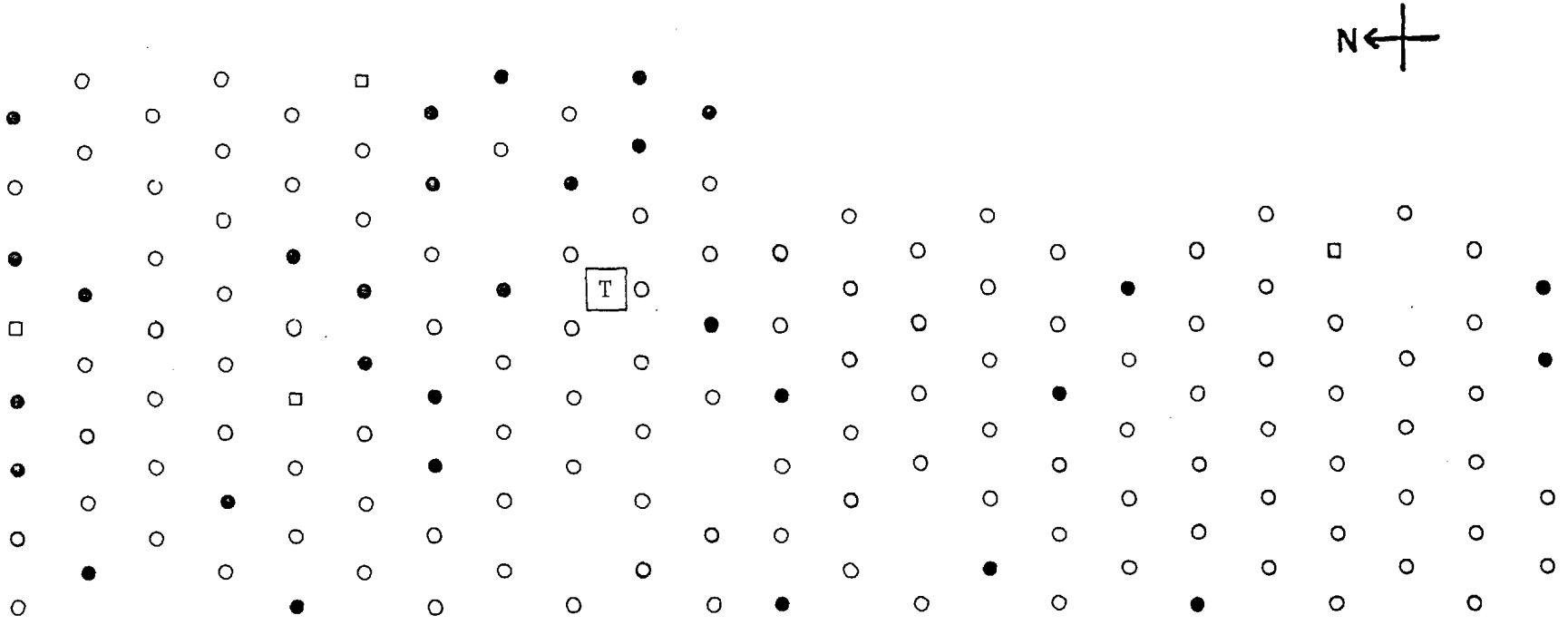
(4) 7 November, at leaf fall.

Tests of the conidia used in these inoculations indicated 94%, 78%, 94% and 89% germination respectively. On each occasion branches on 4 control trees were inoculated using spore-free discs.

Samples of branches inoculated with *C. corticale* and a corresponding control were taken at 6 month intervals in a sequence pre-determined from tables of random numbers. These branches were examined for staining and the presence of *C. corticale*. The cut stubs on the tree were sealed with Vaseline.

Inoculation with *C. corticale* often caused increased radial expansion of the sapwood resulting in bark splitting (Fig. 19) or more rarely production of a ridge extending vertically above and below the point of inoculation. The line of splitting and/or the ridge was rarely more than 1 cm wide and its position corresponded with a more extensive underlying area of stained wood colonised by the fungus; this stained area was also generally less than 1 cm wide. The bark splitting usually involved only

Fig. 18 : Plot plan for time of inoculation field experiment.



Key: T. Thermograph.

□ Control

● Inoculated tree.

○ Other trees.

Fig. 19 : Bark splitting following inoculation with *C. corticale*.

Fig. 20 : Distorted sapwood growth (→) beneath the disc of inoculum.

the outermost bark but occasionally extended to the cambium. The line of split bark varied in length from 1-10 cm from the inoculation point and the longest ridge extended for 8 cm. Growth of the sapwood beneath the disc of inoculum was sometimes distorted (Fig. 20).

A few of the inoculations were unsuccessful because due to the inaccessibility of certain branches only the upper bark layers were removed and inoculum was placed on the inner bark. This was only evident after sampling and such inoculations were omitted from the analyses of results. The fungus failed to colonise one branch although distortion of the sapwood occurred beneath the disc of inoculum, but other branches on this tree were inoculated successfully at other times. Occasionally when the fungus had made little progress no stain was observed.

A thermograph at canopy height (4 m) recorded temperature throughout the experiment (Table 7, Appendix Table 6). The temperatures over the 6 month period from inoculation to the first sampling were lowest for trees inoculated in November and highest for ones inoculated in February and May. After 12, 18 and 24 months there was little difference in the mean temperatures over the period from inoculation to sampling. In the summers of 1978 and 1979 five of the summer months (June, July, August) were particularly cool with mean daily maximum temperatures less than 20^{o} (Appendix Table 12). July 1979 was warmer with a mean daily maximum temperature of 21.5^{o}. However, Young (1978) considers disease is unlikely to become acute unless the monthly maximum exceeds 23^{o}.

The results are summarised in Table 8 and detailed in Appendix Tables 13 and 14. Means for the samples taken after 6, 12, 18 and 24 months for each time of inoculation suggest that the fungus makes some growth but is then virtually stopped, i.e. there is no apparent progression of fungal

TABLE 7 : Mean monthly temperatures for time of inoculation experiment.

Months after inoculation	Time of inoculation			
	February	May	August	November
1	6.5	13.4	14.4	6.3
2	7.0	13.7	12.8	3.3
3	10.4	15.2	10.9	1.7
4	14.1	14.5	6.2	2.8
5	13.9	12.4	2.9	4.8
6	15.3	10.3	1.6	8.6
Mean after 6 mth:	11.20	13.25	8.13	4.58
7	14.1	5.9	3.1	12.3
8	14.7	1.7	4.9	14.3
9	10.4	1.6	8.9	16.0
10	3.1	3.8	12.5	14.0
11	2.0	5.9	14.6	12.2
12	1.4	9.5	15.8	10.5
Mean after 12 mth:	9.41	9.0	9.05	8.9
13	4.6	12.2	14.2	7.0
14	7.7	15.0	12.2	5.2
15	9.8	15.8	9.7	3.2
16	13.4	13.2	7.3	6.1
17	15.1	12.3	4.6	5.9
18	15.2	8.2	3.9	8.7
Mean after 18 mth:	9.93	10.25	8.92	7.94
19	13.1	8.1		
20	11.9	3.2		
21	6.6	4.8		
22	7.7	5.6		
23	3.1	6.7		
24	5.5	9.4		
Mean after 24 mth:	9.44	9.27		

TABLE 8 : Growth (cm) of *C. corticale* in sycamores inoculated at different times.

	Inoculation time	Sampling time (months after inoculation)			
		6	12	18	24
a) Stain[a]	Feb.	13.8	6.9	6.2	7.8
	May	9.5	5.8	9.0	6.2
	Aug.	6.5	7.2	5.7	-
	Nov.	2.5	2.6	8.3	-
b) Reisolation[a]	Feb.	15.5	7.3	5.3	7.2
	May	5.1	4.3	7.0	2.8
	Aug.	3.7	5.7	3.1	-
	Nov.	1.9	0.2	3.2	-
c) Confirmation[ab]	Feb.	8.2	3.0	3.5	6.0
	May	2.7	3.5	5.8	1.2
	Aug.	1.4	4.4	2.2	-
	Nov.	1.4	0.2	1.0	-

Key: a. Mean of total growth in all branches sampled, but excluding failed inoculations.

b. Sporulation on sterile wood.

growth with time. On this basis the data for the 6 month sample only were analyzed first.

Analyses of the results for staining (Table 9a) showed that there were no differences with respect to the time at which the trees were inoculated with *C. corticale*. A similar analysis of data for re-isolation of characteristic mycelium showed that the fungus grew more extensively from inoculations in February than from those at any other time (Table 9b). However, certain values obtained for this inoculation after 6 months did appear unusually high compared to the other samples in this series.

Possible bias due to this high value was minimized by analyzing the data as a whole. These analyses (Table 10) showed that growth of the fungus, as indicated both by the extent of staining and re-isolation of characteristic mycelium, was significantly less ($P \leq 0.05$) following inoculations in November than at any other time. The data for re-isolation of mycelium also indicated that fungal growth was most extensive following inoculations in February ($P \leq 0.05$). This was not so apparent in the data for staining mainly because the extent of staining following inoculations in May and August was somewhat greater than the corresponding figures for re-isolation of mycelium. Indeed, re-isolations from inoculations other than those in February tended to be erratic (Table 11).

The growth of the fungus above and below the inoculation point was also examined. The data for all samples following each inoculation were bulked (Table 12) since there was no real progression of fungal growth with time (see Table 8). No difference was found in the extent of growth above and below the inoculation point, even following the February inoculation. A t-test on the data for re-isolation of mycelium for this inoculation date indicated no difference ($t = 1.66$, n.s. at 22 df).

TABLE 9 : Analysis of mean growth (cm) of *C. corticale* 6 months after inoculation at different times.

a) Stain

ANOVA

	DF	SS	MS	F
Times of inoculation	3	465.32	155.11	1.444 n.s.
Error	27	2901.26	107.45	

Duncans new multiple range test[a]

Time of inoculation	Nov.	Aug.	May	Feb.
Ranked means	2.6	6.5	9.4	13.8

b) Reisolation of characteristic mycelium

ANOVA

	DF	SS	MS	F
Times of inoculation	3	734.86	244.95	5.96**
Error	27	1109.33	41.086	

Duncans new multiple range test[a]

Time of inoculation	Nov.	Aug.	May	Feb.
Ranked means	1.9	3.7	5.1	15.5

Key: a. Any means not underscored by the same line are significantly different.

** $P \leq 0.01$

TABLE 10 : Analysis of mean growth (cm) of *C. corticale* after inoculation at different times.

a) Stain

ANOVA

	DF	SS	MS	F
Times of inoculation	3	269.30	89.767	1.685 n.s.
Error	96	5114.82	53.279	

Duncans new multiple range test[a]

Time of inoculation	Nov.	Aug.	May	Feb.
Ranked means	4.11	6.72	7.65	8.72

b) Reisolation of characteristic mycelium

ANOVA

	DF	SS	MS	F
Times of inoculation	3	680.22	226.74	8.728***
Error	96	2494.02	25.979	

Duncans new multiple range test[a]

Time of inoculation	Nov.	Aug.	May	Feb.
Ranked means	1.30	4.21	4.76	8.91

Key: a. Any means not underscored by the same line are significantly different.

*** $P \leq 0.001$

TABLE 11 : Reisolation of characteristic mycelium from stained tissue.

Time of inoculation	Percentage reisolation[a]				
	6 mth	12 mth	18 mth	24 mth	Mean
February	100	100	86	92	95
May	54	75	78	46	63
August	66	76	54	-	65
November	75	8	38	-	41

Key: a. (Mean reisolation/mean stain) x 100

TABLE 12 : Mean growth of *C. corticale* above and below the inoculation point.

	Feb.	May	Aug.	Nov.
a) Stain				
Above inoc. pt.	4.72	4.40	3.38	2.22
Below inoc. pt.	4.00	3.29	3.37	1.89
b) Reisolation				
Above inoc. pt.	5.43	2.60	2.07	0.74
Below inoc. pt.	3.48	2.24	2.14	0.83

In some instances, tissues stained and colonised by *C. corticale* were found beneath a layer of new sapwood. Where this occurred the fungus generally appeared to have grown more extensively than from other inoculations where growth was at the surface of the sapwood cylinder. This was examined in a t-test using all the available data from 6, 12 and 18 month samples. A preliminary analysis indicated that for each type of colonisation there was no progression of the fungus with time (Appendix Table 15). Colonisation beneath sapwood occurred in 17 inoculations, compared with 38 occasions where the fungus grew at the surface of the sapwood (Appendix Table 16). The comparison indicated that in the deeper tissues growth of the fungus was significantly more extensive ($\underline{P} \leq 0.05$).

(4) MODE OF ENTRY

Conidia were applied to intact and damaged sycamore tissue to determine possible points of entry in the infection of healthy trees by *C. corticale*.

Infection of young sycamore. The ability of *C. corticale* to infect sycamore by various routes was investigated. A 6 mm millipore disc (Type SM, 5 µm pore size) charged with conidia (c. 2.3×10^3, 94% germination) collected from Laleham Park was used as a standard inoculum. Six sycamores (1-1.5 m, from Silwood Park) were used to investigate penetration via autumn leaf scars. Four sycamores were inoculated on 3 November 1977 by placing spore discs over leaf scars exposed by gentle downward pressure on the stalk of leaves about to abscise, and 2 control trees were inoculated by positioning discs over apparently undamaged bark of stem internode regions. The discs were held in position by parafilm and the trees were placed in a glasshouse maintained at normal autumn temperatures (daily means between 9 and 17° with an overall mean of 12.5°, Appendix Table 17). Two trees and the controls were examined for stain and presence of *C. corticale* after 14 weeks. None of the 32 leaf scar inoculations had caused infection and only one of the 22 control inoculations resulted in colonisation (2 cm). This contrasts with trees inoculated by placing a spore disc beneath the bark on 22 November 1977 and kept in the same glasshouse, where colonisation was evident in all trees sampled 4 or more weeks after inoculation (see Table 18). The remaining 2 trees were placed in a cool shady place and sampled after 30 months, but colonisation did not occur.

A second experiment investigated other possible routes of entry. Eight young sycamores (2-2.5 m high, from Silwood Park) were inoculated

on 15 May 1978 with conidia from Laleham Park (82% germination). The millipore disc with the conidial inoculum was applied to pairs of these trees in one of four ways:(a) on undamaged bark, (b) over 3 parallel scalpel cuts, (c) over the cambium, and (d) over the sapwood. The outer tissues were replaced in treatments (c) and (d) and the wounds sealed with parafilm. In treatments (a) and (b) stems were inoculated at 2 points and the discs were held in place with parafilm.

All trees were kept in a glasshouse at 25° (Appendix Table 5) for one year and then cut at soil level. The stems were examined for stain and presence of *C. corticale*.

The fungus did not penetrate through intact bark and grew only a few millimetres through scalpel wounds. It grew extensively where inoculum was placed on the cambium (48, 57 cm) and sapwood (>65, 51 cm). The limited growth resulting from infection via scalpel cuts suggests that penetration by many spores may be necessary for the rapid colonisation observed following inoculation of wounds 6 mm in diameter.

In a further experiment a camel hair brush was used to apply spores more closely to the potential penetration site. Eight young sycamores (2-2.5 m high, from Silwood Park) were inoculated on 1 June 1978 with conidia from Laleham Park (90% germination). Each tree was inoculated at several points along the stem at one of the following sites (2 trees/site): (a) unwounded bark; (b) 3 parallel scalpel cuts, c. 6 mm long; and at scars resulting from removing (c) small twigs and (d) leaves. The conidia were sealed in position using parafilm and the trees were placed in a glasshouse at c. 25° (Appendix Table 5) until sampling 5 weeks later. Examination for stain and *C. corticale* (Table 13) showed no evidence of infection through undamaged bark. Extensive colonisation resulted from brushing

TABLE 13 : Mode of entry of *C. corticale* into young sycamore.

Inoculation site	Tree	Inoculation point	Stain[a]	Reisolation[a]
Scored bark	1	a	37.0	38
		b	38.0	37
	2	a	27.0	26
		b	25.0	25
Broken twig	1	a	0.3	2
		b	10.0	12
		c	13.0	13
		d	0	1
	2	a	3.5	2
		b	7.0	6
		c	9.0	10
		d	9.0	9
Leaf scar[b]	1	a	0	0
		b	0	1
		c	0	0
		d	0	0
	2	a	0	3
		b	0	0
		c	0	0
		d	0	0
		e	0	1
Under bark (control)		a	5.5	4

Key: a. Growth (cm) after 5 weeks at 25°.

b. Exposed by tearing off leaf.

conidia over scored bark. The greater number of spores in contact with susceptible tissue compared with the disc technique used in the previous experiment may account for the more extensive colonisation. *C. corticale* gained entry through wounds produced by tearing off twigs and growth was well established after 5 weeks. Where leaves had been torn off the fungus was able to colonise from only a few inoculations, and made only limited growth. However, other experiments suggest that *C. corticale* is unable to infect via fresh leaf scars in autumn, probably because of changes associated with formation of the abscission layer.

Infection of sycamore under field conditions. A sycamore tree (c. 10 m) growing at the edge of the plot at Silwood Park was inoculated on 13 November 1978 to investigate infection of leaf scars in autumn under field conditions. Leaves ready to abscise and other firmly attached ones were pulled off and the resulting scars were inoculated with conidia from Laleham Park (89% germination) using a camel hair brush. Some scars were left exposed and others were sealed using "Scotch" pressure sensitive tape. Mean daily temperatures are recorded in Appendix Table 6. The inoculated twigs were harvested after 11 months and examined for stain and presence of the fungus. *C. corticale* did not colonise the tissues via any of the 30 inoculated leaf scars.

To investigate the possibility of transmission by root contact young sycamore seedlings (c. 30 cm) growing near the stump of a diseased tree were cut at soil level and examined for infection by *C. corticale*. The infected tree had been felled in October 1977 at Sunninghill Park, Ascot and the seedlings were inspected 8 months later. The young trees were free of infection but it was not established whether their roots had been in contact with those of the diseased tree. Sucker growth (c. 1 m) which

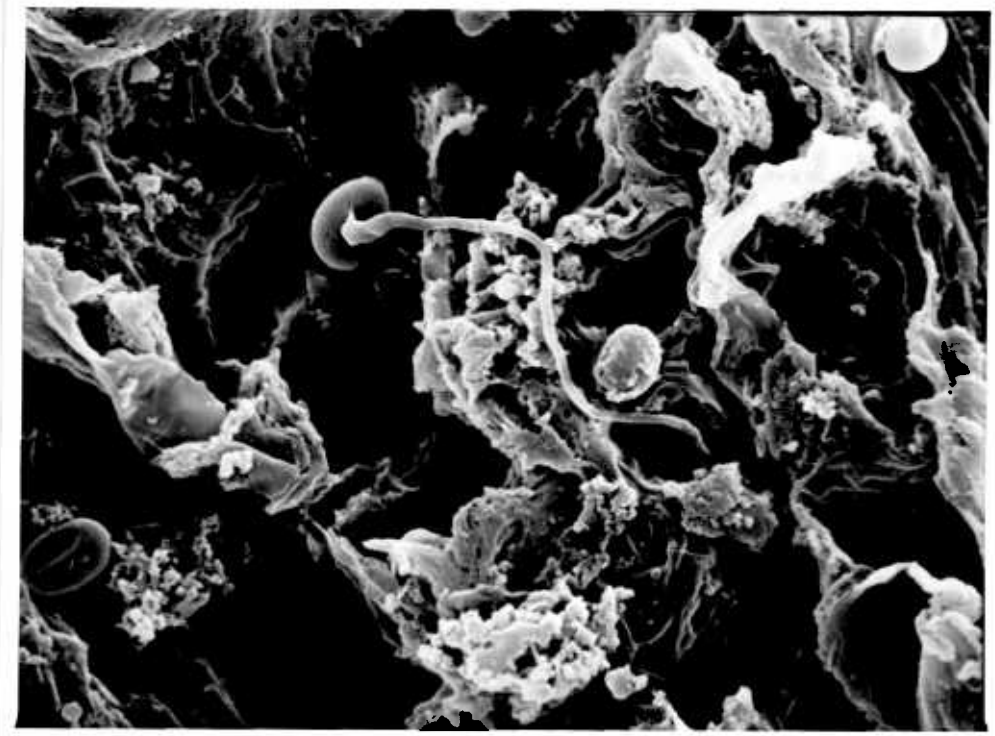
had developed on some stumps at the same site was examined 29 months after felling and found to be free of infection.

Penetration of sycamore wood at 25°. Five young sycamores (1-1.5 m) from a single seed stock were placed in a glasshouse at 25° for 7 weeks under extended daylength (15 h light, 9 h dark) to break their dormancy. On 29 January 1980 each tree was inoculated with conidia of the Laleham Park isolate produced in culture (79% germination) using the millipore disc technique. The sycamores were inoculated at c. 6 evenly-spaced points along the stem; after 9, 12, 15, 18 and 24 h a tree was cut and samples were immediately fixed in FAA.

Pieces of fixed wood from the points of inoculation were dehydrated and coated with gold for examination with a scanning electron microscope (SEM). Many conidia in samples taken only 9 h after inoculation had germinated and produced long germ tubes (up to c. 50 µm; Fig. 21). A few conidia produced shorter, wider germ tubes (Fig. 22). After 12 h some germ tubes appeared to fork (Fig. 23). Penetration occurred within 15 h, either through pits (Fig. 24) or directly through the cell wall (Fig. 25). The germ tube was often swollen near the penetration point, and the host tissue appeared to have been chemically dissolved where the hypha passed through (Fig. 24 & 25). When the specimen was subjected to high voltage (25 kV) it tended to break up and the fracture points were generally along the path of hyphae, suggesting that the fungus weakens the cell wall (Fig. 26).

Fig. 21 : Long narrow germ tube; 9 h after inoculation. X 2500.

Fig. 22 : Short, wide germ tube; 9 h after inoculation. X 10000.



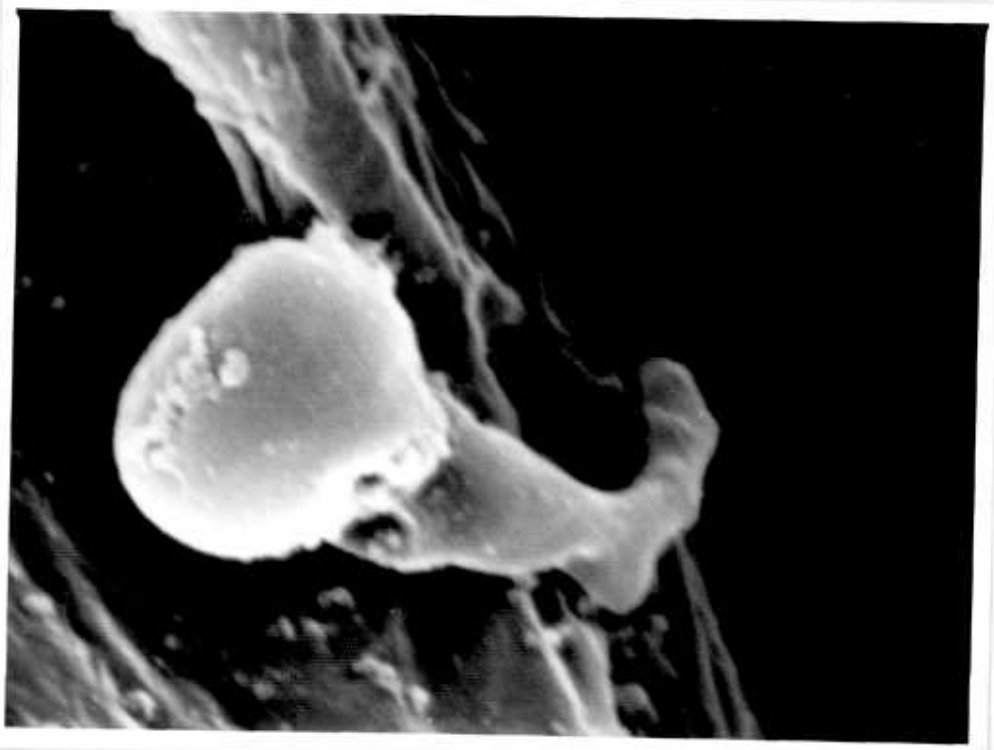
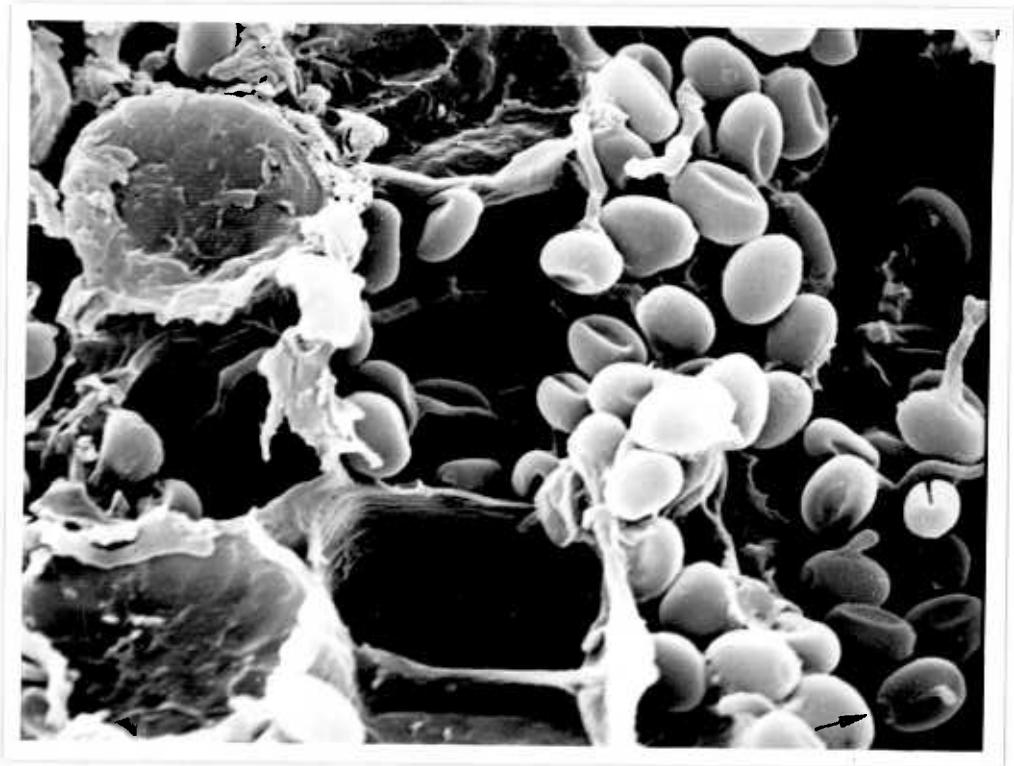


Fig. 23 : Forked germ tubes; 12 h after inoculation. Scars are visible where conidia were joined (↑). X 2500.

Fig. 24 : Penetration via pits; the membrane has dissolved where the hypha passed through. X 5000.



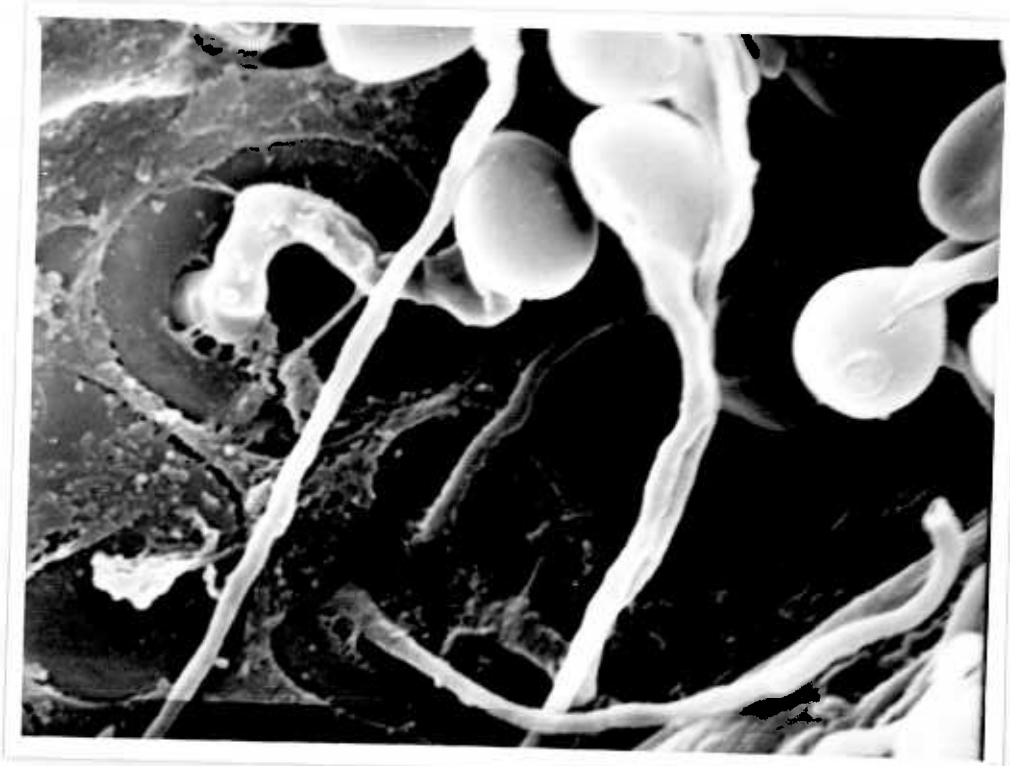
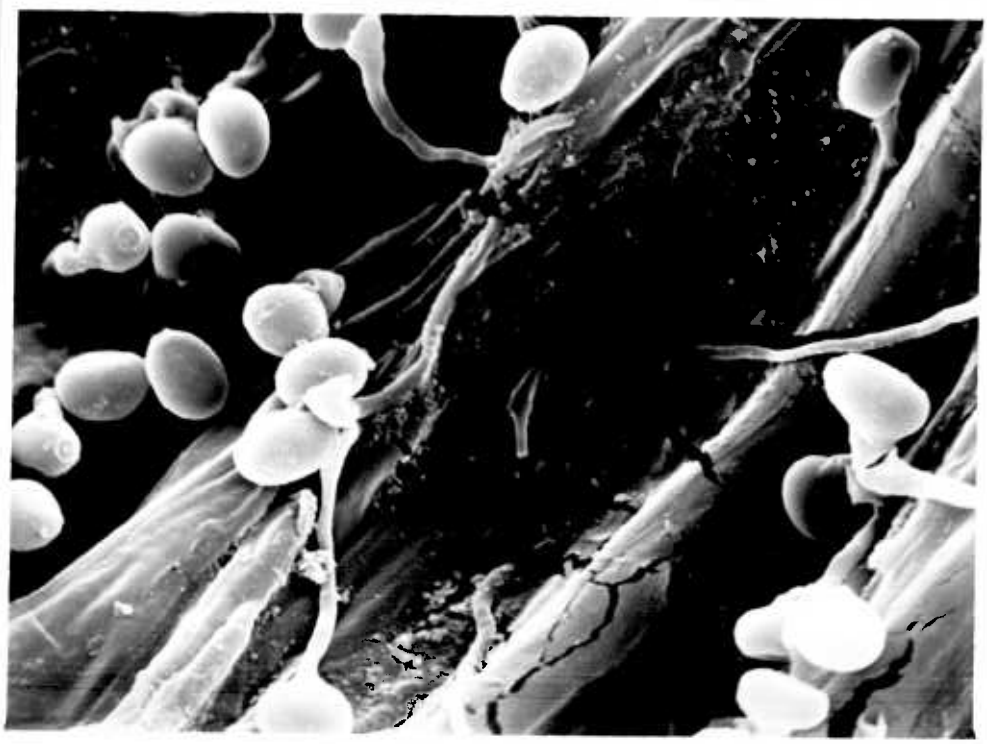


Fig. 25 : Penetration of the cell wall which has been degraded where the hypha passed through. X 20000.

Fig. 26 : Fracturing of cell walls under high voltage; cell walls appear weakened beneath hyphae. X 2500.



(5) COLONISATION OF SYCAMORE TISSUES

Infected sycamores were examined microscopically to determine which tissues are colonised by *C. corticale*.

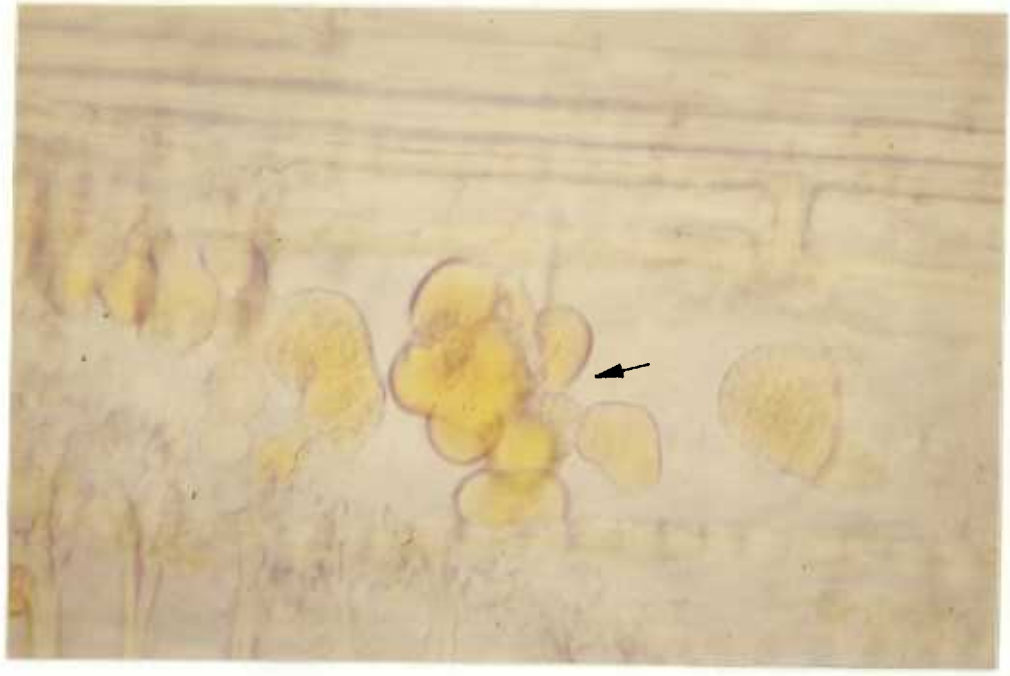
Light microscopy. Fresh or FAA-fixed tissue from infected sycamores was sectioned at 20 or 40 µm longitudinally and radially using a Reichert microtome (p. 23). Sections were usually stained with safranine and picro-aniline blue (Gurr, 1965). Hyphae appeared deep blue and lignified walls deep pink. Thionin and Orange G (Stoughton, 1930) was also used: cellulose walls stained yellow or green, lignified tissue blue and hyphae violet. With this procedure, the host tissue stained less deeply than with safranine picro-aniline blue but the hyphae were less distinct. All sections were mounted in Canada balsam. Fresh tissue was also examined unstained and mounted in glycerol. Material from uninoculated controls was also examined.

In sapwood colonized by *C. corticale* many vessels (Fig. 27, 28), fibres (Fig. 29) and ray cells (Fig. 30) were filled or partially filled with gum-like material, usually of granular appearance. Such cells were observed along the length of stain produced by *C. corticale* and were thus not associated solely with a wound reaction at the point of inoculation.

Hyphae of *C. corticale* were fairly sparsely distributed throughout the sapwood. Zones of relatively dense infection (Fig. 31) were infrequent, and examination of most of the stained wood did not reveal any fungus. Hyphae were most commonly observed in vessels and fibres, where the diameter of individual hyphae varied considerably (Fig. 32, 33). Single hyphae often ran vertically for long distances in individual vessels or fibres (Fig. 34). Occasionally hyphae penetrated the cell wall (Fig. 35) and this generally took place through pits (Fig. 36). In unstained

Fig. 27 : Gum (→) in vessel of infected sycamore wood. RLS X 1400.

Fig. 28 : Gum (→) filling vessel of infected sycamore wood; stained with safranine picro-aniline blue. RLS X 2100.



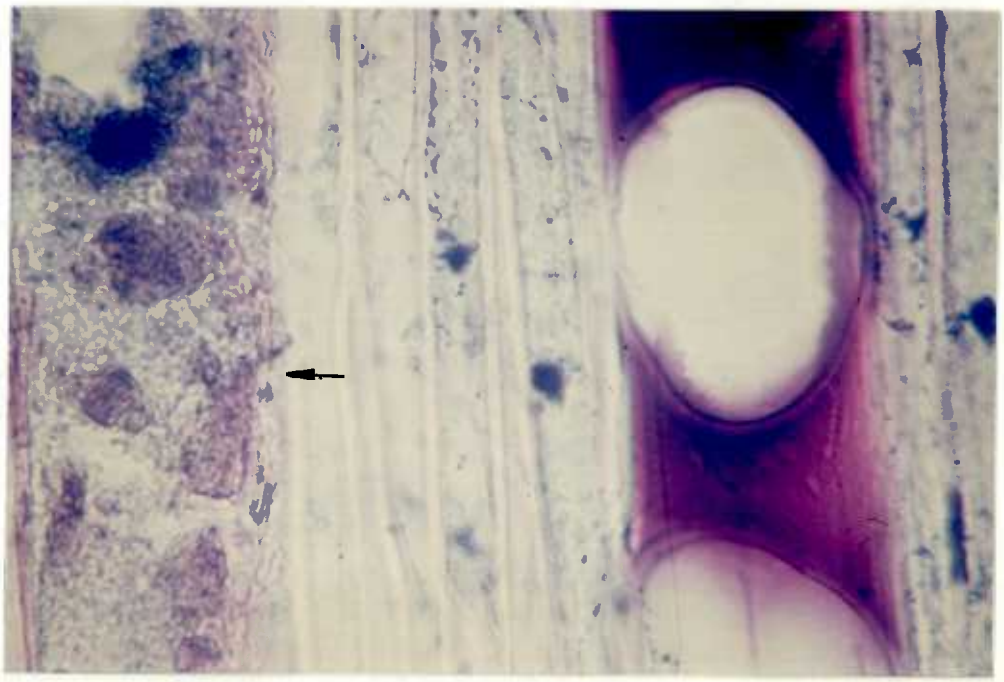
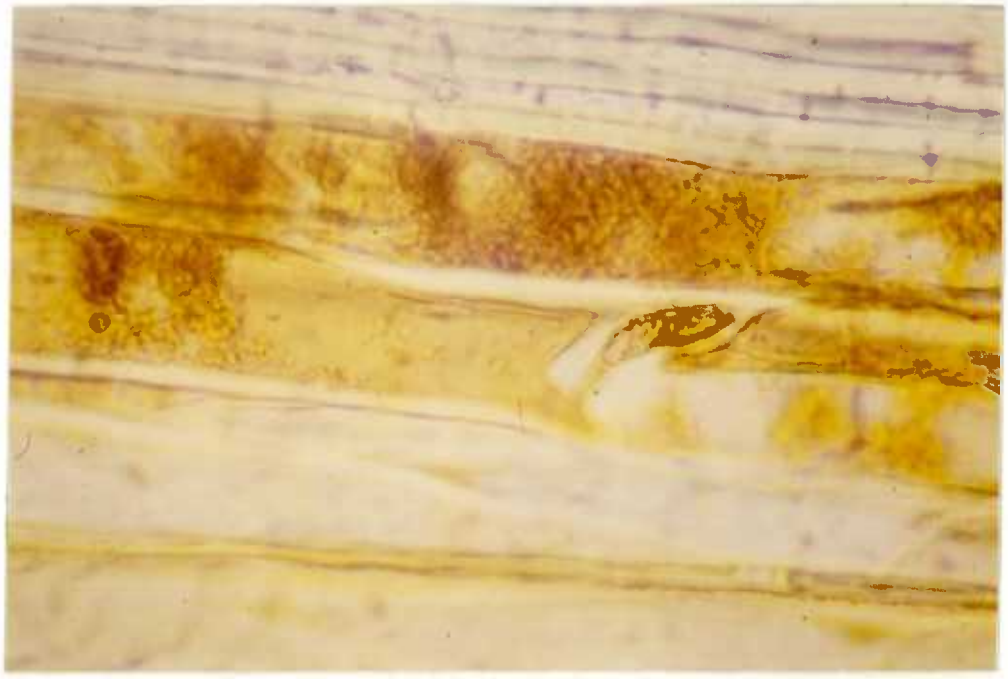


Fig. 29 : Gum filling fibres of infected sycamore wood. RLS X 2100.

Fig. 30 : Gum in ray cells of infected sycamore wood. RLS X 3500.



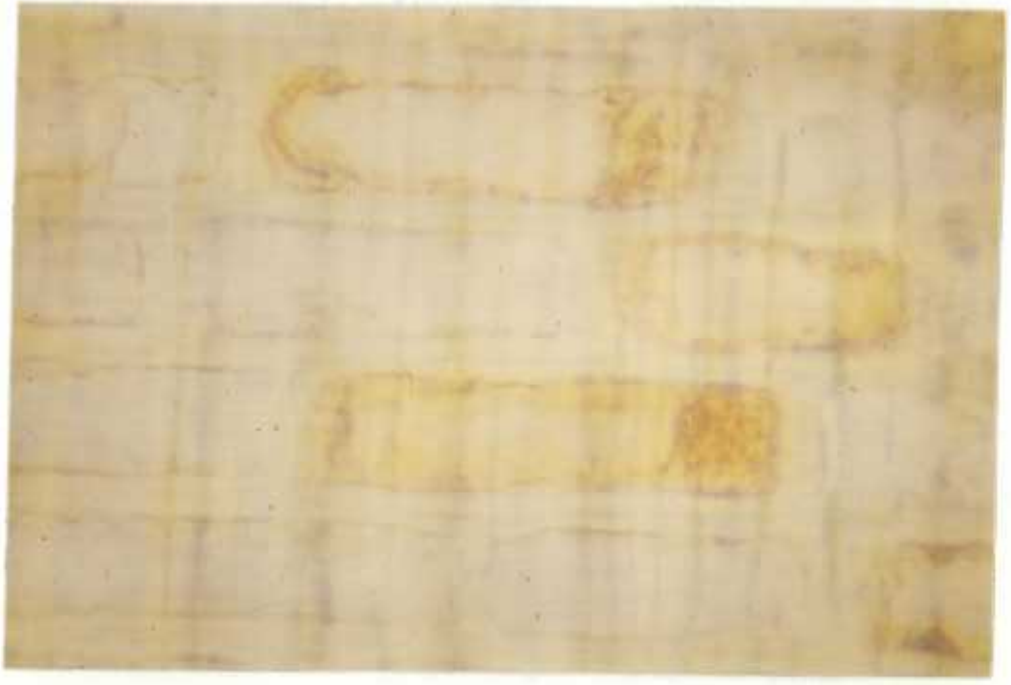
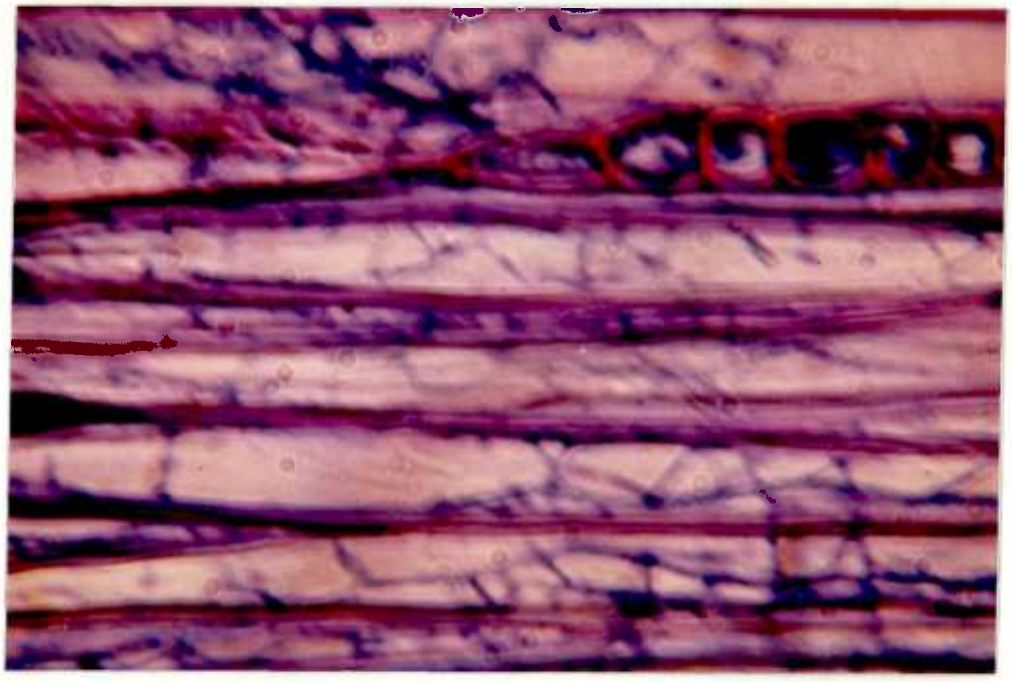


Fig. 31 : Fibres containing numerous hyphae; stained with safranine picro-aniline blue. TLS X 3500.

Fig. 32 : Thick hypha in vessel and thin hypha in fibre; stained with safranine picro-aniline blue. TLS X 1400.



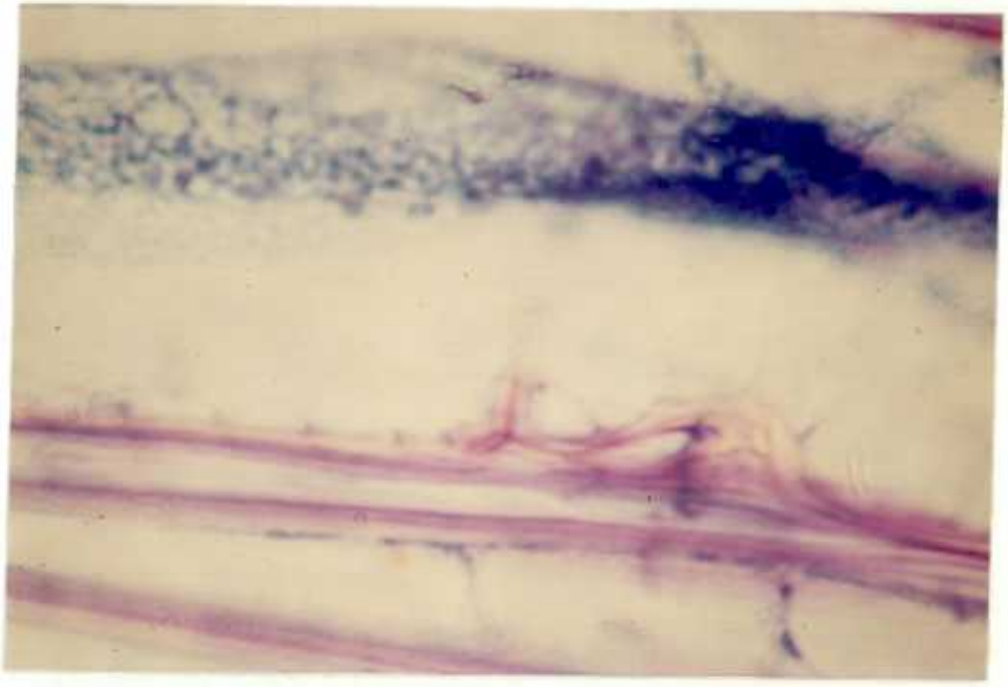
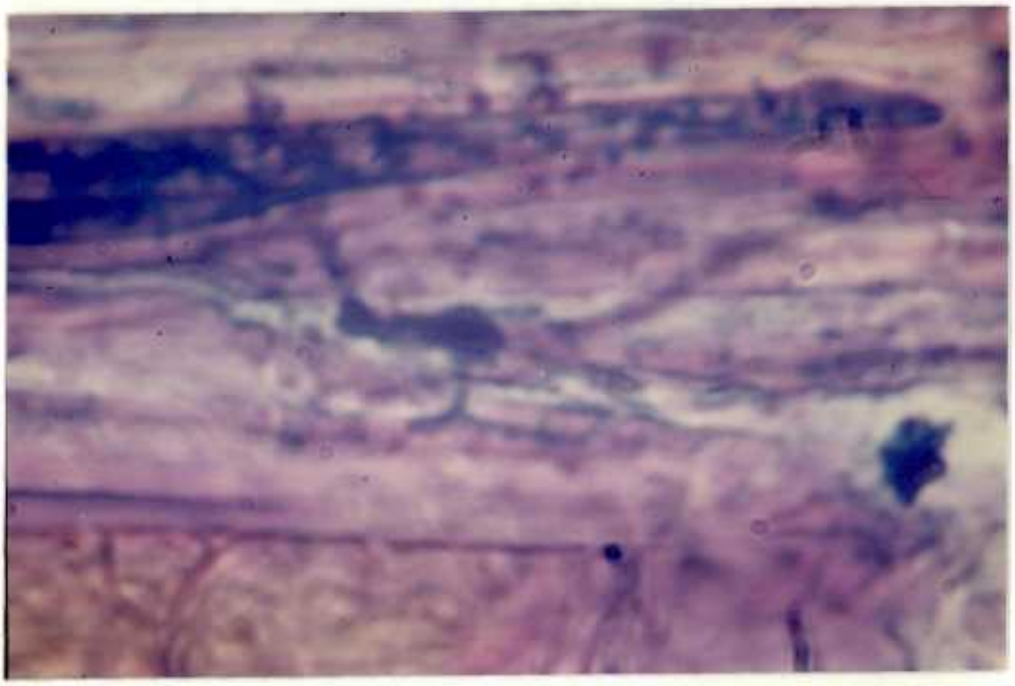


Fig. 33 : Thick hypha with narrower branch which passes through the cell wall to colonise the adjacent fibre; stained with safranine picro-aniline blue. RLS X 3500.

Fig. 34 : Long hypha with short, side branches attaching it to the fibre wall; stained with safranine picro-aniline blue. TLS X 1400.



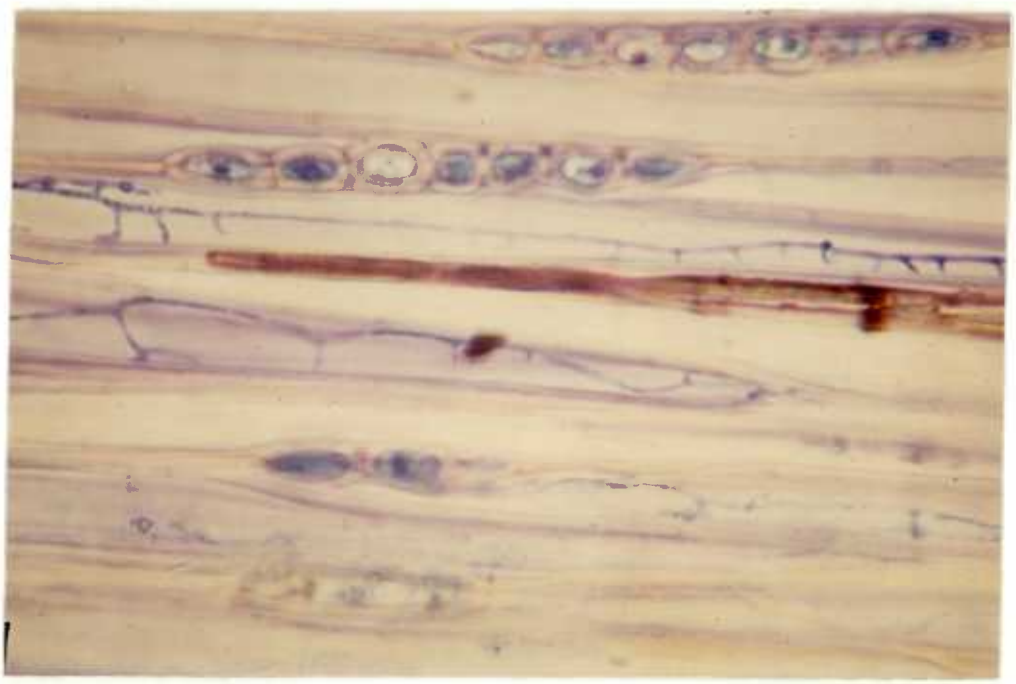
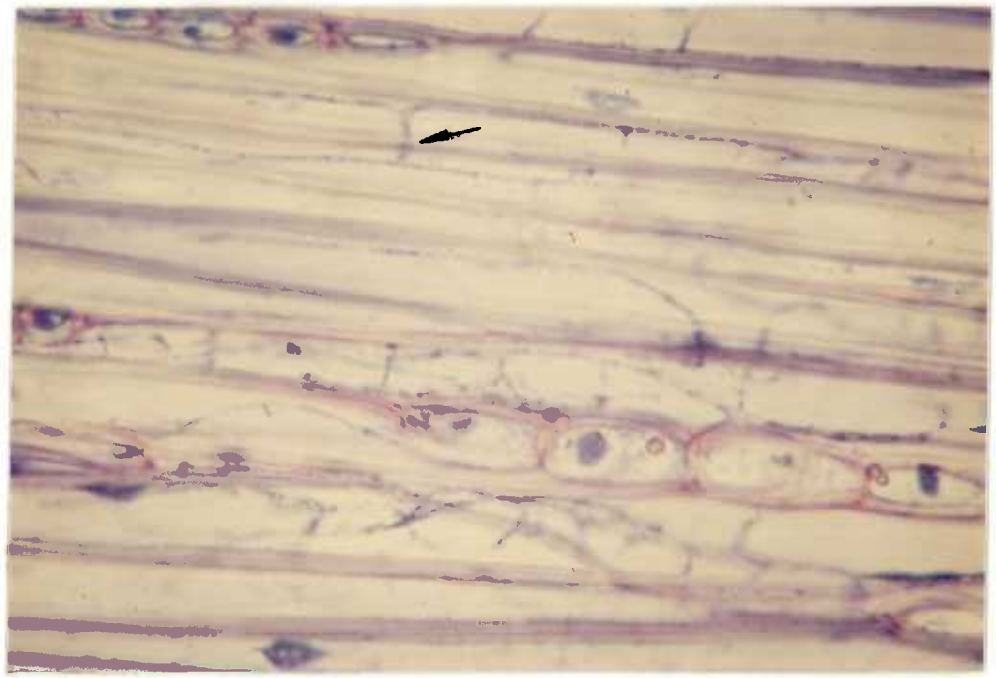
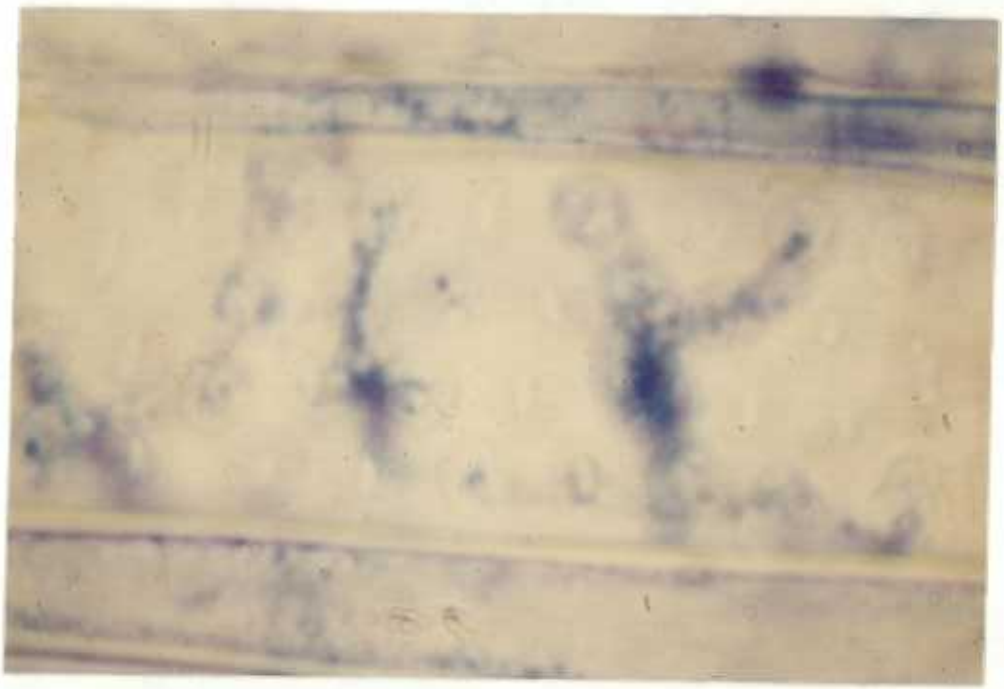


Fig. 35 : Hyphae passing through cell walls (→); stained with safranine picro-aniline blue. TLS X 1400.

Fig. 36 : Hyphal branches terminating directly over pits, through which they probably continue into the adjacent cell; stained with safranine picro-aniline blue. TLS X 3500.





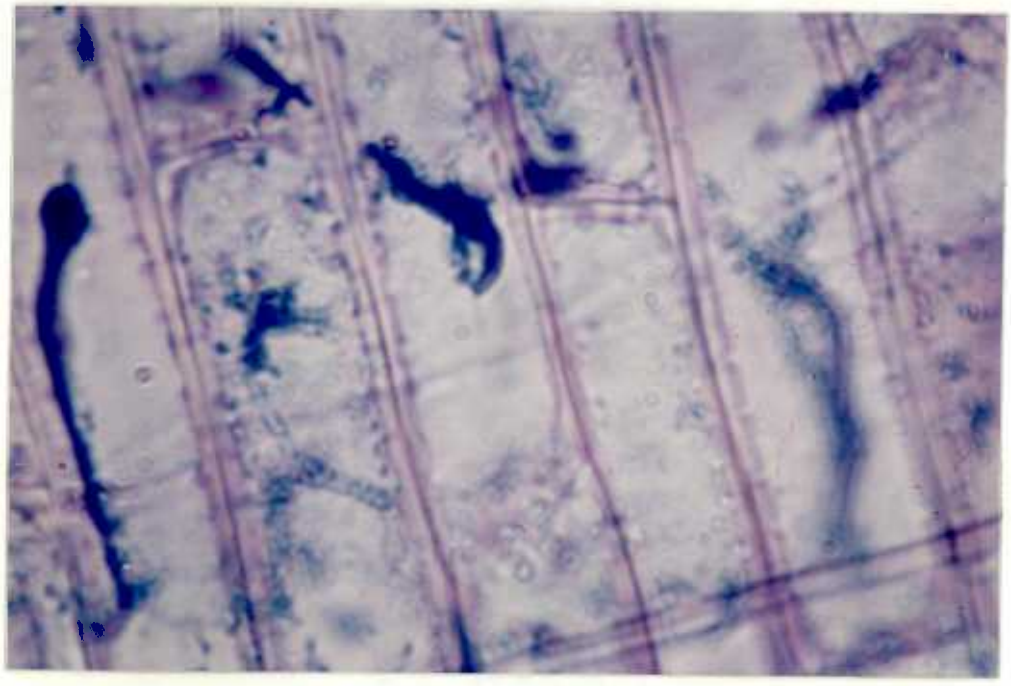
preparations hyphae were observed within material blocking vessels (Fig. 37) and in one section a hypha was discoloured and appeared to have small brown particles deposited on it. Hyphae were occasionally observed in ray parenchyma cells (Fig. 38).

The fungal hyphae were distributed in all cell types in the colonised sapwood, but were not observed in adjacent tissues. No fungal spores were detected. Tissues beyond the limit of the stain and new growth above it were examined. No discoloured or blocked cells were observed in these areas, nor fungal hyphae. This is in agreement with the failure of attempts to reisolate *C. corticale* from above the stain or more than a few cm beyond its limit.

SEM. Examination of stained and control sycamore tissue using the SEM confirmed the fungal distribution observed with the light microscope. Hyphae were fairly smooth-walled and often solitary hyphae extended unbranched for considerable distances along vessels (Fig. 39). Single hyphae were commonly observed growing through vessel lumina, contacting the wall only by occasional minor branches which appeared to be functioning mainly as anchorages (Fig. 40). Hyphae were sometimes attached more closely to the vessel wall by tiny branches (Fig. 41). The surface of the hyphae occasionally showed minor bumps which seemed to be an integral part of its structure (Fig. 41). However, other hyphae showed irregularities which appeared to be material deposited onto the hypha (Fig. 40). In some vessels hyphae and parts of the wall adjacent to them were covered by numerous small globular masses of material and an accompanying fibrous or mucous deposit (Fig. 42, 43, 44). Blistering of vessel and hyphal walls sometimes occurred (Fig. 45) possibly following rupture of the globules (Fig. 46). In other vessels hyphae were not covered by globules and few

Fig. 37 : Hyphae (→) in vessel filled with gum. RLS X 2100.

Fig. 38 : Hyphae in ray cells, passing from cell to cell; stained with safranine picro-aniline blue. RLS X 3500.



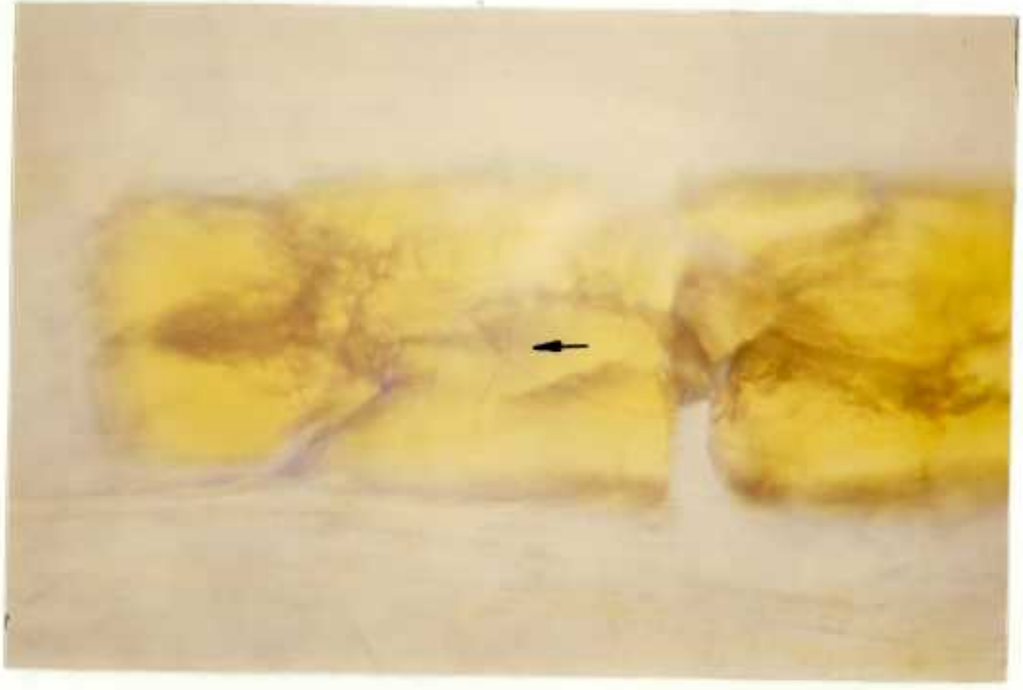
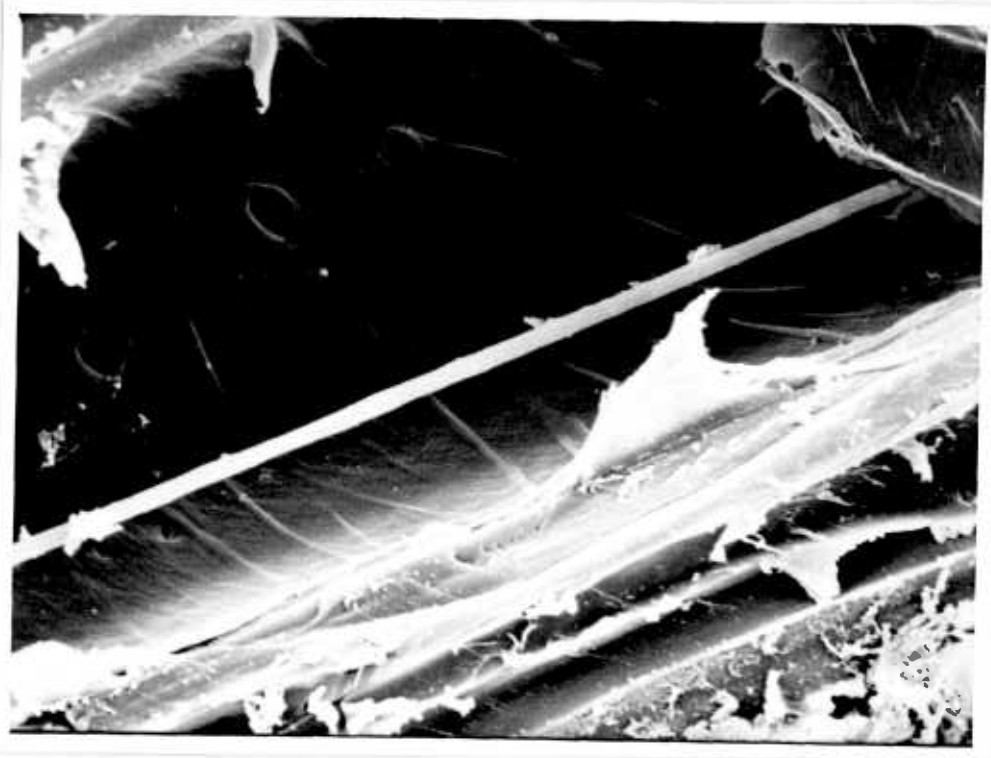


Fig. 39 : Long unbranched hypha in vessel. TLS X 1250.

Fig. 40 : Hypha with small side branches apparently functioning as anchorages. The hypha appears to have material deposited on it. TLS X 1250.



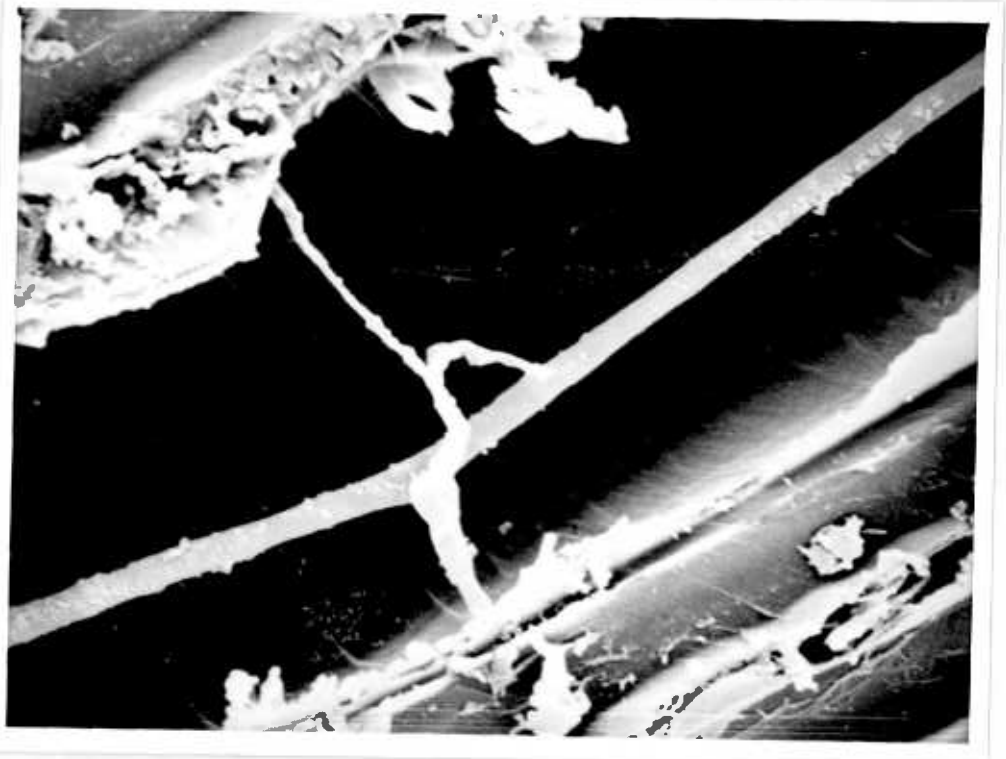
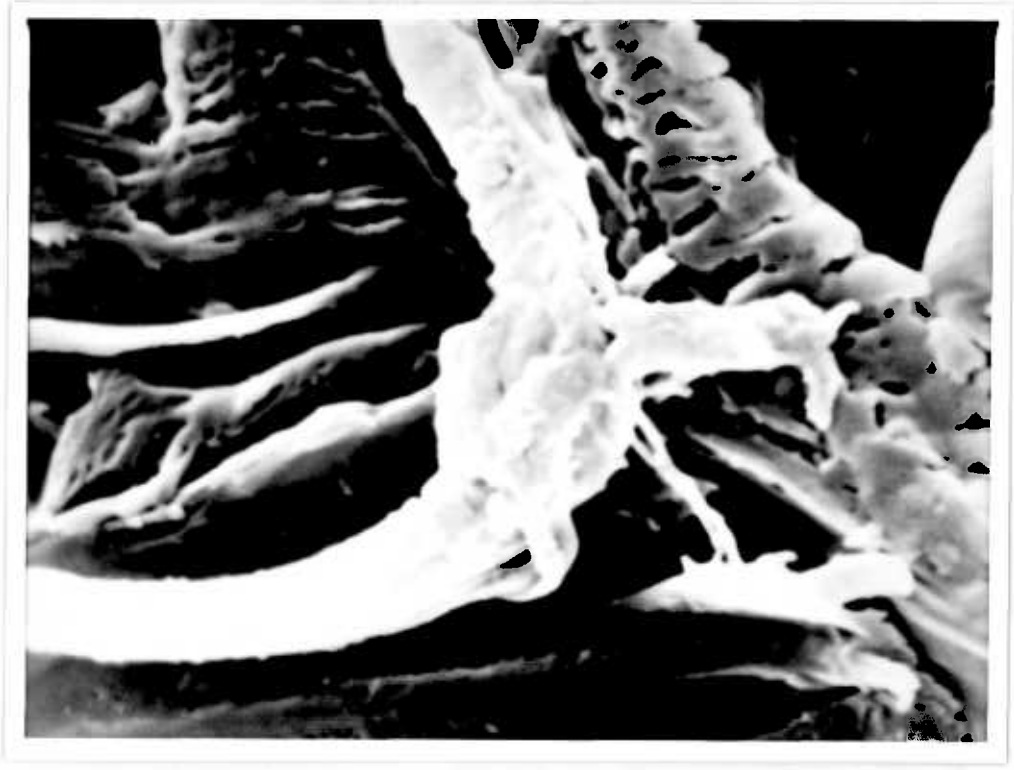


Fig. 41 : Hypha attached to vessel wall by small branches. The bumps on the hypha seem to be part of its structure. TLS X 10000.

Fig. 42 : Hypha and adjacent vessel wall covered by globular masses of material. TLS X 2500.



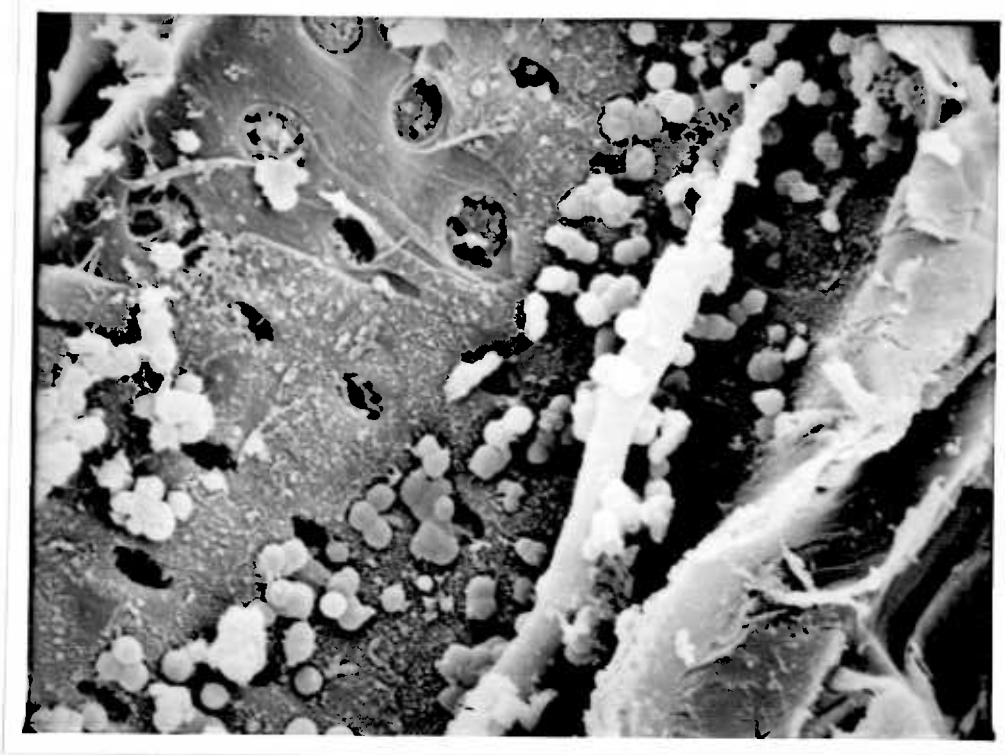
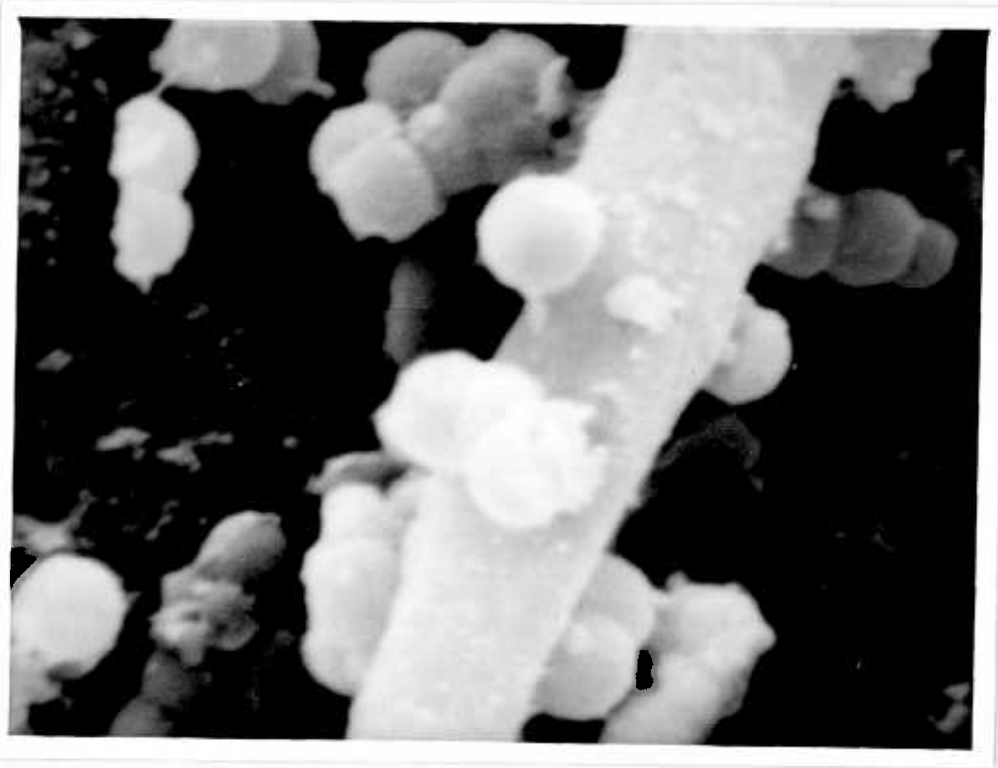


Fig. 43 : Detail of Fig. 42 showing globules on hypha and vessel wall. TLS X 10000.

Fig. 44 : Hyphae and vessel wall covered by globules and a fibrous or mucous deposit. TLS X 2500.



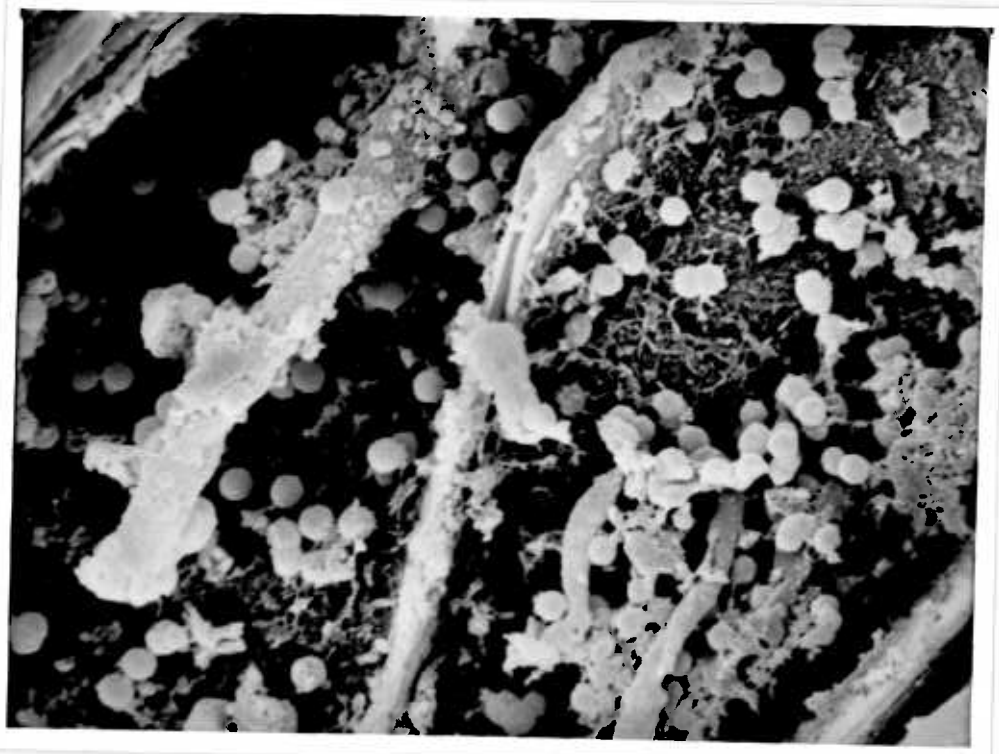
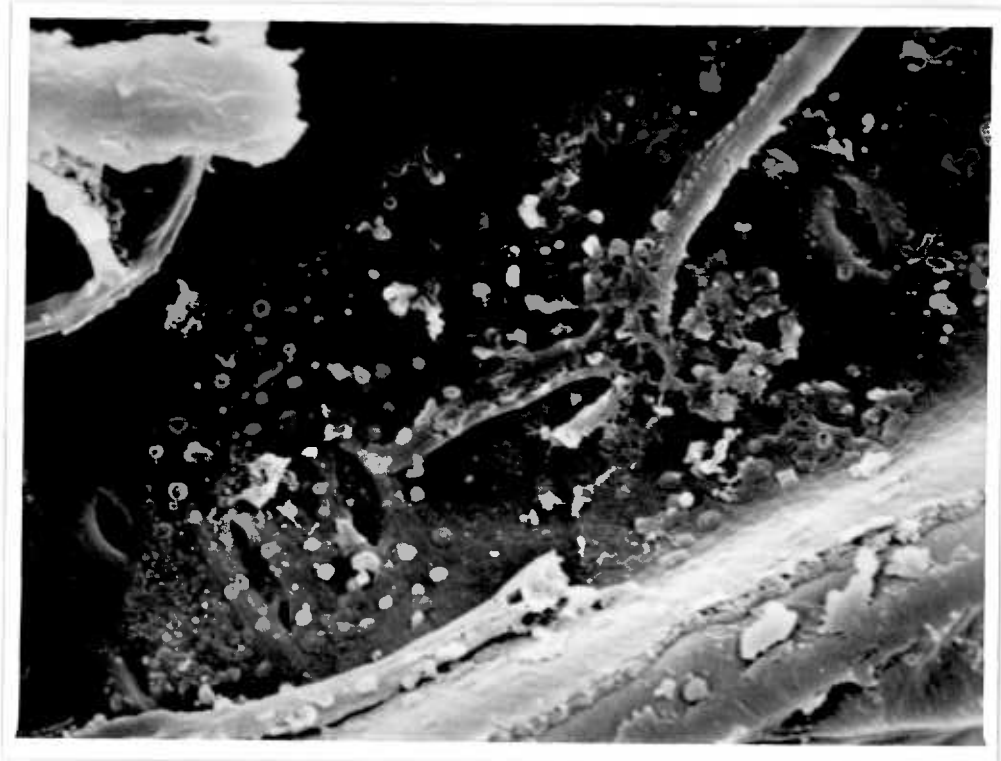
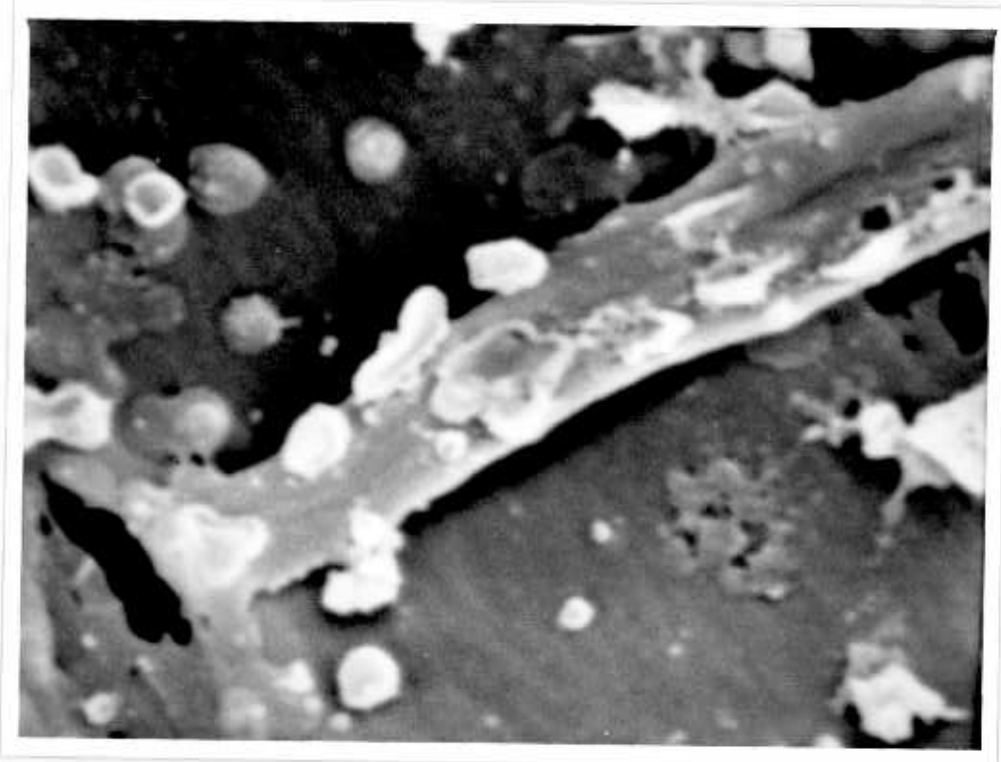


Fig. 45 : Hypha and vessel wall with blisters which may be ruptured globules. TLS X 2500.

Fig. 46 : Detail of Fig. 45. Hypha with blisters which may be ruptured globules. TLS X 10000.





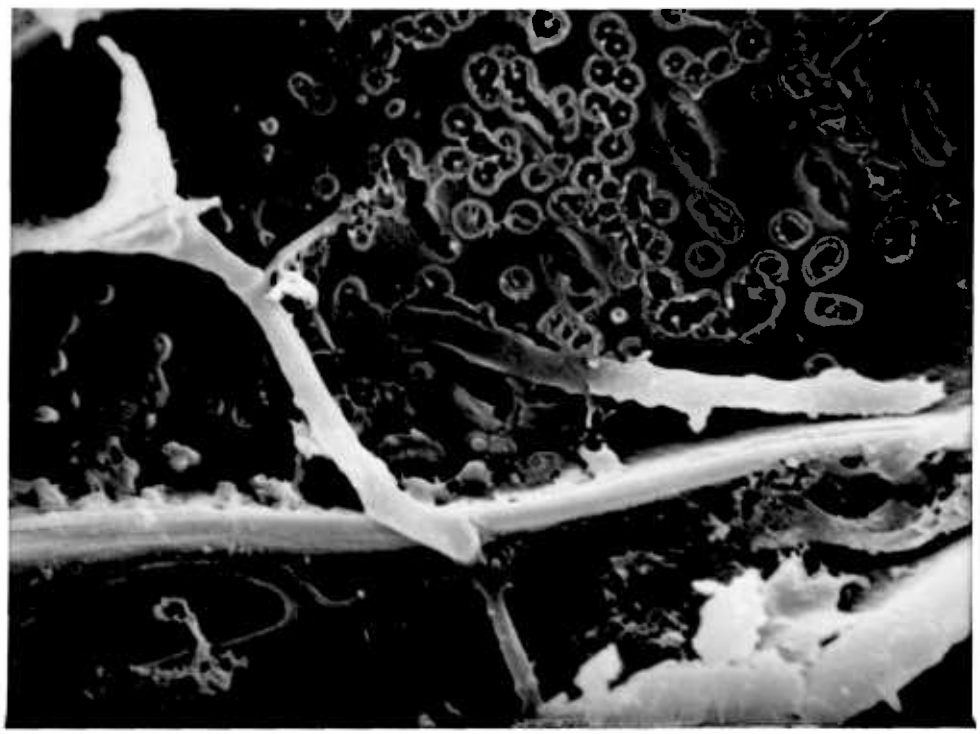
intact globules were apparent on the vessel wall (Fig. 47); blisters on the wall appeared to be superficial and sometimes seemed to contain mucous, they resembled globules in size and outline and were probably derived from them. The globules on vessel walls were similar in appearance to the warty layer which covers the lumen side of the tertiary wall of most differentiated wood cells. However, the globules could be distinguished since the distribution of warts is mostly regular and local crowding is rare, also the warty layer is laid down just prior to death of the cell protoplast and would therefore be distributed independently of *C. corticale* hyphae. The size of warts ranges from 0.01 µm to 1 µm (Liese, 1965) and those in sycamore appeared to be considerably smaller than the globules (Fig. 48). The globules and fibrous material which were present on both certain areas of the vessel wall and associated hyphae seem likely to be the product of a host reaction to *C. corticale*.

Dissolution of the vessel wall was evident both at the penetration point and beneath hyphae in contact with the wall (Fig. 47). Gum- or gel-like material was observed blocking fibres (Fig. 49). Fibres and parenchyma cells often contained numerous small, smooth-walled, balloon-like structures (Fig. 50, 51, 52) which are probably gums (see Fig. 27). Hyphae appeared to be present in the same fibres as gums (Fig. 52), in one fibre particularly the hypha had globules at one end but further along the hyphal structure seemed to be disintegrating, suggesting that the gums are associated with a host reaction detrimental to *C. corticale*. The gums were not observed in control tissue.

Investigation of the apparent limitation of growth in sycamore. Results from various inoculation experiments (e.g. Tables 18 and 20) suggested that after an initial period of rapid colonisation lasting c. 4-6 weeks the

Fig. 47 : Vessel wall with numerous blisters and some globules. A few globules may be developing on the hyphae. Dissolution of the vessel wall is visible where a hypha has penetrated the vessel wall, and also around the hypha growing below the perforation plate. TLS X 2500.

Fig. 48 : Uninoculated control in which the warty layer is visible (→). TLS X 640.



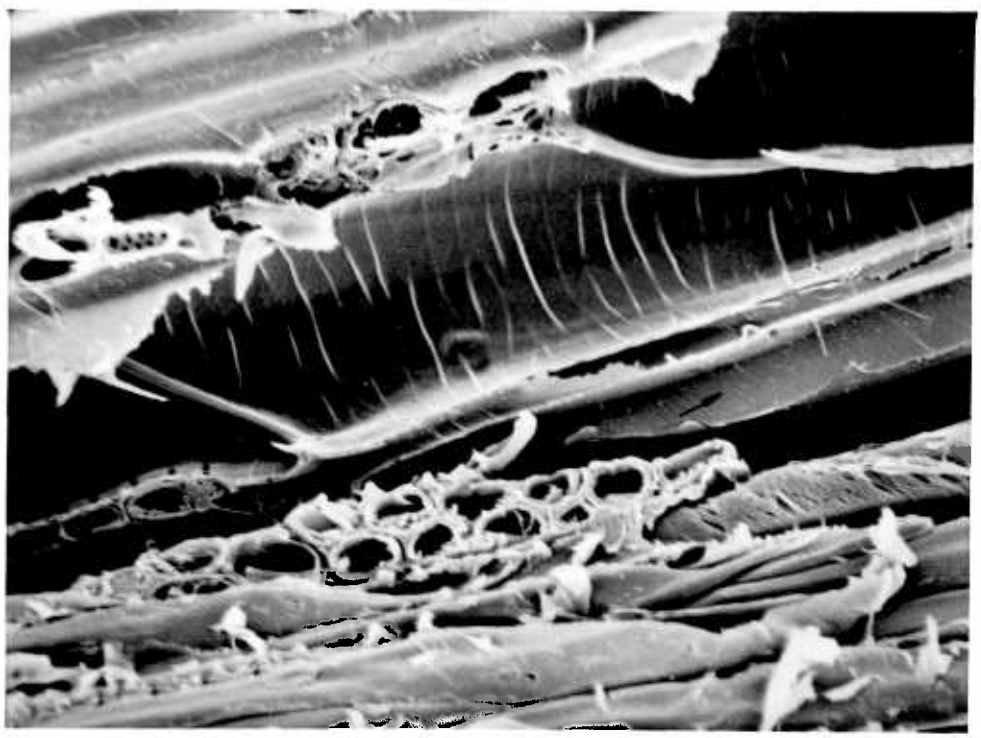
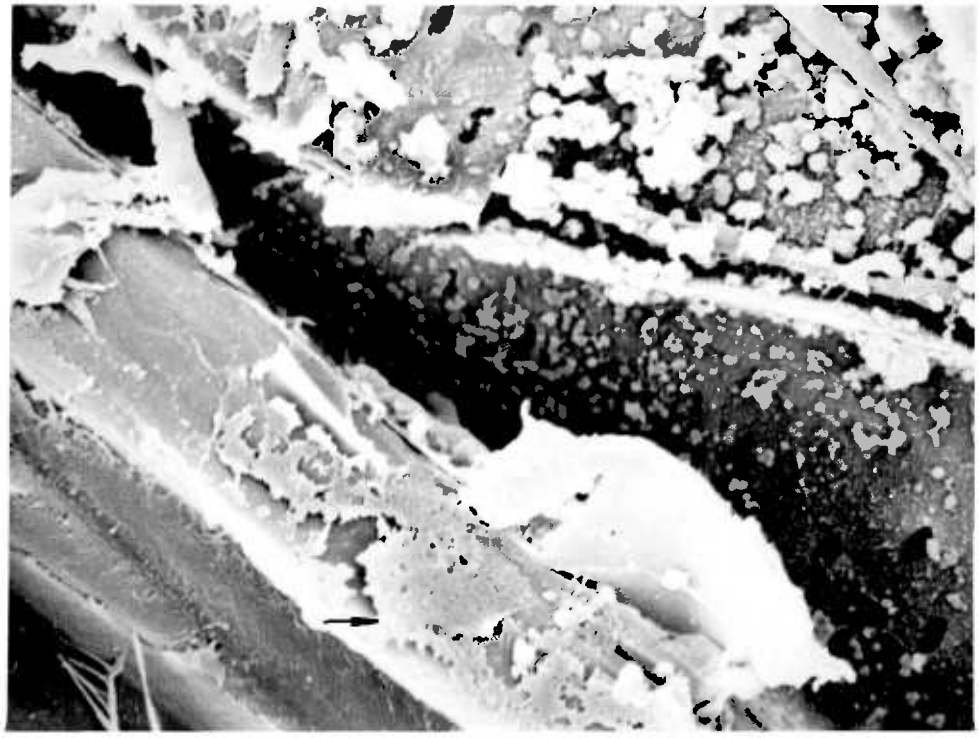


Fig. 49 : Gum- or gel-like material occluding a fibre (→). TLS X 1850.

Fig. 50 : Fibres containing balloon-like structures which may be gummy material. Oblique section X 640.



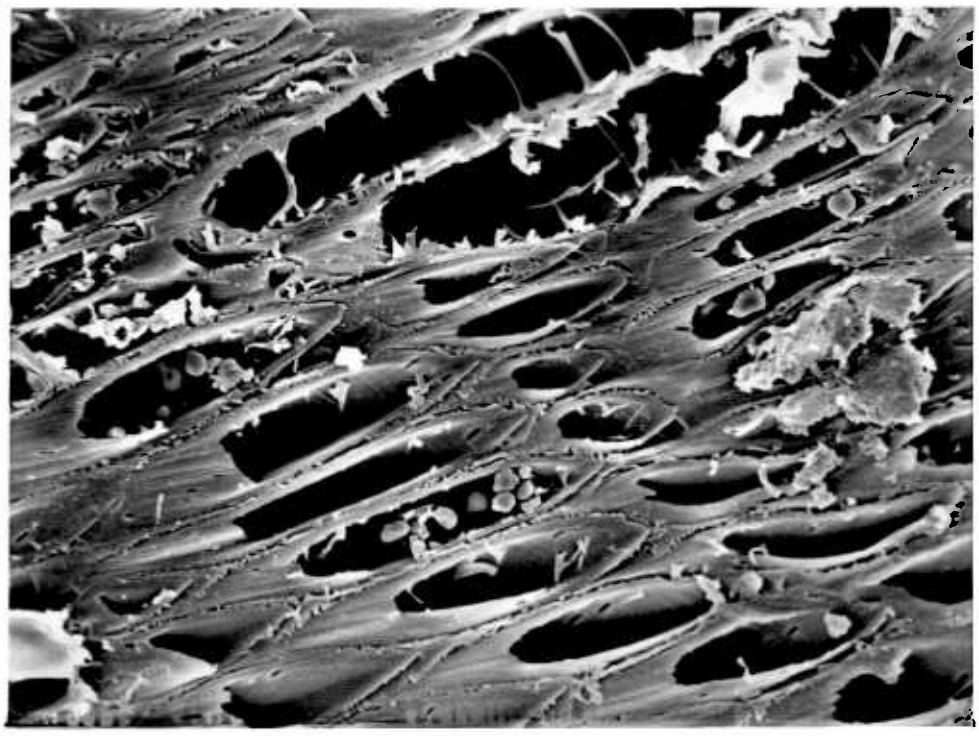


Fig. 51 : Ray cells containing swellings which may be gums. TLS X 2500.

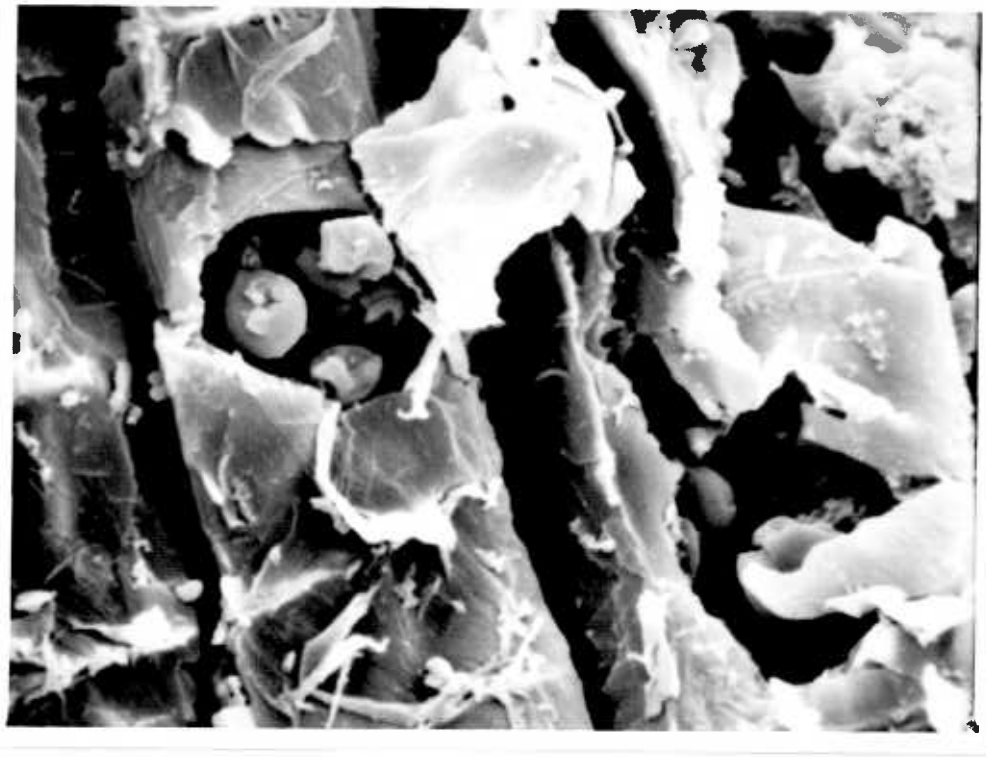


Fig. 52 : Fibres containing swellings which may be gums. Hyphae appear to be present in some fibres, and in one case there are globules on one section of a hypha (→) but further along its structure seems to disintegrate. TLS X 1250.

rate of spread by *C. corticale* is reduced or halted, possibly following a host reaction. To investigate this 26 young sycamores (1.5-2 m) from the Ascot area were placed in a glasshouse at c. 25° for 7 weeks under extended daylength (15 h light, 9 h dark) to break their dormancy. On 28 January 1980 the trees were inoculated with conidia of the Laleham Park isolate produced in culture (c 79% germination) using the millipore disc technique, and sampling dates were randomly assigned to them. During the experiment the mean daily glasshouse temperature ranged from 21°-29° with an overall mean of 24.8°. Four or five trees were cut at soil level after 2 weeks and 1, 2, 3, 4 and 5 months. In trees sampled more than 3 months after inoculation a ridge was commonly observed extending vertically above and below the inoculation point as a result of stimulated radial growth of the sapwood associated with colonisation by *C. corticale* (see Appendix Table 18), and the heartwood of one tree was colonised for several cm (Fig. 53). Results of examination for staining and reisolation of *C. corticale* are given in Table 14 and Appendix Table 18. Analysis of results (Table 15) by analysis of variance showed no differences due to sampling times i.e. no progression of the fungus with time. The lack of a significant result in this analysis is partly due to the considerable variation in results at each sampling. Further analysis using Duncan's new multiple range test showed that the fungus had grown further ($P \leq 0.05$) after 4 and 5 months than in trees sampled after 2 weeks or 1 month. However, growth in trees sampled after 3 months was less than after 2 months, and the test showed no significant progression with time after the first month. Thus results indicate that *C. corticale* made little growth after the initial month, possibly following a host reaction.

No difference was found in the extent of growth above and below the inoculation point (for stain $t = 1.11$ n.s., for reisolation $t = 0.99$ n.s.).

Fig. 53 : Colonisation of stem heartwood by *C. corticale*. Infection has caused growth of the stem to become distorted.

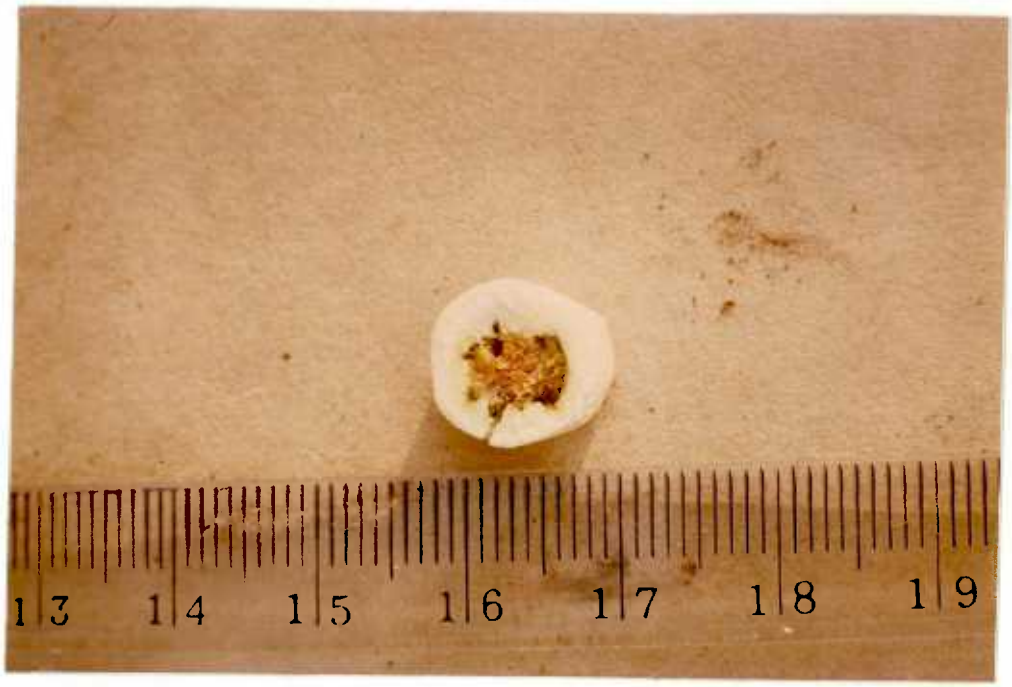

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TABLE 14 : Growth[b] of *C. corticale* in sycamore at 25°.

Time[a]	Stain	Reisolation
0.5	7.3	6.6
1	9.7	8.6
2	14.9	13.8
3	14.1	13.2
4	17.1	17.2
5	20.5[c]	20.0[c]

Key: a. Months after inoculation.

b. Mean growth in 5 replicate trees (cm).

c. Mean growth in 4 replicate trees.

TABLE 15 : Analysis of growth by *C. corticale* in sycamore at 25°.

a) Stain

ANOVA

	DF	SS	MS	F
Sampling times	5	533.002	106.600	1.031 n.s.
Error	23	2377.412	103.366	

Duncans new multiple range test[a]

Sampling time (months)	0.5	1	3	2	4	5
Ranked means	7.34	9.7	14.1	14.9	17.1	20.5

b) Reisolation of characteristic mycelium

ANOVA

	DF	SS	MS	F
Sampling times	5	589.2	117.84	0.982 n.s.
Error	23	2758.8	119.948	

Duncans new multiple range test[a]

Sampling time (months)	0.5	1	3	2	4	5
Ranked means	6.6	8.6	13.2	13.8	17.2	20.0

Key: a. Any 2 means not underscored by the same line are significantly different.

The correlation between staining and colonisation by the fungus was highly significant ($P \leq 0.001$) with a coefficient of 0.983.

Investigation of changes resulting from colonisation by *C. corticale*.

a) Histochemical tests were performed in an attempt to determine any changes in host tissue infected by *C. corticale*. Sycamores inoculated with *C. corticale* and kept at 25° for 2 weeks were sampled and fresh material taken from the stained area was sectioned at 20 or 40 µm. i) The iodine and sulphuric acid method was used to test for cellulose (see Jensen, 1962). Walls containing cellulose stain dark blue and lignin stains orange to yellow. After a short time the acid dissolved the cellulose and the tissue disintegrated. No difference was observed between inoculated and control tissues. ii) A saturated solution of aniline sulphate was used to test for lignin, which turns bright yellow. Gummy material stained yellow. However, all the cells in both infected and control tissue were also stained. iii) Sudan III (0.5 g/100 ml 70% ethanol) stains oils red. The material blocking certain vessels turned pink, but other cells were unstained in both inoculated and control tissue. iv) True gums are carbohydrate substances, soluble in warm water but not in ethanol, and are usually formed by the breakdown and degredation of wood cell walls. The substances blocking vessels did not dissolve in warm water and thus are not true gums. v) Tannins turn blue-black when treated with a 1% solution of ferric chloride in 0.1 M hydrochloric acid. No blue-black colour was observed in either inoculated or control tissue.

b) *In vitro* experiments were set up to determine whether *C. corticale* readily degrades cellulose. The fungus may produce cellulolytic enzymes most readily when it is well established on another nutrient source. To investigate this sterile cellophane discs were placed to cover the surface

of eight 3% MEA plates and inoculated centrally with a mycelial plug (6 mm) from a culture of isolate LP on 3% MEA. Controls were inoculated with a disc of agar and other plates were inoculated with *Corticium praticola* for comparison. After 7 and 14 days at 25° results were assessed by testing the relative strengths of cellophane from the different treatments. *C. praticola* had degraded the cellophane and after 7 days only small pieces could be pulled up before tearing away from the remainder of the disc. The force required to tear cellophane over which *C. corticale* had grown only sparsely seemed comparable with controls. Thus *C. corticale* appeared unable to produce cellulolytic enzymes in significant quantity when growing well on another carbon source, although the pH of c. 5 was suitable for production of fungal cellulases (optimum pH 4-7). However, fungi generally produce cellulases *in vitro* much more abundantly when the only readily available carbon source in the medium is cellulose (Wood, 1967) i.e. most cellulases are adaptive enzymes. Two further experiments using cellulose as the major carbon source failed to detect cellulolytic activity (Appendix p. 163). However, it seems likely that *C. corticale* produces cellulose-degrading enzymes under conditions provided by the host since the fungus can penetrate cell walls directly and SEM studies show evidence of chemical dissolution of walls in contact with hyphae. Cellulases have been reported to be induced by substances other than cellulose or cellulose derivatives e.g. *Trichoderma viride* produces cellulase very effectively in response to sophorose (2-O-β-D-glucopyranosyl-D-glucose) (Wood, 1967); possibly *C. corticale* cellulase is best induced by a substance other than cellulose.

(6) TEMPERATURE AND WATER STRESS

Disease outbreaks have followed years with especially warm summers, suggesting that there is a link between the incidence of sooty bark and high summer temperatures (Peace, 1955; Young, 1978). The present study examined initially the effect of temperature on the growth of *C. corticale in vitro* and in young sycamore and then the effect of water stress on its growth in sycamore at 25°.

Effects of Temperature *in vitro*

Longevity of conidia. The longevity of conidia collected from Laleham Park, Sunninghill Park and Thamesmead was examined in two experiments. The conidia were stored in stoppered specimen tubes (5 x 1 cm) at temperatures ranging from -20° to 30° and their viability was tested at intervals on 3% MEA (standard test, see p. 18). The results are given in Table 16; data for any particular vial show some inconsistencies due to variability among the many conidia therein, including occasional sampling of pockets of non-viable spores. However, this does not mask the general trends. Conidia stored at 20° or below retained their viability for many weeks and those stored at 5° or less germinated almost as well after 2 years. In contrast, after 32 weeks less than 20% of the conidia stored at 25° germinated and none of those stored at 30°. Conidia from Thamesmead stored at 15° and at 20° lost viability more rapidly than conidia from other sources.

Growth on agar. (a) Germination. Conidia from Laleham Park were streaked onto a thin layer of 3% MEA on glass slides which were then incubated in damp chambers at 5°, 10°, 15°, 20°, 25° or 30° for either 18 h or 24 h when germination was arrested by adding drops of lactophenol. This gave better results than germination in water (Appendix p. 165). Percentage

TABLE 16 : Longevity of *C. corticale* conidia.

Expt. 1[a]

Storage time (weeks)	Mean % germination[c]							
	Laleham Park[d]				Sunninghill Park[d]			
	-20°	5°	10°	15°	-20°	5°	10°	15°C
4	92	94	35	61	92	81	71	17
8	82	93	68	63	72	39	14	14
12	71	65	46	40	95	76	62	28
16	79	88	53	60	92	74	76	59
28	78	82	56	75	87	71	70	44
33	64	90	-	54	87	71	81	69
41	89	94	-	82	82	38	53	29
108	77	75	-	3	59	-	52	<1
129	74	79	-		83	-	67	0

Expt. 2[b]

Storage time (weeks)	Mean % germination[c]							
	Laleham Park[d]				Thamesmead[d]			
	15°	20°	25°	30°	15°	20°	25°	30°C
1	-	93	90	92	-	93	86	91
2	-	89	87	84	-	83	84	71
4	-	85	79	61	-	55	60	46
5	-	84	90	57	-	78	79	61
7	86	89	82	5	80	75	45	50
18	82	83	44	0	78	68	83	4
32	54	27	0	-	59	29	17	0
53	35	23	-	-	0	1	0	-

Key: a. Initial germination: Laleham Park 94%; Sunninghill Park 97%.

b. Initial germination: Laleham Park 93%; Thamesmead 95%.

c. Mean of 4 replicate counts of 100 conidia after 18 h incubation at 25°.

d. Source of conidia.

germination of each temperature was assessed from counts of 100 conidia on each of 4 replicate slides and mean germ tube lengths from measurements of 10 germinated conidia on each of 6 replicate slides. (b) Mycelial growth. Radial growth on 3% MEA was measured, the method is described on p. 24.

About 90% of the conidia incubated at 20°, 25° and 30° germinated after 18 h but less than 1% of those at 10° or 15° germinated and none did so at 5° (Table 17). Extending the incubation period to 24 h increased germination at 10° and 15°. Both germ tubes and mycelium grew best at 25°; they grew rather less well at 30° and 20° and only slowly below 20°. (Mycelial growth of a range of isolates at different temperatures is shown in Table 1).

Effects of Temperature *in vivo*

Experiment 1. Eleven pairs of trees (1-1.5 m) from Silwood Park were matched for size and appearance. The dormant trees were inoculated using millipore discs charged with conidia from Laleham Park (93% germination) on 22 November 1977. Immediately after inoculation one member of each pair was transferred to one of two compartments in a glasshouse. One compartment was maintained as near as possible at 15° and the other at 25°. Thermographs were placed in both compartments and these indicated the following mean daily ranges (and overall means) over the 13 weeks of the experiment: 11°-16° (12.2°); 17°-26° (21.4°). At intervals of 1-2 weeks growth of *C. corticale* was assessed in one pair of trees, but the last 2 pairs were kept for subsequent observation. The order in which pairs were sampled was predetermined using random numbers at the start of the experiment.

Superficial bark splitting extending 2-9 cm from the inoculation point

TABLE 17 : Germination of *C. corticale*[a] on 3% MEA.

	°C					
	5	10	15	20	25	30
Germination after 18 h (%)[b]	0	≤1	≤1	89	90	90
Germination after 24 h (%)[b]	<1	60	99	*	*	*
Germ tube length (µm)[c]	0	5†	1.3†	10.8	14.4	11.5
Mycelial growth (mm/day)[d]	1.0	1.9	2.9	4.8	7.1	5.0

Key: a. Laleham Park isolate.

b. Means of 400 conidia.

c. Means of 60 germ tubes.

d. Means of 3 cultures.

* Extensive germ tube growth made counts impossible.

† These values represent only the 1% of conidia which germinated.

was observed on trees sampled after 10, 11 and 13 weeks at 21°. The tops of 3 trees kept at this temperature were dead at the time of sampling and this masked the limits of staining, but it did not prevent reisolation of *C. corticale* from the dead tissues. At each sampling date the fungus had grown more extensively at 21° than at 12° (Table 18, Appendix Table 19) and an analysis of variance based on reisolation of characteristic mycelium (Table 19) indicated that, overall, the differences in growth were significant ($P \leq 0.001$). While there were also obvious (and significant) differences in growth of *C. corticale* in trees from the same treatment sampled at different times, there was no consistent progression in fungal growth with time. For example, the fungus had grown less in trees sampled after 12 and 13 weeks than it had in those sampled after 4, 6, 8 or 9 weeks.

The correlation between stain and colonisation as determined by reisolation of characteristic mycelium was highly significant ($P \leq 0.001$), the coefficients for 12° and 21° were 0.832 and 0.821 respectively. Generally, growth of *C. corticale* above and below the inoculation point was similar (Appendix Table 20). An analysis of data for staining at 21° showed significantly greater growth below the inoculation point, but this was not reflected in the reisolation data.

Experiment 2. Rate of spread of *C. corticale* from equally established infections was compared at 15° and 25°. On 10 April 1978 conidia from Laleham Park (76% germination) were used to inoculate 12 pairs of matched trees (1.5-2 m, from Silwood Park). The trees were kept in an illuminated growth room at 25° for a week to allow the fungus to establish infection; one tree of each pair was then transferred to an illuminated growth room at 15°. The growth of *C. corticale* was assessed in two predetermined trees

TABLE 18 : Growth (cm) of *C. corticale* in young sycamores.

	Mean temperature[a]	Weeks after inoculation								
		2	4	6	8	9	10	11	12	13
Stain	12.2	0	6.0	2.5	13.0	8.0	6.5	2.0	1.0	2.0
	21.4	7.0	27.5	45.0	>44	(45)	40.0	(20)	>45	(25)
Reisolation	12.2	0	13	8	17	15	2	2	2	3
	21.4	9	26	46	36	45	25	20	25	25

Key: a. Over 13 weeks.

() Estimate (top of shoot dead).

TABLE 19 : Analysis of growth of *C. corticale* in young sycamore at 12 and 21°.

a) ANOVA based on staining[a]

	DF	SS	MS	F
Temperature	1	2685.021	2685.021	27.775**
Sampling times	5	776.854	155.37	1.607 n.s.
Error	5	483.354		

b) ANOVA based on reisolation of characteristic mycelium

	DF	SS	MS	F
Temperature	1	2090.89	2090.89	55.54***
Sampling times	8	11.56	144.5	3.84
Error	8	301.11	37.64	

Key: a. 9, 11 and 13 week results omitted.

* Significant at $P \leq 0.05$.

** Significant at $P \leq 0.01$.

*** Significant at $P \leq 0.001$.

from each temperature regime at 1-3 week intervals, and the final pair was retained.

Bark splitting associated with colonisation was observed on one tree at 15° (3 cm) and several at 25° (4-76 cm) sampled after 4 or more weeks. Growth of the fungus (Table 20, Appendix Table 21) as indicated by reisolation of characteristic mycelium was significantly more extensive at 25° than at 15° ($P \leq 0.05$, Table 21). Again there was no consistent relationship between the extent of fungal growth and the time allowed for this.

Correlation between stain and reisolation at 15° and 25° was highly significant ($P \leq 0.001$), the respective coefficients being 0.954 and 0.996. Growth above and below the inoculation point was analysed by a t-test (as this allows individual treatment of trees), and no difference was found (Appendix Table 22).

Pairs of trees from each experiment were kept for c. 2 years, but even at 25° disease did not become acute. Examination for *C. corticale* showed total growth extending 9 cm and > 109 cm in trees from Experiment 1, and > 49 in the tree from the second experiment. After c. 18 months trees kept at c. 15° were transferred to a warm glasshouse for several months, but growth in trees from the first experiment was only 4 cm in each of the trees. The tree kept at 25° for a week to allow initial establishment and then at 15° for c. 1 year was also transferred to a warm glasshouse for several months before sampling, growth in this tree totalled 35 cm. These observations support the hypothesis that the fungus makes some growth but then is virtually stopped. Even prolonged exposure to warm temperatures did not appear to encourage further growth by *C. corticale* in trees which had been at c. 15°.

TABLE 20 : Growth (cm) of *C. corticale* in young sycamore from infections established for one week at 25°.

	Mean temperature[a]	Weeks after inoculation											
		1		2		3		4		5		8	
Stain	15°	-	-	14	10	14	14	13	17	47	15	79	17
	25°	7	14	17	14	>89	28	30	>105	42	46	83	37
Reisolation	15°	-	-	11	4	14	7	12	14	47	14	55	6
	25°	4	9	22	13	>90	27	31	>105	42	45	80	32

Key: a. Over weeks 2 to 8.

TABLE 21 : Analysis of growth of *C. corticale* in young sycamore from infections established for one week at 25°.

a) ANOVA based on stain

	DF	SS	MS	F
Sampling time	4	3865.70	966.425	1.177 n.s.
Temperature	1	2904.05	2904.050	3.536 n.s.
Interaction	4	2147.70	536.925	0.654 n.s.
Error	10	8213.50	821.350	

b) ANOVA based on reisolation of characteristic mycelium

	DF	SS	MS	F
Sampling time	4	2393.20	598.30	0.77 n.s.
Temperature	1	4590.45	4590.45	5.95*
Interaction	4	1657.80	414.45	0.537 n.s.
Error	10	7715.50	771.55	

Key: * Significant at $P \leq 0.05$

Effects of Water Stress

Experiment 1. Eight pairs of trees (1.5-2 m, from Silwood Park) were inoculated with *C. corticale* conidia (from Laleham Park, 90% viability) by the millipore disc technique on 15 May 1978. The soil was brought to field capacity and the pots were placed in a glasshouse at c. 25° (23°-26°, with an overall mean of 24.2°). One tree of each pair was watered frequently (non-stressed trees), the other was only watered when its leaves began to wilt (water-stressed trees). The soil moisture in each pot was monitored from the resistance of a porous gypsum block buried at root level (Slavik, 1974). The blocks were calibrated using a pressure plate apparatus. For stressed trees the mean soil matric water potential immediately before watering was -10.24 ± 0.47 bar, for non-stressed trees the corresponding value was < -1 bar. The growth of the fungus was assessed in 2 predetermined pairs of trees after 2 and 4 weeks and in the remaining 4 pairs of trees at 6 weeks.

The results (Fig. 54, Appendix Table 23) were examined in two ways: by analysis of variance to determine the overall effects of water stress and sampling time (Table 22) and by t-tests to compare fungal growth above and below the point of inoculation in trees within the same treatment. Analysis based on staining showed that the fungus grew more extensively in trees subjected to water stress ($P \leq 0.05$), analysis based on reisolation of characteristic mycelium showed this difference as highly significant ($P \leq 0.01$). In the stressed trees *C. corticale* grew further down the stem from the point of inoculation than it grew up the stem (for staining $t = 5.167$, $P \leq 0.01$; for reisolation $t = 3.390$, $P \leq 0.05$). In watered trees the difference in fungal growth up and down the stem was not significant when based on staining ($t = 1.834$); however when data for

Fig. 54 : Growth (cm) after 2, 4 and 6 weeks of *C. corticale* up (+) and down (-) the stems of inoculated sycamores subjected, or not, to water-stress. Paired trees are indicated by the letters A to H.

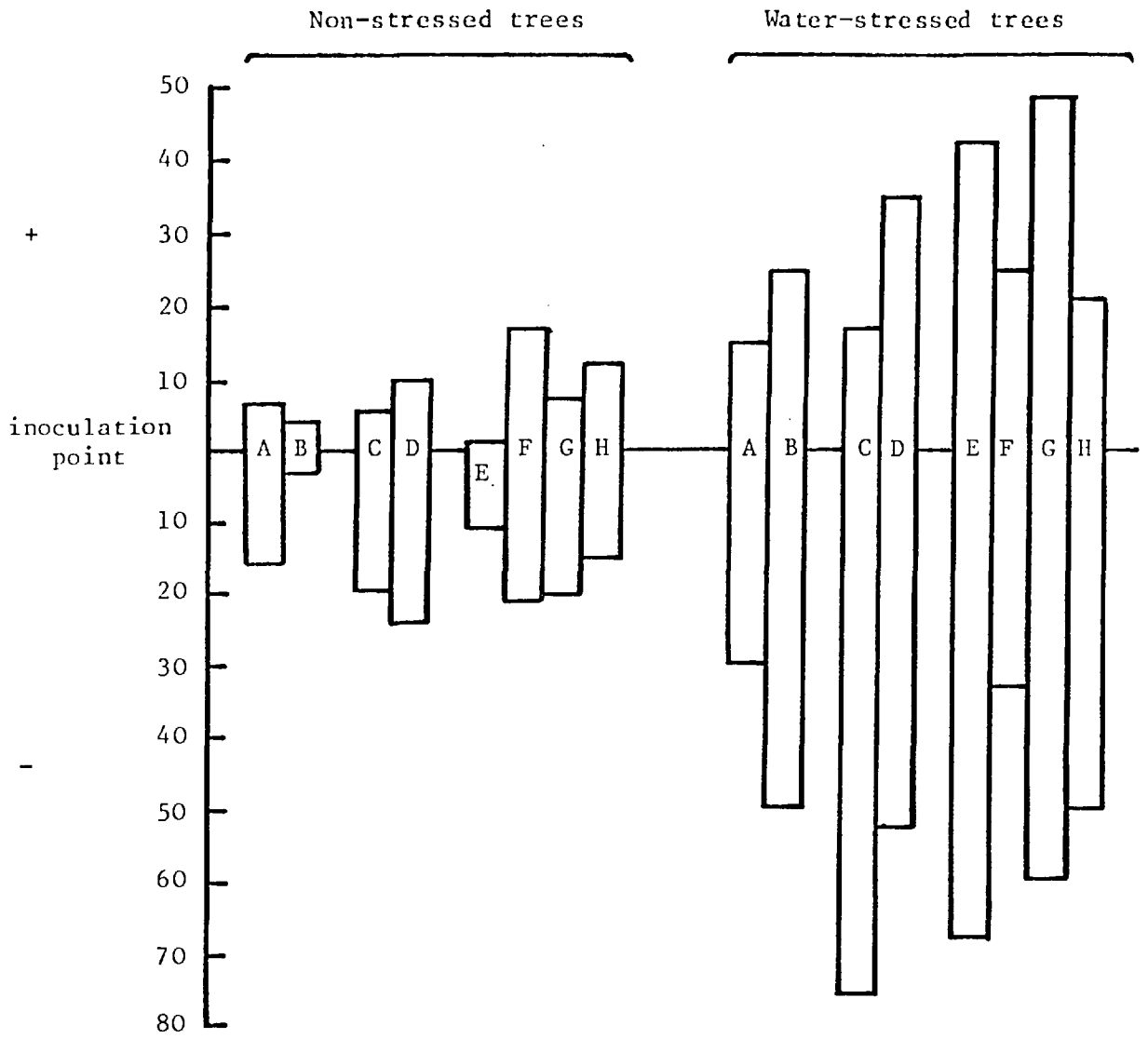

TABLE 22 : Analysis of growth of *C. corticale* in water-stressed and non-stressed sycamores.

a) ANOVA based on staining

	DF	SS	MS	F
Watered v. stressed	1	3570.940	3570.940	63.043*
Sampling times	2	951.162	475.581	8.396 n.s.
Error	2	113.286	56.643	

b) ANOVA based on reisolation of characteristic mycelium

	DF	SS	MS	F
Watered v. stressed	1	4676.041	4676.041	106.172**
Sampling times	2	591.583	295.792	6.716 n.s.
Error	2	88.084	44.042	

Key: * Significant at $P \leq 0.05$

** Significant at $P \leq 0.01$

reisolation was analysed the result was significant ($t = 2.887$; $P \leq 0.05$), this was because the extent of staining above the inoculation point was somewhat greater than the corresponding figures for reisolation of mycelium (see Appendix Table 23).

Experiment 2. The trees in Experiment 1 were subjected to a severe water stress that would be rare in the field. In this experiment, polyethylene glycol 4000 (PEG) was used in an attempt to induce lower levels of water stress. PEG solutions (-2.5 and -5 bar) were prepared using the calibration curve of Lawlor (1970). There were 18 container-grown trees (1.5-2 m), matched in 6 sets of three, to give 6 trees per PEG treatment and 6 controls. All trees were placed in a glasshouse at c. 25° (daily means 19°-28°, with an overall mean of 25.7°), and watered 2 days before applying the PEG solutions. These were poured on the soil at dusk, to minimise shock, in 0.5 l aliquots at 10 min. intervals until the soil was saturated. This procedure was repeated the following morning and the pots were placed in trays lined with capillary matting saturated with the test PEG solution or water. The trees were inoculated using millipore discs charged with conidia from Laleham Park (76% germination) on 13 July 1979.

In the PEG treatments some phytotoxic symptoms (necrotic spots) were observed on a few leaves of 2 trees after 10 days and necrotic margins and leaf spots (Fig. 55) developed on some other trees towards the end of the experiment. The leaf area affected was recorded (Table 23). Stomatal resistance was monitored with a Lambda porometer; water-stressed trees showed greater stomatal resistance than controls and -5 bar PEG caused more stomatal closure than -2.5 bar PEG (see Table 23). Other methods attempted for measurement of stress in individual trees are described in the Appendix (p. 166).

Fig. 55 : Necrotic spots which are a phytotoxic symptom sometimes observed in sycamores treated with solutions of polyethylene glycol 4000 at -2.5 or -5 bar.

TABLE 23 : Effects of polyethylene glycol (PEG) on sycamore.

Treatment	Phytotoxicity*	Leaf resistance†	
		10 days	17 days
Water (control)	0	6.8	7.2
-2.5 bar PEG	< 4.3	8.5	8.5
-5 bar PEG	< 3.0	12.5	18.4

Key: * Mean % leaf area affected at 17 days

† Mean stomatal resistance (sec cm^{-1})

Colonisation of *C. corticale* was assessed in all trees after 17 days (Fig. 56; Appendix Table 24) and t-tests on these data indicated that the fungus had grown more extensively in trees treated with -2.5 bar PEG ($\underline{P} \leq 0.01$) and -5 bar PEG ($\underline{P} \leq 0.05$) than in the controls. Differences between the 2 PEG treatments were not significant. This was largely owing to the considerable variation within the -5 bar PEG treatment where in some trees growth of the fungus was extensive and continued into the roots but in one tree particularly growth was poor. In the -2.5 bar treatment only, growth of the fungus down the stem was significantly greater than its upward growth ($\underline{P} \leq 0.05$). Again, this was a feature in some trees of the -5 bar treatment but not others.

The correlation between staining and colonisation was highly significant ($\underline{P} \leq 0.001$) for both water stress experiments, the coefficients being respectively 0.975 and 0.999.

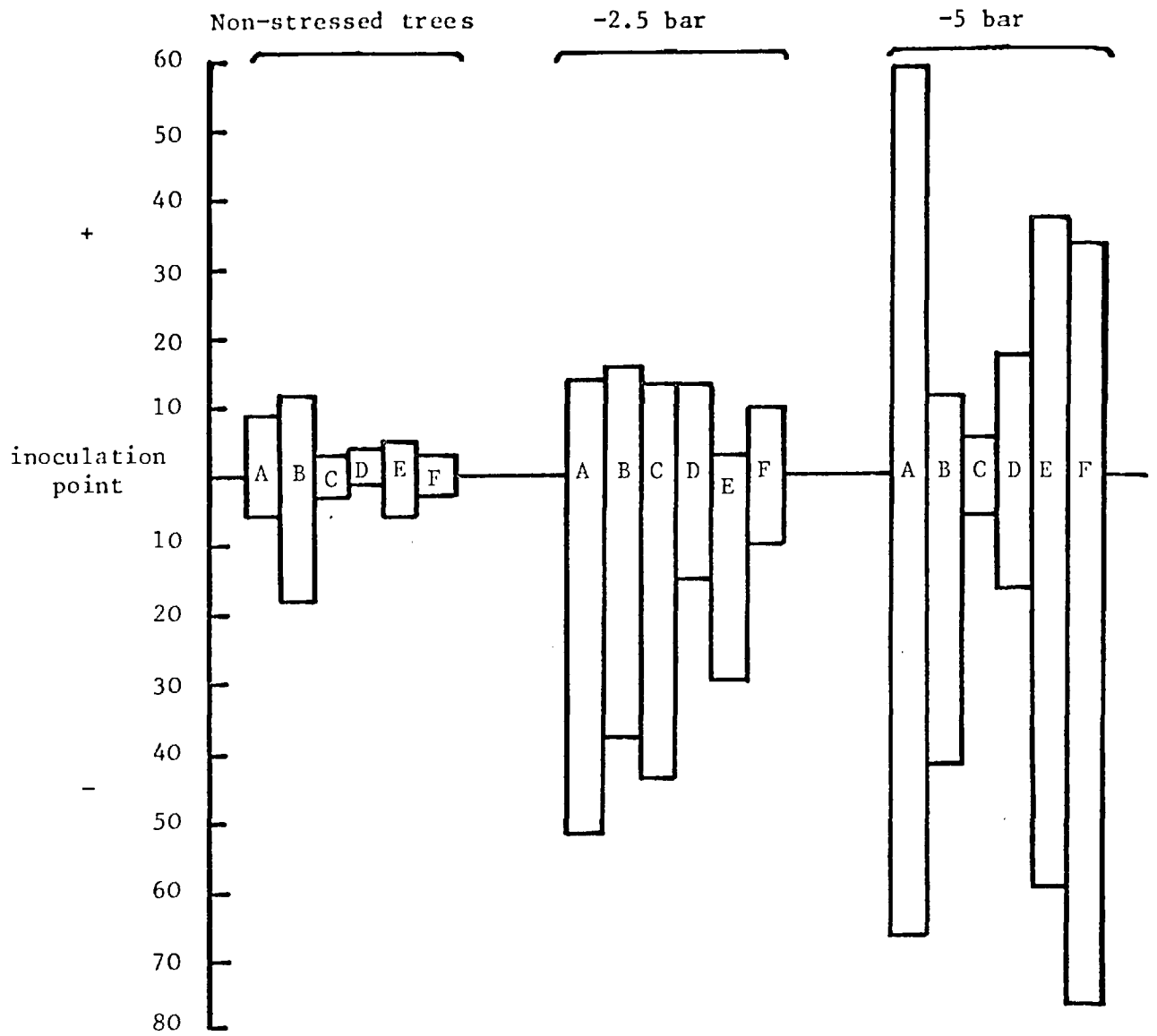

Fig. 56 : Growth (cm) after 17 days of *C. corticale* up (+) and down (-) the stems of inoculated sycamores subjected to water stress by polyethylene glycol (PEG). Matched trees are indicated by the letters A to F.

DISCUSSION

Sooty bark has appeared somewhat sporadically in Britain following its discovery in 1945. Until 1975 limited outbreaks occurred, particularly in the London area, and there were also a few reports elsewhere. However, in 1976 and 1977 the disease developed into a major and widespread epidemic. The pattern of disease outbreaks has followed years with especially warm summers and this supports the hypothesis that there is a link between the incidence of sooty bark and high summer temperatures (Peace, 1955; Young, 1978).

The present experiments, like those of Townrow (1954), indicate that 25° is the optimum temperature for conidial germination, germ tube growth and mycelial extension of *C. corticale* on an agar medium. Whether it is also the optimum temperature for its growth in sycamore remains to be established but certainly the fungus grew more extensively in trees kept at c. 25° than in those at c. 15° in situations where trees were placed at these temperatures immediately following inoculation (Table 18) or where the infection was first allowed to establish by keeping all trees at 25° for one week (Table 20).

However, other factors besides temperature obviously influence the growth of *C. corticale* in sycamore, not least of which is the host itself. In both temperature experiments there was clearly considerable variation between the trees to the extent that progression of the infection with time was obscured. The data suggest that fungal growth rate may decrease after c. 4–6 weeks (Tables 18 and 20) possibly due to a host reaction. It is interesting to compare the overall growth of the fungus (based on mycelial reisolation) at the higher temperatures after 4 weeks within the two experiments. In Experiment 1 there were 2 trees in which collectively

the fungus grew 35 cm in a total of 6 weeks, based on the time at which each tree was sampled (2 & 4 weeks). This gives a mean growth of 5.8 cm/wk. Similar calculations for Experiment 2 (8 trees, 301 cm growth, 20 weeks) give a mean of 15.1 cm/wk. These figures suggest that the fungus was able to grow more rapidly in the trees in Experiment 2 which were in full leaf than in those of Experiment 1 which were dormant. However, the faster growth rate in the second experiment may be partly attributable to the difference in temperature (25^{o} cf. a daily mean of 21.4^{o} in Experiment 1) since mycelial growth at 25^{o} is faster than at 20^{o} (Table 17). A similar comparison over the first 4 weeks between trees at the lower temperatures in the 2 experiments indicates that the fungus had a mean growth rate of 2.2 cm/wk in Experiment 1 (2 trees, total fungal growth 13 cm) and 3.4 cm/wk (6 trees, total fungal growth 62 cm) in Experiment 2.

These figures are less easy to interpret because in Experiment 2 a week at 25^{o} preceded the period at 15^{o} but they indicate a similar growth rate in the two experiments. While it would be unwise also in view of tree-to-tree variation to place too much reliance on such figures, they suggest that high temperatures are not so important for the initial establishment of the fungus as for its continued growth in sycamore. *C. corticale* can infect young sycamores at temperatures ranging at least from 12^{o} to 25^{o}, and its subsequent spread is markedly affected by prevailing temperatures. Tree bark areas exposed to direct insolation have higher temperatures than the ambient air e.g. in summer the south side of a pine tree may reach 55^{o} in North America (Smith, 1970). Up to c. 30^{o} these higher values favour *C. corticale* development.

The apparent reduction in fungal growth rate after c. 4-6 weeks was investigated at 25^{o} in a further experiment sampled at intervals over 5

months. This experiment showed no significant progression of *C. corticale* with time after the first month (Table 14, Appendix Table 18), supporting observations in previous experiments and strongly suggesting that growth may be limited by some host reaction. Overall growth after 4 weeks calculated as before (10 trees, 76 cm growth, 30 weeks) was only 2.5 cm/wk, a result closer to the growth at c. 15° than at c. 25° in the temperature experiments. However, the fastest growth rate in a previous experiment (15.1 cm/wk) was achieved in a controlled temperature room, and it may well be that temperature fluctuations as experienced in a glasshouse compartment reduce the growth rate of *C. corticale* considerably. This seems likely as growth on agar is less at 20° or 30° than at 25°. Although the mean temperatures in the glasshouse were 21.4° (Temperature Experiment 1) and 24.8° (progression with time experiment), over much of the experimental periods the temperature was above or below the optimum for growth of the fungus. The difference in result between the 2 glasshouse experiments for trees kept at c. 25° is probably due to the apparent tree-to-tree variation in susceptibility (Table 18, Appendix Table 18) and emphasises both the need for sufficient replication and for using matched trees for treatments wherever possible. This in itself presents problems because reisolating *C. corticale* and establishing its identity by inducing sporulation is not only time consuming but logistically is difficult with many replicate trees. The high degree of correlation obtained between staining and the reisolation of *C. corticale* suggests that in some experiments of this type staining alone might be used to determine the extent to which the fungus grows. However, such an approach must be counselled with caution because staining in sycamore may result from other causes.

In the field high summer temperatures, especially if prolonged, impose various degrees of water stress and the effect may be compounded

because during drought the tree suffers from overheating as well as dehydration (Henckel, 1964) so higher temperatures than normal may prevail in the tissues. The water stress experiments suggest that under these conditions *C. corticale* would grow even faster in the tissues. Again, it is interesting to compare, by the method outlined above, the overall growth of the fungus where water stress was induced either by droughting (W.S. Experiment 1, see Appendix Table 23) or by watering with PEG solutions (W.S. Experiment 2, see Appendix Table 24). The respective figures, as cm fungal growth per week, are: W.S. Experiment 1 after 4 weeks, watered 7.4, droughted 25.1; W.S. Experiment 2 after 17 days, watered 5.0, -2.5 bar PEG 17.7 and -5 bar PEG 29.6. Growth in both watered controls was less than the comparable figure (15.1 cm/wk) obtained in the second temperature experiment but more than in the experiment to investigate progression with time (2.5 cm/wk), where trees in full leaf were also used. Also, it would appear that either the PEG treatments imposed overall as great (-2.5 bar PEG) or a greater (-5 bar PEG) water stress as lack of watering or this material has other effects on sycamore that facilitate the growth of *C. corticale*.

Despite the inherent difficulties in relating water stress applied to young sycamores in pots to a field situation the apparent effect of water stress at 25^{o} on fungal growth is impressive; the growth rates obtained are in excess of 2 cm per day. Also the greater part of this growth is down the stem. Why this should be so is not clear. Further information on how water stress in sycamore favours growth by *C. corticale* is required to interpret this difference. Drought damage is in fact one of the commonest predisposing factors to fungal attack and there is evidence that cells which are not fully turgid may be more easily invaded by some fungi (Peace, 1962). However, the living cells of the sapwood invaded by *C.*

corticale have rigid walls and turgidity of the protoplast is unlikely to be important in determining rate of colonisation. Other tree pathogens favoured by water stress include *Botryodiplodia theobromae* which killed stressed but not non-stressed 2 year old *Platanus occidentalis* (Lewis & Arsdel, 1978), *Diplodia pinea* on pine and *Dothichiza populea* on poplar (Peace, 1962).

Plant growth is controlled directly by plant water stress, which modifies the anatomy, morphology, physiology and biochemistry of the plant. As the soil water content decreases, water deficits become more severe; breakdown of RNA, DNA and proteins increases, photosynthesis decreases and respiration increases. As stress increases, synthesis and translocation of growth regulators is probably inhibited, and translocation of other compounds is hindered. Carbohydrates and proteins are hydrolysed, and there is often an increase in soluble sugars and nitrogen compounds (Kramer, 1969) which is likely to favour growth of *C. corticale*. The amount of nitrogen in wood is rarely greater than 0.3% by weight and may well limit the growth rate of the fungus. Indeed, the carbon/nitrogen ratio of common artificial laboratory media is approximately 10/1 - 20/1, while that of wood may vary from 350/1 to 1250/1 (Merrill & Cowling, 1966). Growth of *C. corticale* in stressed trees may also be enhanced because alterations to the normal metabolism and particularly to enzyme systems of the tree could reduce host resistance.

The degree of which *C. corticale* is favoured by periods of water stress may vary as the physiological state of the tree changes over the growing season. Water stress is likely to be most severe at the end of hot, dry summers and results from the water stress experiments suggest that disease outbreaks following such summers are due to the effects of water stress as

well as high temperatures. This is supported by the observation of disease attacks on an island in Wanstead Park (Gregory & Waller, 1951) where the water table could have been about 60 cm below the surface of the soil (Peace, 1955) and more recently (1979) on an island at Thamesmead in south east London. Also, Bevercombe and Rayner (1978) observed that trees on freely drained ground were extensively infected, unlike those on poorly drained ground. Thus there is considerable support for the hypothesis that *C. corticale* is likely to be most damaging to sycamores during warm summers when periods of water stress are likely to occur, as in 1975 and 1976.

Most experiments which attempt to unravel the effects of temperature and water stress have to be carried out using young trees in pots, to allow control of their environment. In the field sooty bark has generally been reported on older sycamores, although in 1978 the disease was observed on young trees (c. 3 cm stem diameter) near Henley in Oxfordshire. Ideally older trees should be used for experiments, but in the field weather is unpredictable, and experiments set up in a hot summer are likely to give better results than in a cool summer. Basic to all studies of tree pathogens in the field is the variability of host material since the time required prohibits growth of relatively uniform trees for experiments, and it is necessary to piece together whatever information is available to discover which factors affect the disease.

An alternative hypothesis which could account for the sudden severe outbreak of sooty bark in 1976-7 is the development or introduction to Britain of a new highly pathogenic strain of *C. corticale*. Growth rates of a range of isolates on agar and in sycamore (Table 2) showed that there is considerable variation within the species. Although the pathogenicity

of isolates was in some cases significantly different, isolates of intermediate pathogenicity exist and there was no indication of the occurrence of more than one strain of *C. corticale*. Isolates from 1976-7 were certainly no more pathogenic than an isolate obtained in 1962 (Table 2 and Appendix Table 7) and there is no evidence that a new strain was responsible for the severe disease outbreak. The fungus is extremely variable, as is clearly evidenced by examination of isolates collected from Holland Park. Isolates from this one site cover the entire range of pathogenicity e.g. isolate 3 (collected in 1976) grew an average of 16.3 cm in sycamore over a 4 week period at c. 25° while isolate 108 (collected in 1975) grew 104.5 cm.

No sexual (perfect) state of *C. corticale* has so far been found and the mechanism of variation is unknown. Mutation and parasexuality are both possibilities, and although mutations affecting pathogenicity are rare this could be an effective mechanism and is perhaps the more likely considering the enormous numbers of conidia produced.

There was also considerable variation in the growth of *C. corticale* in sycamores within the various experiments, despite the standardisation of inoculum achieved by mycelial plugs or conidia on millipore discs and the use (in some experiments) of trees from a single seed stock. Thus a single isolate grew to different extents in trees of similar age kept under the same experimental conditions. Occasionally the fungus sporulated near the inoculation point within the experimental period, but other apparently identical inoculations did not cause lesion development. The colonisation of individual trees from two inoculations on opposte sides of the trunk was also variable (Appendix Table 7). The reason for the variability is not clear but it emphasises again the need for adequate

replication in inoculation experiments. It perhaps also reflects some weakness in the ability of the fungus to colonise sycamore which hinders the development of sooty bark epidemics.

Until this investigation the susceptibility of other *Acer* species was largely unknown. In Britain, sooty bark is usually found on sycamore though it has also been recorded on *A. campestre* (Anon, 1952). In France, also, sycamore appears to be the main host for *C. corticale* but it has been reported on *A. campestre*, *A. negundo* and *A. platanoides* though Moreau and Moreau (1954) consider these less susceptible. There is also a record of *C. corticale* on *A. platanoides* in Norway (Anon, 1961).

The scarcity of reports on other *Acer* species suggests that they are much less susceptible and rarely succumb to sooty bark. This is not entirely supported by the results of the inoculation experiment where *C. corticale* became established in all 13 types of ornamental acers and in one species, *A. nigrum*, caused wilting and sporulated on the tree within 19 days of inoculation.

Lesions of *C. corticale* were found on one, unidentified ornamental acer at Laleham Park (Staines) in 1977 indicating that some natural infection can occur. However, the lack of reports on these trees is surprising considering their apparent susceptibility and the frequency with which they are planted in some parks, especially in London, where sooty bark on sycamore has been severe.

C. corticale was recently observed growing saprophytically on *Aesculus hippocastanum* (horse chestnut) by Young (1978) and has been reported on species of *Carya* and *Tilia*. Inoculations of a range of trees and shrubs failed to infect most trees, although limited establishment occurred in a

few instances. On these results, it seems unlikely that *C. corticale* can cause disease in genera other than *Acer*.

Reports of sporulation on other trees and the apparent ability of the fungus to produce conidia on any sterile wood suggest that in nature *C. corticale* may be capable of sporulating on trees killed by other agencies. These could provide a reservoir of inoculum for infection of susceptible acers growing nearby and thus sometimes play a role in the disease epidemiology.

Two key questions remain largely unanswered: the mode of entry of *C. corticale*, and the times of year when infection is likely to occur. The results of the present investigation suggest that bark is an effective obstacle to *C. corticale* and that the fungus infects only through fairly substantial wounds. It seems incapable of entering natural openings such as the autumn leaf scars and this may be a further factor limiting its ability to develop rapidly within a sycamore population. Failure to infect via leaf scars could be due to changes associated with the formation of the abscission layer; possibly the number of conidia that can enter a leaf scar are insufficient to establish infection, or there are relatively few nutrients available at this site to give the inoculum sufficient potential for breaching cell walls. The histological studies indicate that once established in the tissues, *C. corticale* is capable of penetrating cell walls directly as well as through pits so, presumably, it is capable of producing some cellulases *in vivo*. However, if this is so, the response is essentially to cellulose materials in the host. All attempts to demonstrate cellulolytic activity *in vitro* with other forms of cellulose failed.

The problem of determining when infection is most likely to occur is

one of linking field experiments with hot summers, since all the evidence suggests that summer temperatures are most likely to determine the extent to which *C. corticale* will become established. Unfortunately, during the present field experiments the summers were cool and so the results must be interpreted with some caution. Nevertheless, they indicate that *C. corticale* can probably become established through wounds at any time of year but that its progress in the tree is likely to be more substantial following inoculations in early spring.

Growth of *C. corticale* seems to slow down after an initial period of six months or rather less, creating further problems in the interpretation of results. When a pathogen has successfully established itself in host tissue its subsequent spread is influenced by many factors including its aggressiveness, susceptibility of the tissue, and environmental conditions. Whether the reduction in growth is due to a host response to *C. corticale* remains in doubt. Histological studies provided no direct evidence of a host reaction limiting growth, although colonisation was associated with generalised production of gums, and sometimes unidentified globules and blisters were closely associated with hyphae. Gummy substances are often produced when plant tissue is damaged and may be effective barriers against some pathogens, especially in woody plants. Occasionally discoloured hyphae were observed embedded in gummy material, suggesting a fungicidal effect. The limitation of *C. corticale* within infected sycamore may depend on the rate at which the pathogen spreads through the tissues and thus avoids, or otherwise, the chemical or morphological defences of the host. This rate of spread will depend partly on temperature and water stress in the host and also on the inherent vigour of the pathogen, as well as on the speed of reaction of the invaded tissue. It seems likely that for about a month, or longer if conditions are optimal, *C. corticale* can

outstrip the host reaction. This is evidenced by frequent reisolation of the fungus from a few centimetres beyond the stained area during the first few weeks after inoculation.

In view of the history of sooty bark in Britain and the evidence supporting the apparent relationship to summer temperature, it seems unlikely that the disease will ever cause an epidemic on the scale of Dutch elm disease. The available evidence indicates that unusually hot summers are required as a primary factor for disease outbreaks, and sooty bark is also limited by the apparent dependence on wounding without any evidence of an associated vector. The fungus-eating beetles *Enimus brevicornis* and *Diplocoelus fagi* are commonly found in the spore layers of *C. corticale*, but there is no evidence that they play any part in spreading the disease. Grey Squirrels strip bark from healthy sycamores and may thus provide entrances for *C. corticale*, but such damage is not essential for major disease outbreaks. Conidia can be carried on the claws of squirrels which have been feeding on *C. corticale* and they may sometimes, through damage of sycamore tissues, transmit the fungus (Abbott, Bevercombe & Rayner, 1977). However, evidence is lacking for their involvement in most disease outbreaks and the fungus is dependent on chance wounding by animal, insect or climatic stress agents. Also, there is no evidence, so far, of a strain or race of *C. corticale* which can readily establish and cause acute disease in all inoculations.

The success of *C. corticale* depends so much on climate and biotic factors that sooty bark disease seems likely only to flare up following hot summers, and then die down again. Previous outbreaks have subsided naturally and the 1976-1977 epidemic has now died down. Cool summer weather appears to act as a natural control of the disease. If the apparent

relationship with summer temperature holds, the disease should remain largely confined to the south of the rivers Humber and Mersey. Control by felling and burning trees, as advocated after the original outbreak, is unlikely to be successful and is probably unnecessary in view of the natural control of disease in cool years. However, felling and burning diseased trees has some merit in view of the longevity and continued production of conidia. These could still infect fresh wounds several years after the appearance of disease. In the London area, which is the warmest part of Britain and thus the area in which disease outbreaks are most likely, acers should be mixed with other tree species to prevent sooty bark creating large gaps in the stands. Elsewhere in Britain less favourable conditions should restrict the expression of the pathogenicity of *C. corticale* in all but the most exceptionally hot summers.

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APPENDIX

TABLE 1 : Preparation of agar media[a].

Agar medium	Constituents	Distilled water
Cellulose	NH_4 SO_4 0.5 g L-aspargine 0.5 g KH_2 PO_4 1.0 g K Cl 0.5 g Mg SO_4 0.2 g Ca Cl_2 0.1 g Yeast extract 0.5 g Cellulose[c] 10 g Agar[b] 20 g	To 1 ℓ
3% malt extract	Malt extract 3.0 g Agar[b] 2.0 g	0.1 ℓ
5% malt extract	Malt extract 5.0 g Agar[b] 2.0 g	0.1 ℓ
Potato dextrose	Scrubbed potatoes (boiled and sieved) 20 g Dextose 2.0 g Agar[b] 2.0 g	0.1 ℓ
Rose bengal	Dextrose 1.0 g Yeast extract 0.05 g KH_2 PO_4 0.05 g K_2H PO_4 0.05 g Mg $SO_4.7H_2O$ 0.05 g Peptone 0.05 g Rose bengal 0.005 g Streptomycin 0.003 g Agar[b] 2.0 g	0.1 ℓ
V8-juice	Cambell's tomato and vegetable juice 0.01 ℓ Agar[b] 2 g	0.09 ℓ

TABLE 1 : Continued.

Agar medium	Constituents	Distilled water
Water Agar[b]	2 g	0.1 ℓ

The following media were prepared from standard Oxoid products:

Corn Meal

Czapex-Dox

Nutrient

Sabouraud dextrose

Key: a. Autoclaved at 125° for 15 min.

b. Davis or BDH.

c. Whatman's standard grade cellulose powder for chromatography.

TABLE 2 : Growth[a] of *C. corticale* in sycamores at 25°.

RANKED ISOLATES[b]	STAIN								REISOLATION								CONFIRMATION[d]								GIRTH[e]			
	A[c]		B		C		D		A		B		C		D		A		B		C		D		A	B	C	D
	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+				
57	3	3	0	0.3	2	1.5	1	1.2	1	1	0	0	1	0	1	3	−	−	−	−	−	−	−	−	41	45	40	39
Cr	11	4.5	5	13	5	9	3	6	11	5	5	4	1	2	0	1	−	−	−	−	−	−	−	−	42	34	43	40
L10	2	4	3	5	3	4	7	7	2	2	6	8	1	0	8	7	−	−	−	−	−	−	−	−	42	49	45	45
3	3	3	9	7	12	14	9	5	2	3	9	7	12	14	13	5	−	20	−	−	−	−	−	−	35	47	44	39
L13	22	11	4	10	8	5	11	13	21	10	4	10	10	4	11	13	−	−	−	−	−	−	−	−	41	38	40	42
113	8	5	10	4	33	23	5	8	2	5	9	4	34	20	4	6	−	−	−	−	−	−	−	−	44	43	44	39
251	16	12	21	17	13	19	21	25	15	12	21	18	1	19	21	25	−	−	−	−	−	−	−	40	43	39	45	45
114	19	20	17	13	38	26	14	24	18	20	16	13	30	20	15	26	−	−	−	−	−	−	−	−	35	43	48	39
250	51	50	6	4	28	23	22	31	40	40	5	4	38	20	22	29	−	53	−	1	20	10	−	−	50	33	40	44
WP	9	12	64	38	43	43	22	33	8	9	64	38	30	48	22	29	−	−	20	30	−	−	−	−	40	47	44	44
112	23	13	43	16	45	69	25	26	23	13	44	16	48	>69	25	20	10	−	30	10	30	50	−	10	44	41	40	37
HP	18	12	22	15	43	56	70	59	18	11	22	15	44	57	73	30	10	1	12	20	10	−	20	20	40	43	49	44

TABLE 2 : Continued.

RANKED ISOLATES[b]	STAIN								REISOLATION								CONFIRMATION[d]								GIRTH[e]			
	A[c]		B		C		D		A		B		C		D		A		B		C		D		A	B	C	D
	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+				
SP	20	11	33	15	26	14	78	79	20	10	33	15	26	17	72	79	−	−	30	1	10	1	−	−	42	38	49	47
L16	36	14	41	25	39	43	39	29	36	16	42	26	38	45	39	31	30	−	10	20	−	10	20	31	48	47	42	40
H	28	24	67	36	30	15	41	30	28	23	67	38	29	10	44	36	−	−	60	20	−	−	−	−	38	36	46	36
D	13	11	31	18	10	17	>100	71	13	12	31	18	13	16	>100	73	−	−	−	−	1	−	−	10	38	35	48	41
LP	18	18	62	36	75	29	24	56	18	19	70	39	76	30	26	60	10	18	−	−	20	−	−	−	47	37	39	49
108	79	29	44	12	60	35	106	63	79	29	44	12	50	36	105	63	50	10	40	10	10	20	40	−	44	34	38	44
WC	53	46	70	66	>100	60	62	50	55	46	70	75	>99	63	65	49	40	46	−	50	60	60	60	20	42	43	46	42
Control	0	0	0	0	0.2	1	1	0.1	0	0	0	0	0	0	0	0	−	−	−	−	−	−	−	−	48	46	46	43

Key: a. Growth (cm) below (−) and above (+) the inoculation point.

b. Based on *C. corticale* reisolation.

c. Replicate trees A, B, C, D.

d. Sporulation on sterile wood.

e. At inoculation point (cm).

TABLE 3 : Growth of *C. corticale* in sycamore trees at 25°.

ANOVA[a]: reisolation of characteristic mycelium

	DF	SS	MS	F
Isolates	18	86877.6	4826.53	4.75***
Residual	57	57882.5	1015.48	

Key: a. This ANOVA was the basis for Duncan's new multiple range test.

*** Significant at $P \leq 0.001$

TABLE 4 : Girth of sycamores inoculated with *C. corticale* isolates.

Isolate	Tree girth (cm)[a]			
	a	b	c	d
57	22.5	27	31	28
108	26	38	21	22.5
L13	30	37	32	19.5
114	33.5	36	29	27
SP	32	20	30	30
L10	39	33.5	35	30
112	20	22.5	40	22.5
WP	19	27	24.5	13.5
WC	34	30.5	27	22.5
3	31.5	30	34	29
LP	19	30.5	31	32
L16	24	19	29.5	22.5
Cr	35	27	15.5	21
113	22	25.5	38.5	32.5
250	33	32	27.5	13.5
251	28	34	16	24.5
$\bar{x}$	28.03	29.34	28.84	24.41
± (S.E.)	1.59	1.44	1.75	1.46

Key: a. Girth at the points of inoculation on 9 May 1980.

TABLE 5a : Mean daily temperature in glasshouse maintained at c. 25°[a].

DATE	1977		1978											
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1	-	24.5	*22*[b]	21.0	22.5	21.0	19.0	24.0	21.5	18.5	17.0	18.0	18.0	18.0
2	15.5	23.5	*22*	*19.0*	25.0	21.0	19.0	23.5	21.5	21.5	23.0	17.5	19.0	19.5
3	13.5	23.5	*22*	*17.0*	*25.0*	20.0	25.0	23.0	20.0	24.0	17.0	15.5	17.0	19.0
4	16.5	23.5	*21*	15.5	*25.0*	19.0	22.0	22.0	21.0	23.5	20.0	16.5	17.5	20.0
5	17.0	23.5	21	13.5	*25.0*	22.0	*21.0*	23.0	21.0	21.5	*19.0*	18.5	16.5	20.5
6	16.0	24.0	20.5	16.0	25.0	27.5	20.0	22.5	21.0	20.5	18.0	18.0	16.5	19.0
7	17.0	24.0	20.0	*17.0*	25.5	21.0	20.5	22.0	21.0	22.5	18.0	18.0	17.5	20.5
8	18.0	24.0	21.5	*17.0*	23.0	20.5	21.0	22.0	21.5	22.5	19.0	17.5	17.0	21.0
9	18.5	24.5	20.5	*17.0*	24.0	21.0	22.5	21.0	23.0	21.5	20.0	17.5	17.0	21.0
10	19.0	24.5	20.5	18.0	24.5	21.5	21.0	17.0	24.0	22.5	22.0	20.0	15.5	21.0
11	17.0	24.5	18.0	18.5	26.0	23.0	23.0	23.0	23.5	21.0	17.5	19.0	16.5	21.5
12	13.0	24.5	18.5	19.0	23.0	20.5	20.0	21.0	24.5	21.5	19.0	17.5	17.0	21.5
13	14.0	25.0	21.5	20.5	*22.0*	20.0	21.0	21.0	26.0	22.0	19.0	16.0	17.0	21.5
14	14.5	24.5	21.0	21.0	21.5	19.0	21.0	21.0	22.5	22.0	17.5	16.5	17.5	21.5
15	17.5	24.0	20.5	21.0	21.5	16.0	21.0	21.0	22.5	21.5	19.5	15.0	16.5	21.5
16	19.0	24.0	20.0	21.0	20.5	17.0	21.5	20.0	23.5	21.5	19.0	14.0	17.5	21.5
17	18.5	24.0	18.0	19.0	22.5	17.0	21.5	24.0	25.5	21.5	17.0	15.5	*20.0*	21.0
18	18.0	23.0	18.0	19.0	23.0	15.0	17.0	24.0	24.0	21.5	16.5	15.5	*18.0*	20.5
19	21.0	25.0	20.0	20.0	21.0	17.0	22.0	24.0	20.0	22.0	17.0	17.0	*18.0*	20.5
20	20.0	22.5	20.0	20.0	20.0	17.5	23.0	22.0	19.0	23.0	18.5	16.5	*17.0*	21.0
21	25.0	23.0	21.0	22.0	*21.0*	16.0	21.0	21.5	21.5	23.5	19.0	17.0	18.0	21.5
22	26.0	24.0	19.0	22.5	22.0	17.0	21.5	21.0	20.5	21.5	21.0	15.0	*17.5*	21.5
23	24.5	23.5	21.5	23.5	21.0	15.0	21.0	21.5	19.5	21.5	20.0	15.5	17.0	21.5
24	24.5	21.0	21.5	22.5	22.0	23.0	22.0	23.0	21.0	21.0	16.0	16.5	16.5	21.5
25	24.0	*23.0*	22.0	23.5	22.0	20.0	23.0	22.0	23.0	21.0	17.5	17.0	14.0	21.5
26	24.0	*22.0*	19.5	23.5	22.0	19.0	24.0	20.5	22.0	21.0	15.5	17.5	13.0	21.5
27	23.5	20.5	21.5	23.0	22.5	20.0	25.0	21.5	23.0	21.5	15.5	16.0	13.5	21.5
28	23.5	*21.0*	20.5	23.5	21.5	19.0	25.5	23.5	21.0	21.5	16.0	16.0	14.0	21.5

TABLE 5a : Continued.

DATE	1977		1978											
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
29	23.5	21.0	19.0		*22.0*	*19.0*	25.5	21.5	21.5	22.0	14.5	16.5	20.0	22.0
30	24.0	21.0	19.0		23.0		26.0	23.5	20.5	20.0	16.5	16.5	20.5	*22.0*
31		22.5	21.0		20.5		23.5		20.0	18.0		17.5		*21.0*

Key: a. From thermograph at canopy height (1 m).

b. Figures in italics are estimates.

TABLE 5b :

DATE	1979									1980					
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Jan.	Feb.	Mar.	Apr.	May	June
1	*21.0*[b]	22.0	22.5	25.0	22.5	21.5	23.5	25.5	22.0	-	*25.0*	25.0	27.5	23.5	25.0
2	21.0	21.0	22.0	*24.0*	24.0	23.0	20.0	26.0	22.5	-	*25.0*	25.0	28.0	23.0	27.0
3	21.5	21.0	23.0	*23.0*	22.0	23.0	23.5	*25.0*	22.5	-	*24.5*	24.0	27.0	24.0	29.0
4	20.0	21.0	2.15	*22.0*	25.0	22.5	24.0	*24.0*	22.0	-	24.5	24.0	24.0	23.0	28.5
5	20.5	22.0	23.5	*21.0*	29.0	20.0	25.0	*23.5*	22.0	-	24.5	*26.0*	23.5	22.0	27.0
6	21.5	21.0	24.0	20.0	26.0	21.5	24.0	23.0	23.0	-	25.0	*26.0*	23.0	22.0	26.5
7	22.0	21.0	23.0	18.0	24.5	23.0	21.0	23.0	-	-	25.5	26.0	23.0	22.0	25.5
8	22.0	20.5	21.5	18.5	24.0	23.0	23.5	22.0	-	-	25.0	26.0	22.0	22.0	25.0
9	22.0	*20.5*	19.0	21.0	27.0	23.5	24.0	21.5	-	-	26.5	26.5	21.0	27.0	25.0
10	22.0	20.5	21.5	21.0	23.0	23.0	24.0	22.0	-	-	25.0	26.0	24.0	26.5	24.0
11	22.5	20.5	23.5	22.5	25.0	24.0	22.5	23.0	-	-	*25.0*	27.5	25.0	27.0	26.0
12	22.0	20.0	20.5	25.0	28.0	23.0	24.0	24.0	-	-	*25.0*	26.0	26.0	26.0	27.5
13	21.5	20.5	20.0	24.5	28.0	23.0	22.0	22.0	-	-	25.0	23.5	26.0	26.0	26.0
14	21.5	20.0	20.0	24.5	26.0	22.0	23.0	22.0	-	-	26.0	24.0	24.0	27.0	26.0
15	21.5	20.0	20.5	25.0	23.5	22.0	21.5	21.0	-	-	25.0	24.0	24.0	*26.0*	25.0
16	21.5	21.5	*20.5*	20.5	23.0	22.0	23.0	21.0	-	-	25.5	24.5	24.0	25.0	
17	21.5	20.5	*21.0*	21.5	23.0	23.5	25.5	19.0	-	-	26.0	24.0	24.0	26.0	
18	21.5	21.0	21.5	24.0	22.0	25.0	23.5	20.0	-	-	25.5	24.0	25.5	25.0	
19	22.0	21.0	19.5	24.0	20.0	25.5	23.5	20.5	-	-	25.5	23.0	23.5	23.5	
20	21.5	21.0	23.0	*20*	19.0	26.0	22.0	22.0	-	-	26.0	*24.0*	23.0	23.5	
21	21.5	21.0	22.0	23.5	19.5	24.0	18.5	23.0	-	-	25.0	24.0	24.5	22.0	
22	21.5	21.0	24.0	25.0	21.5	24.0	21.5	22.0	-	-	24.0	24.5	23.0	*23.0*	
23	21.5	22.5	24.0	23.0	22.5	23.5	23.0	22.0	-	-	25.0	24.5	23.5	23.0	
24	21.5	24.0	25.0	23.0	21.0	24.0	24.5	21.0	-	-	24.0	24.0	24.0	23.5	
25	22.5	23.0	25.0	25.0	19.0	23.5	26.0	22.0	-	-	24.5	24.0	23.0	25.0	
26	21.5	23.5	23.0	22.0	18.0	22.0	27.0	19.0	-	-	24.5	24.0	23.0	25.0	
27	21.5	22.5	23.0	24.0	21.0	24.5	27.5	19.5	-	-	26.0	24.0	23.5	25.0	
28	21.5	23.0	22.5	19.0	20.5	23.5	25.5	21.0	-	*25.0*	25.0	26.0	23.0	23.5	

TABLE 5b : Continued.

DATE	1979									1980					
	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Jan.	Feb.	Mar.	Apr.	May	June
29	21.5		21.5	25.0	23.0	22.5	24.5	23.0	-	*25.0*	25.0	26.0	23.0	25.0	
30	21.0		21.5	21.0	23.0	22.5	23.5	24.0	-	*25.0*		26.0	23.5	24.0	
31	21.5		23.0		23.0		24.5	23.5	-	*25.0*		26.5		24.0	

Key: a. From thermograph (1 m).

b. Figures in italics are estimates.

TABLE 6a : Mean daily temperature within field plot[a].

DATE	1978											1979		
	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
1	-	*8.0*[b]	8.5	8.0	19.0	15.5	15.0	11.5	9.0	13.5	1.0	-4.5	*6.0*	4.0
2	-	5.0	8.5	8.0	18.0	15.0	15.0	12.0	9.0	13.0	4.0	-2.0	*2.0*	10.5
3	-	*5.5*	6.5	13.5	19.0	12.0	15.0	13.0	8.0	9.5	5.0	-2.0	*1.0*	8.5
4	-	*3.5*	6.5	11.0	16.0	13.0	*15.0*	14.0	11.0	11.0	3.0	-1.5	*2.0*	5.0
5	-	*3.0*	7.0	13.0	15.5	12.0	*15.0*	14.0	12.5	11.0	*1.5*	*-2.0*	*2.0*	7.0
6	-	4.0	4.0	13.0	15.0	12.0	*14.0*	15.0	10.0	9.0	*1.5*	2.0	*-0.5*	6.0
7	-	9.0	7.0	11.0	14.5	14.0	*13.5*	13.5	13.0	12.5	*5.0*	6.5	*3.0*	7.0
8	-	6.0	7.0	12.0	14.0	14.0	*14.0*	15.5	13.5	9.0	9.5	4.0	*2.0*	8.5
9	-	5.0	6.5	13.0	12.5	14.0	*14.0*	16.0	14.0	8.5	11.0	1.5	*3.0*	2.5
10	-	10.0	5.0	12.0	12.0	13.0	*15.0*	18.0	15.0	8.0	10.0	*3.5*	*2.5*	9.0
11	-	12.0	3.0	*11.5*	14.0	*14.0*	*14.0*	15.0	14.0	9.5	10.0	*2.0*	*1.5*	8.0
12	-	8.0	5.0	10.0	11.0	*14.0*	*13.0*	*15.0*	13.0	8.5	8.0	*0.0*	*0.0*	6.0
13	-	7.5	2.5	8.5	11.0	16.0	*16.0*	16.0	11.0	9.0	7.0	*-2.5*	2.0	3.0
14	-	6.0	5.0	9.0	9.0	15.0	16.0	13.5	12.0	13.0	6.0	2.0	2.0	2.0
15	-	7.0	5.0	11.5	10.0	14.0	15.0	15.5	11.0	9.0	6.0	4.0	2.0	1.0
16	-	4.0	7.0	11.5	13.5	14.0	15.0	16.0	11.5	8.5	3.0	4.0	-1.0	4.5
17	-	2.0	7.0	11.0	14.0	16.0	14.5	11.0	7.0	12.0	0.0	2.0	-1.0	5.0
18	-	6.5	*6.0*	11.0	14.0	15.5	14.0	12.0	10.0	14.5	- 2.5	*1.0*	-1.0	5.5
19	-	8.5	8.0	10.5	16.5	15.0	15.0	11.0	11.0	8.0	0.0	3.0	-1.0	3.0
20	-	5.5	10.0	11.5	18.0	13.5	*14.0*	14.0	11.0	8.0	0.5	1.0	*1.0*	3.0
21	-	5.5	9.0	11.0	13.5	14.5	*18.0*	13.0	11.5	12.0	1.0	1.5	*1.5*	*3.0*
22	-	*6.0*	9.0	11.0	12.0	14.5	*16.0*	16.0	9.0	11.5	0.0	0.0	*3.5*	*3.0*
23	-	*5.0*	11.0	14.0	10.5	14.5	*15.0*	15.0	9.5	11.5	1.0	1.0	*5.0*	3.0
24	8.5	*6.5*	10.0	11.0	12.0	15.0	*14.5*	10.0	12.0	5.5	7.0	*-1.5*	*1.5*	10.0
25	8.0	*7.5*	7.0	11.0	12.5	16.0	15.0	12.5	13.0	2.0	7.0	*2.5*	*0.0*	8.5
26	7.5	*6.0*	5.5	11.0	10.0	16.5	14.0	11.0	13.0	- 2.0	9.0	3	*0.0*	6.0
27	*8.4*	*10.0*	5.5	14.0	13.0	18.0	15.0	12.5	8.0	- 0.5	9.0	*-4.0*	*4.0*	6.0
28	*8.0*	8.0	7.0	15.0	16.5	18.0	15.0	*16.0*	7.0	- 2.0	9.0	*0.0*	*4.0*	4.5

TABLE 6a : Continued.

DATE	1978											1979		
	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.
29		8.5	9.0	15.5	13.5	18.5	14.0	9.0	9.0	*- 1.0*	5.0	*1.0*		5.0
30		7.0	11.0	18.0	13.0	17.5	13.0	10.5	11.0	*- 2.0*	- 2.5	*1.0*		4.5
31		8.0		19.0		15.0	11.5		10.0		- 6.0	*3.0*		*5.0*

Key: a. From thermohydrograph at canopy height (4 m).

b. Figures in italics are from Silwood Park meteorological records.

TABLE 6b :

DATE	1979									1980				
	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
1	*6.5*[b]	4.0	14.0	13.0	16.0	16.5	10.0	8.0	11.0	*0.5*	4.0	7.0	7.0	11.0
2	*3.5*	5.0	14.0	13.0	15.5	15.0	14.0	8.5	11.0	-1.0	4.0	5.0	7.5	9.0
3	*2.5*	5.0	16.0	15.0	14.5	14.0	15.0	13.5	9.0	7.0	6.5	2.0	5.5	8.0
4	*1.5*	5.0	15.0	17.0	14.0	14.0	10.0	9.0	10.5	5.5	7.0	4.0	7.0	8.5
5	*2.0*	4.5	13.0	21.5	16.0	16.0	9.0	10.0	11.0	6.0	2.5	6.5	8.0	7.5
6	5.0	7.5	12.0	17.0	17.0	16.0	13.5	9.0	11.5	6.0	8.0	6.0	6.0	7.0
7	5.5	10.0	11.0	16.0	15.0	15.0	13.0	9.0	10.0	4.5	8.0	5.5	8.0	7.0
8	6.5	11.0	13.5	17.5	14.0	17.0	16.5	7.0	10.0	3.5	9.0	5.0	6.0	6.0
9	11.0	11.0	14.0	15.0	13.0	16.0	16.0	3.0	9.0	2.5	8.0	5.5	8.0	7.0
10	12.0	11.0	13.0	16.0	15.5	14.0	12.5	3.0	4.5	0.0	7.0	6.0	6.5	8.0
11	11.5	13.5	13.0	15.0	17.5	13.0	13.5	9.0	9.5	-1.0	6.0	8.5	8.0	12.0
12	10.0	11.0	13.0	16.0	18.5	13.0	15.5	0.0	8.5	0.0	6.0	6.0	10.0	14.0
13	12.0	16.0	13.5	17.5	17.0	9.0	12.0	4.0	*9.5*	-1.5	7.0	3.5	11.5	16.0
14	13.0	18.0	11.0	16.5	14.5	10.5	10.0	4.5	8.5	0.5	8.5	4.5	12.0	14.0
15	13.0	18.0	12.0	14.0	14.0	10.0	10.5	4.0	6.0	3.0	5.0	5.0	12.0	13.0
16	13.5	12.0	14.0	17.0	14.0	13.0	11.5	3.0	7.5	2.0	5.0	4.0	13.0	13.5
17	5.0	11.5	16.0	19.0	12.5	15.0	12.0	6.5	5.5	1.5	8.0	3.0	11.0	
18	9.0	10.0	16.0	16.0	13.5	16.5	10.5	6.0	3.5	0.5	7.0	4.5	11.0	
19	11.0	10.5	18.0	14.5	13.0	14.5	12.5	2.0	3.5	4.0	6.0	2.5	8.0	
20	9.0	10.0	19.0	14.0	16.0	10.5	9.0	2.5	3.0	4.0	9.0	1.5	5.5	
21	8.5	10.5	15.0	12.5	12.0	8.0	10.5	3.0	1.5	6.0	9.0	1.0	8.5	
22	10.0	11.5	13.5	13.0	11.0	8.0	9.0	9.0	2.5	5.0	5.0	4.0	8.5	
23	8.0	10.0	13.0	15.0	10.5	10.0	8.0	5.0	1.5	*5.5*	3.5	6.5	8.0	
24	7.5	10.0	12.0	16.0	11.0	11.0	11.0	5.0	3.0	*5.5*	4.5	7.0	9.5	
25	8.0	9.0	13.0	17.0	11.5	16.0	11.0	10.5	7.0	*3.0*	1.5	6.0	5.5	
26	8.5	9.0	14.0	18.0	11.5	15.0	8.5	11.5	3.5	*2.5*	3.5	9.0	7.5	
27	9.0	10.5	13.5	21.5	10.0	9.0	5.0	11.0	2.5	*0.0*	5.0	9.5	8.5	
28	8.0	13.0	16.5	19.0	12.0	9.0	6.5	7.0	*8.0*	*-2.0*	6.0	9.0	8.0	

TABLE 6b : Continued.

DATE	1979									1980				
	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May
29	10.0	13.0	13.0	16.0	14.0	11.5	5.0	8.5	*4.0*	*3.0*	8.0	7.5	6.5	
30	7.0	15.0	11.0	15.5	15.0	11.0	11.0	10.5	*3.5*	*5.0*		7.5	10.0	
31		12.0		15.0	17.0		8.0		*2.0*	9.5		10.0		

Key: a. From thermohydrograph at canopy height (4 m).

b. Figures in italics are from Silwood Park meteorological records.

TABLE 7 : Growth[a] of *C. corticale* in sycamore under field conditions.

RANKED ISOLATES[b]	YEAR OF COLLECTION	STAIN																REISOLATION													
		Aa[c]		Ab		Ba		Bb		Ca		Cb		Da		Db		Aa		Ab		Ba		Bb		Ca		Cb		Da		Db	
		−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+
57	1948-50	0	0.5	0	0	0.5	1	0.5	0.5	1	0	0	3	3	1	7	4	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
108	1975*	4	5	5	3	0	1	0	1	0.2	0.2	3	2	3	4	1	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
L13	1976	1	2	5	1	0	4	0	4	1	4	4	2	†	†	9	5	0	0	0	0	0	0	0	0	0	1	5	0	0	0	8	5
114	1975*	3	6	2	6	5	4	0	3	2	1	0	0.5	4	5	1	4	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0
SP	1977	1	1	6	3	2	8	0.5	0.5	5	2	1	1	7	2	1	6	0	0	0	3	0	0	0	0	5	0	0	0	0	0	2	1
L10	1976	4	1	0	1	1	1	4	5	6	8	1	2	9	4	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
112	1975*	0	6	0	1.5	4	6	0	1	4	4	0	1	10	9	1	5	0	5	0	1	0	0	0	0	4	5	0	0	7	4	0	2
WP	1978	2	2	2	2	9	5	1	2	2	3	1	3	3	7	1	9	0	0	1	0	2	2	0	0	0	3	1	2	2	5	0	6
WC	1962	3	7	3	2	2	2	0	1	12	4	1	2	2	4	5	12	0	0	0	0	0	0	0	0	0	0	0	0	2	4	5	12
3	1976*	0	1	0	1	3	7	2	6	4	10	1	2	8	10	3	11	0	0	0	0	0	0	0	0	3	8	1	2	9	0	0	0
LP	1977	8	4	2	1	4	1	5	16	2	4	4	4	7	6	3	4	4	0	0	0	0	0	6	15	0	5	0	0	0	0	0	0
L16	1976	c. 0.5	8	0	c. 20	2	4	10	2	4	9	5	3	1	2	2	2	8	0	5	13	0	2	9	0	0	0	1	0	0	0	0	0

TABLE 7 : Continued.

RANKED ISOLATES[b]	YEAR OF COLLECTION	STAIN																REISOLATION													
		Aa[c]		Ab		Ba		Bb		Ca		Cb		Da		Db		Aa		Ab		Ba		Bb		Ca		Cb		Da		Db	
		-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Cr	1978	4	2	10	8	3	5	3	4	1	3	0	0	5	5	13	9	0	0	8	5	0	0	0	0	0	3	0	0	5	4	4	2
113	1975*	0	0	0	1	16	10	10	5	4	4	2	4	3	8	5	6	0	0	0	0	10	0	0	0	0	0	2	2	0	1	0	0
250	1976	9	4	0	1	4	10	8	1	2	9	2	5	7	6	4	6	1	0	0	0	0	0	11	2	0	3	2	6	7	6	0	5
251	1976*	0	6	9	10	12	9	10	6	3	1	10	5	8	1	2	7	0	6	1	5	0	0	10	6	0	0	10	5	0	1	10	6
Control	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Key: a. Growth (cm) below (-) and above (+) the inoculation point.

b. Based on stain.

c. Replicate trees A, B, C, D with inoculations a and b.

† Stripped by squirrels.

* Collected from Holland Park.

TABLE 8 : Growth of *C. corticale* in sycamore under field conditions.

ANOVA[a]: stain induced by *C. corticale*

	DF	SS	MS	F
Isolates	15	734.53	48.97	1.706 n.s.
Residual	112	3215.25	28.71	

Key: a. This ANOVA was the basis for Duncan's new multiple range test.

TABLE 9 : Susceptibility of *Acer* spp. to *C. corticale*.

	Height (cm)	Girth (cm)[a]	Stain[b]		Reisolation[b]		Confirmation[bc]	
			-	+	-	+	-	+
A. nigrum (1)	87	24	>11	15	11	48	11	48
A. saccharum	250	53	1	18	3	18	3	17
A. negundo	260	82	8	7	1	6	1	6
A. rubrum	180	49	7	1.5	6	2	1	2
A. saccharinum	150	49	1.5	5	1	4	1	4
A. platanoides	220	54	1	2	1	2	1	0
A. hersii	200	34	11	9	11	6	11	6
A. cappadoicum	140	40	4	4	2	4	2	2
A. campestre	130	48	4	1	4	1	2	1
A. japonicum	70	53	0	5.5	4	1	4	1
A. palmatum	95	36	0	3	3	3	3	3
A. palmatum dissectum atropurpureum	40	42	0	1	1	1	1	1
Sycamore (1)	200	45	2	3	2	3	1	3
*A. griseum**	90	33	4	8	5	7	-	8
A. nigrum (2)*	90	31	2	5	2	4	-	-
Sycamore (2)*	90	29	1	1	1	1	-	-

Key: a. Girth at the inoculation point on the day of sampling.

b. Growth below (-) and above (+) the inoculation point after 5 or 6 (*) weeks at 25°.

c. Sporulation on sterile wood.

TABLE 10 : Stain induced by inoculation with *C. corticale* in *A. platanoides* and in *A. psuedoplatanus*.

a) ANOVA

	DF	SS	MS	F
Host	3	58.712	19.571	0.587 n.s.
Error	7	233.333	33.333	

b) Modified t-test

F	DF (t)	t
1.905	9	1.266 n.s.

TABLE 11 : Growth of *C. corticale* in *A. saccharum* and in *A. pseudoplatanus*.

a) Stain

ANOVA

	DF	SS	MS	F
Host	1	166.046	166.046	1.307 n.s.
Error	11	1397.837	127.076	

2-tailed F-test

F	DF (d^a)	d^a
24.75***	5.4	1.057 n.s.

b) Reisolation of characteristic mycelium

ANOVA

	DF	SS	MS	F
Host	1	161.556	161.556	1.341 n.s.
Error	11	1325.213	120.474	

2-tailed F-test

F	DF (d^a)	d^a
15.415***	5.6	1.075 n.s.

Key: *** Significant at $P \leq 0.001$.

a. Equivalent to student's t.

TABLE 12 : Mean daily maximum temperatures[a].

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1978	-	5.7	10.6	10.1	16.7	18.9	19.5	19.5	18.7	15.8	11.5	6.8
1979	3.0	3.9	8.3	12.0	15.2	18.4	21.5	19.7	18.4	15.3	9.8	8.7
1980	5.3	9.1	8.3	13.9	16.8							

Key: a. From Silwood Park meteorological records.

TABLE 13 : Summary of growth of *C. corticale* in sycamores inoculated at different times.

Inoculation time	Number of observations				Total stain				Total reisolation				
	6	12	18	24	6	12	18	24	6	12	18	24	months
Feb.	6	6	6	5	83	42	37	39	93	44	32	36	
May	7	6	6	6	66	36	54	37	36	26	42	17	
Aug.	10	10	9	-	65	79	52	-	37	57	28	-	
Nov.	8	9	6	-	21	24	50	-	15	2	19	-	

TABLE 14a : Growth[a] of *C. corticale* in sycamores inoculated at different times.

Sampling time[b]	Branches inoculated February 1978								Branches inoculated May 1978							
	Tree	Girth[c]	Stain		Reisolation		Confirmation[d]		Tree	Girth[c]	Stain		Reisolation		Confirmation[d]	
			-	+	-	+	-	+			-	+	-	+	-	+
6	17	194	2.0	4.0	1	6	-	-	23	97	0.7	1.8	1	2	-	-
	5	84	2.0	2.0	6	6	-	-	3	66	4.0	4.0	3	7	-	-
	4	124	5.0	29.0	8	30	-	30	2	95	17.0	20.0	0	0	-	-
	6	103	0	0	3	3	-	-	5	57	2.0	1.2	2	0	2	-
	1	87	1.5	1.0	1	5	-	-	12	85	0	0	2	0	2	-
	26	196	20.0	16.5	5	19	-	19	15	120	8.0	5.0	9	5	9	5
	C2	115	9.0	5.0	0	0			28	91	2.0	1.0	1	4	1	-
									C3	58	0	0	0	0	-	-
12	24	98	0.5	0.5	4	5	-	-	16	75	3.5	3.0	3	0	3	-
	27	144	10.0	6.0	9	6	9	6	24[F]	87	-	-	-	-	-	-
	7	54	2.5	3.0	2	2	1	-	13	86	2.0	2.5	2	3	2	3
	11	70	4.0	4.0	4	2	2	-	9	75	4.0	9.0	3	7	3	7
	8	92	4.0	5.0	2	5	-	-	14	55	1.0	2.0	1	1	1	1
	28	75	1.0	1.0	1	2	-	-	10	94	2.5	5.0	2	4	1	-
	C3	83	0	0	0	0	-	-	30	92	0	0	0	0	-	-
									C1	58	0	0.5	0	0	-	-
18	22	183	9.0	7.0	6	7	-	7	4	73	3.0	2.5	2	2	2	2
	16	188	1.0	1.0	0	0	-	-	25	52	5.0	4.0	2	3	-	-
	15	118	7.0	3.0	7	2	7	2	20	60	3.5	9.0	4	10	4	10
	18	84	2.0	2.0	2	2	-	2	18[F]	109	-	-	-	-	-	-
	13	75	1.5	1.0	1	2	-	1	11	136	3.0	3.0	0	3	-	3
	14	53	1.0	1.5	1	2	1	1	29	117	3.0	5.0	2	4	2	4
	C4	177	1.0	0	0	0	-	-	26	124	4.0	9.0	4	6	2	6
									C2	49	0.5	0	0	0	-	-

TABLE 14a : Continued.

Sampling time[b]	Branches inoculated February 1978								Branches inoculated May 1978							
	Tree	Girth[c]	Stain		Reisolation		Confirmation[d]		Tree	Girth[c]	Stain		Reisolation		Confirmation[d]	
			-	+	-	+	-	+			-	+	-	+	-	+
24	20	196	3.0	4.0	3	4	3	4	1	114	2.0	8.0	0	0	-	-
	29	158	1.0	1.0	0	0	-	-	8	84	2.0	4.0	5	0	-	5†
	30	177	5.0	3.0	5	2	5	2	22	232	4.0	6.0	1	0	-	-
	3[F]	196	-	-	-	-	-	-	19	104	1.0	1.0	3	3	3	-
	9	50	3.0	5.0	3	5	3	5	27	153	5.0	4.0	4	0	4	4†
	10	90	6.0	8.0	6	8	-	8	6	90	0	0	0	1	-	-
	C1	71	2.0	2.0	0	0	-	-	C4	150	0	0	0	0	-	-

Key: a. Growth below (-) and above (+) the inoculation point.

b. Months after inoculation.

c. At inoculation point on day of sampling (cm).

d. Sporulation on sterile wood.

C. Control.

F. Failed inoculation.

† Result obtained after the manuscript had been typed and therefore not included in analyses or other tables.

TABLE 14b :

Sampling time[b]	Branches inoculated August 1978								Branches inoculated November 1978							
	Tree	Girth[c]	Stain		Reisolation		Confirmation[d]		Tree	Girth[c]	Stain		Reisolation		Confirmation[d]	
			-	+	-	+	-	+			-	+	-	+	-	+
6	5	51	3.0	1.0	0	1	-	1	26	148	0	1.0	0	0	-	-
	4	82	3.0	2.5	2	4	1	4	24	100	0	0	0	0	-	-
	24	68	4.0	1.0	4	1	-	-	13	54	0.5	0.5	1	0	-	-
	22	140	6.5	4.0	0	5	-	5	20	57	1.5	1.0	0	0	-	-
	10	107	8.0	17.0	1	1	1	-	15	153	3.0	3.0	4	3	3	3
	27	72	1.0	2.0	1	6	-	-	9[F]	8[†]	-	-	-	-	-	-
	21	71	2.0	3.0	3	2	-	-	30	61	1.0	1.0	0	0	-	-
	1	84	1.0	1.0	1	0	-	-	8	51	4.5	3.0	2	4	-	3
	6	62	0	0.1	0	0	-	-	23	163	0	1.0	1	0	1	-
	16	55	3.0	2.0	2	3	2	-	22[F]	100	-	-	-	-	-	-
	C2	62	0	0	0	0	-	-	C2	50	0	0	0	0		
12	13	68	1.0	1.0	2	1	2	1	16	56	1.0	1.0	0	0	-	-
	14	72	1.0	1.0	1	2	1	2	2	70	1.0	3.0	0	0	-	-
	8	104	20.0	11.0	10	6	7	-	27	81	0.5	0.5	0	0	-	-
	11	118	10.0	10.0	6	10	6	7	14	58	2.0	0.5	0	0	-	-
	9	76	3.0	3.0	3	3	3	3	29[F]	62	-	-	-	-	-	-
	3	59	0.3	0	0	0	-	-	3	65	1.5	2.0	0	0	-	-
	25	66	2.5	4.0	3	2	3	2	5	72	0.5	2.0	1	1	1	1
	12	77	1.0	1.0	1	1	-	1	25	123	1.0	0.5	0	0	-	-
	19	76	3.0	2.0	3	2	3	2	18	150	1.0	5.0	0	0	-	-
	17	48	1.0	2.5	0	1	-	1	17	128	0.5	0	0	0	-	-
	C2	76	0.5	0.5	0	1	-	1	C4	174	0	0	0	0	-	-

TABLE 14b : Continued.

Sampling time[b]	Branches inoculated August 1978								Branches inoculated November 1978							
	Tree	Girth[c]	Stain		Reisolation		Confirmation[d]		Tree	Girth[c]	Stain		Reisolation		Confirmation[d]	
			-	+	-	+	-	+			-	+	-	+	-	+
18	7	82	2.5	2.0	2	2	-	2	6	69	4.0	4.0	4	0	-	-
	26	117	2.0	1.0	2	1	2	1	11	63	2.0	3.0	2	1	2	3†
	28	102	0	10.0	0	0	-	-	4	68	4.0	5.0	4	4	-	4
	18	63	2.0	2.0	2	2	2	-	1	75	1.0	3.0	0	0	-	-
	15	47	5.0	1.0	3	0	-	-	21	124	4.0	1.0	0	4	4†	-
	3	70	5.0	4.0	5	0	4	-	28	212	9.0	10.0	0	0	7†	4†
	30	116	5.0	5.0	5	4	5	4	7[F]	66	-	-	-	-	-	-
	29	75	1.0	2.0	0	0	-	-	10[F]	156	-	-	-	-	-	-
	23[F]	310	-	-	-	-	-	-	12[F]	117	-	-	-	-	-	-
	20	88	1.0	1.0	0	0	-	-	19[F]	133	-	-	-	-	-	-
	C3	73	1.0	0.5	0	0	-	-	C3	97	0	0	0	0	-	-

Key: b. Months after inoculation.

c. At inoculation point on day of sampling (cm).

d. Sporulation on sterile wood.

C. Control.

F. Failed inoculation.

† Result obtained after the manuscript had been typed and therefore not included in analyses or other tables.

TABLE 15 : Relation between length and depth of stain.

a) Modified t-tests to investigate differences in stain 6, 12 and 18 months after inoculation

Sampling time (months)[a]	Deep stain		Surface stain	
	DF	t	DF	t
6 v. 12	4.0	0.52 n.s.	29.2	1.40 n.s.
6 v. 18	3.8	0.17 n.s.	24.8	2.02 n.s.
12 v. 18	3.1	0.64 n.s.	12.0	1.22 n.s.

Key: a. Months after inoculation.

TABLE 16 : Length and depth of stain in sycamore inoculated with *C. corticale*[a].

a) Deep colonisation[c]

Inoc. time	Sample time[b]	Stain Length (cm)									
Feb.	6	6.0	4.0	34.0							
May	6	13.0									
Aug.	6										
Nov.	6										
Feb.	12	16.0	8.0	2.0							
May	12	7.5	4.5	13.0	7.5						
Aug.	12	2.0	3.0	31.0	20.0						
Feb.	18	16.0	10.0								

b) Surface colonisation[d]

Feb.	6	2.5	36.5								
May	6	2.5	8.0	37.0	2.2	3.0					
Aug.	6	4.0	5.5	5.0	10.5	25.0	3.0	5.0	2.0	0.1	5.0
Nov.	6	1.0	1.0	2.5	6.0	2.0	7.5	1.0			
Feb.	12	1.0	5.5	9.0							
May	12	3.0									
Aug.	12	6.0	0.3	6.5	2.0	5.0	3.5				
Feb.	18	2.0	4.0	2.5	3.5						

Key: a. Inoculations which did not cause staining are omitted. Depth of stain was not recorded on other sample dates.

b. Months after inoculation.

c. $\bar{x}$ = 11.6

d. $\bar{x}$ = 6.1

TABLE 17 : Mean daily temperature in glasshouse maintained at c. 15°[a].

DATE	1977		1978	
	Nov.	Dec.	Jan.	Feb.
1		13.5	*11.0*[b]	12.5
2		11.0	*12.0*	12.0
3	12.5	10.5	13.0	13.0
4	15.5	*11.0*	13.5	13.0
5	16.0	11.5	13.0	13.0
6	15.0	12.0	13.5	13.0
7	14.5	15.5	13.0	13.5
8	14.5	14.0	13.0	12.5
9	16.0	14.5	13.5	11.5
10	17.0	15.0	12.5	11.5
11	15.0	*14.0*	11.0	12.0
12	*14.0*	13.5	10.5	12.0
13	*12.0*	14.5	13.5	12.0
14	11.0	13.5	13.5	12.0
15	12.0	13.5	12.5	12.5
16	12.5	13.5	12.0	13.5
17	12.0	*13.5*	9.0	11.5
18	12.0	*13.0*	10.0	12.0
19	*12.5*	13.0	10.5	12.5
20	13.0	13.0	11.0	12.5
21	*12.5*	13.5	12.5	
22	12.0	15.0	10.5	
23	11.0	15.0	12.5	
24	9.0	*14.0*	11.0	
25	9.0	*12.5*	11.5	
26	10.0	11.5	10.5	
27	*10.0*	10.0	12.5	
28	10.0	*10.0*	12.0	
29	10.5	10.5	11.5	
30	14.0	11.0	11.0	
31		10.0	12.0	

Key: a. From thermograph at canopy height (1 m).

b. Figures in italics are estimates.

TABLE 18 : Growth[a] of *C. corticale* in sycamore at 25°.

Time[b]	Tree	Stain		Reisolation		Girth[c]	Ridge[d]	
		-	+	-	+		-	+
0.5	a	3.0	2.5	3	3	36	-	-
	b	1.0	1.0	1	1	35	-	-
	c	2.0	12.0	1	14	36	-	-
	d	3.5	3.2	4	4	30	-	-
	e	6.0	2.5	1	1	40	-	-
1	a	6.0	7.0	6	6	35	-	-
	b	3.0	10.5	3	7	40	-	-
	c	1.5	2.0	2	1	40	-	-
	d	1.0	5.0	1	4	34	-	-
	e	7.0	5.5	7	6	37	-	-
2	a	11.0	8.0	11	8	35	-	-
	b	4.0	11.0	1	8	34	-	-
	c	3.0	7.0	3	7	38	-	-
	d	13.0	12.5	13	13	41	-	-
	e	1.5	3.5	1	4	36	-	-
3	a	8.0	5.0	8	7	44	4	4
	b	20.0	2.5	20	1	37	10	2
	c	3.0	2.0	1	2	34	2	4
	d	3.0	14.0	3	13	35	-	5
	e	3.0	10.0	1	10	33	-	-
4	a	12.0	22.5	12	22	41	7	6
	b	1.0	4.0	1	4	37	-	-
	c	14.5	10.0	15	10	41	11	2
	d	5.0	4.0	5	4	41	-	-
	e	6.5	6.0	7	7	34	-	-
5	a	24.0	26.5	24	26	33	-	-
	b	4.0	4.5	4	5	36	2	3
	c	2.5	10.0	1	9	33	2	10
	d	5.5	5.0	6	5	34	2	-

Key: a. Growth (cm) below (-) and above (+) the inoculation point.

b. Months after inoculation.

c. At inoculation point (cm) on sampling date.

d. Sapwood expansion in ridge extending below (-) and above (+) the inoculation point.

TABLE 19 : Growth[a] of *C. corticale* in young sycamores.

	Sample week[b]	Mean temperature[c] 12.2°		21.4°	
		-	+	-	+
Stain	2	0	0	3.5	3.5
	4	3.0	3.0	15.0	12.5
	6	1.0	1.5	25.0	20.0
	8	6.0	7.0	>26.0	18.0
	9	4.0	4.0	>19.0	†
	10	3.0	3.5	30.0	10.0
	11	1.0	1.0	12.5	†
	12	0.5	0.5	>30.0	15.0
	13	1.0	1.0	25.0	>10.0
Reisolation	2	0	0	6	3
	4	7	6	13	13
	6	6	2	31	15
	8	8	9	15	21
	9	5	10	15	30
	10	2	0	15	10
	11	1	1	5	15
	12	1	1	15	10
	13	2	1	20	7
Confirmation[d]	2	0	0	6	3
	4	4	6	13	13
	6	6	2	31	15
	8	8	9	15	5
	9	-	-	-	20
	10	-	-	5	-
	11	1	-	5	15
	12	1	-	15	10
	13	2	-	20	7

Key: a. Growth (cm) below (-) and above (+) the inoculation point.

b. Time after inoculation.

c. Over 13 weeks.

d. Sporulation on sterile wood.

† Top of shoot dead.

TABLE 20 : Analysis of growth of *C. corticale* above and below the inoculation point.

a) At 12.2°

Stain

	DF	SS	MS	F
Above v. below	1	0.222	0.222	3.364 n.s.
Sampling time	8	69.861	8.733	132.318***
Error	8	0.528	0.066	

Reisolation

Above v. below	1	0.222	0.222	0.082 n.s.
Sampling time	8	170.444	21.306	7.827**
Error	8	21.778	2.722	

b) At 21.4°

Stain

	DF	SS	MS	F
Above v. below	1	212.521	212.52	7.20*
Sampling time	5	572.938	114.59	3.88 n.s.
Error	5	147.604	29.52	

Reisolation

Above v. below	1	6.722	6.722	0.129 n.s.
Sampling time	8	559.778	69.97	1.346 n.s.
Error	8	415.778	51.97	

Key: * Significant at $P \leq 0.05$.

** Significant at $P \leq 0.01$.

*** Significant at $P \leq 0.001$.

TABLE 21 : Growth[a] of *C. corticale* in young sycamore from infections established for one week at 25°.

		Mean temperature[c]			
	Sample week[b]	15°		25°	
		−	+	−	+
Stain	1	−	−	2	5
		−	−	6	8
	2	10	4	10	7
		5	5	6	8
	3	7	7	>59	30
		7	7	13	15
	4	8	5	15	15
		5	12	>74	31
	5	37	10	22	20
		6	9	25	21
	8	39	40	53	30
		13	4	17	20
Reisolation	1	−	−	2	2
		−	−	5	4
	2	7	4	10	12
		3	1	6	7
	3	7	7	59	31
		6	1	12	15
	4	7	5	16	15
		1	13	74	31
	5	37	10	22	20
		5	9	24	21
	8	20	35	50	30
		5	1	17	15
Confirmation[d]	1	−	−	1	0
		−	−	5	4
	2	7	2	6	12
		3	1	6	7
	3	1	7	59	30
		5	1	12	15
	4	1	2	10	10
		1	13	74	31
	5	37	1	10	20
		5	9	24	21
	8	10	35	50	30
		1	1	15	10

Key: a. Growth (cm) below (-) and above (+) the inoculation point.

b. Time after inoculation.

c. Over weeks 2 to 8.

d. Sporulation on sterile wood.

TABLE 22 : Analysis of growth of *C. corticale* above and below the inoculation point from infections established for one week at 25°.

a) At 15°

	DF	t
Stain	9	1.142 n.s.
Reisolation	9	0.008 n.s.

b) At 25°

	DF	t
Stain	9	1.909 n.s.
Reisolation	9	1.881 n.s.

TABLE 23 : Growth[a] of *C. corticale* in water-stressed sycamores.

	Pair	Sample week[b]	Non-stressed		Stressed	
			-	+	-	+
Stain	A	2	17.0	7.0	30.0	14.0
	B		3.5	5.0	53.0	27.0
	C	4	25.0	20.0	76.0	21.0
	D		24.0	29.0	53.0	29.0
	E	6	15.0	7.5	>70.0	44.0
	F		19.0	17.0	34.0	23.0
	G		19.0	9.0	>61.0	45.0
	H		15.0	13.0	>50.0	30.0
Reisolation	A	2	16	7	30	15
	B		3	4	50	25
	C	4	20	5	76	17
	D		24	10	53	35
	E	6	11	1	68	43
	F		21	17	34	25
	G		20	7	60	49
	H		15	12	50	21
Confirmation	A	2	16	7	30	15
	B		1	1	40	25
	C	4	10	5	76	17
	D		24	1	53	35
	E	6	11	-	59	43
	F		21	-	-	25
	G		-	7	-	13
	H		15	10	-	21

Key: a. Growth (cm) below (-) and above (+) the inoculation point.

b. Time after inoculation.

TABLE 24 : Growth[a] of *C. corticale* in sycamores subjected to water stress induced by polyethylene glycol 4000 (PEG).

	Tree	Non-stressed		-2.5 bar PEG		-5 bar PEG	
		-	+	-	+	-	+
Stain	A	6.0	9.0	49.0	13.0	66.0	52.0
	B	14.0	10.0	36.0	16.0	39.0	13.5
	C	2.5	3.0	41.0	13.0	6.0	5.0
	D	2.0	3.5	16.0	12.0	16.0	17.0
	E	6.0	4.5	25.0	7.0	58.0	35.0
	F	3.0	3.0	9.0	10.0	>77.0	33.0
Reisolation	A	6	9	52	14	67	59
	B	18	12	38	16	42	11
	C	3	3	44	13	6	5
	D	1	4	15	13	17	17
	E	6	5	30	3	60	37
	F	3	3	10	10	77	33
Confirmation[b]	A	6	9	52	14	67	59
	B	18	12	38	-	42	-
	C	3	3	44	13	6	5
	D	1	4	15	13	17	17
	E	6	5	29	-	60	37
	F	3	3	9	10	-	33

Key: a. Growth (cm) below (-) and above (+) the inoculation point, after 17 days at 25°.

b. Sporulation on sterile wood.

Further experiments to investigate the ability of *C. corticale* to produce cellulase in vitro

i) The utilisation of cellulose was tested by the method described by Yung Chang, 1967 (modified from Garrett, 1962) where cellulose is the major carbon source. Wads of 10 filter papers (Whatman No. 3, 5.5 cm) were dried to constant weight and placed in 150 ml conical flasks. Fifteen mls of mineral solution were added ($NaNO_3$, 5 g; K_2HPO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $Ca(NO_3)_2 \cdot 4H_2O$, 0.01 g; $FeCl_3$, 0.001 g; yeast extract, 0.5 g; water 1 ℓ). Eight replicates were inoculated with a 6 mm plug from a V8-juice plate culture of isolate LP and 4 controls using discs of blank agar. The flasks were incubated for 3 weeks at 25°, after which time the filter paper pads were dried to constant weight. The net loss in weight of cellulose after subracting the value of the control series was 0.02 g, thus *C. corticale* was scarcely able to use cellulose under the experimental conditions. However, Wood (1967) reported that under certain conditions cellulases are strongly adsorbed by their specific substrates e.g. the insoluble cellulose of filter paper; such bound enzymes would not be free to degrade the substrate.

ii) To estimate the cellulolytic power of fungal cultures, Eggins and Pugh (1962) used a medium in which very finely divided cellulose provides the major carbon source (cellulose agar, Appendix Table 1). When poured into petri dishes the medium is white and opaque and it sets with the cellulose particles evenly dispersed. Growth of cellulolytic fungi is obvious by the clearing of the medium, usually after 7 days at 25°. Attempts to repeat this technique for *C. corticale* isolate LP (using Whatman's standard grade cellulose powder for chromatography) were unsuccessful. Control plates inoculated with *Corticium praticola*, (which

readily degrades cellulose) did not clear, probably because the crystalline cellulose is not easily accessible to cellulolytic enzymes. Ball-milling can be used to increase accessibility and susceptibility of cellulose to enzymic attack (to reduce the average particle size to c. 5 μm) but the available ball-mill was found to be unsuitable.

Germination of *C. corticale* conidia

Germination of *C. corticale* conidia collected from Laleham Park was tested in water and on agar. After 18 h at 25°, germination of conidia streaked over 2 replicate plates of 3% MEA (93%; 94%) was greater than on water agar (61%; 54%), suggesting that the additional nutrients encourage germination. However, germination in drops of water or 0.01% Tween 80 on sterile slides was only c. 2% after incubation in moist chambers. The poor germination in water could be due to a nutrient effect; to test this the experiment was repeated using 0.1% glucose but germination was not improved. This could be due to insufficient diffusion of oxygen into the drop of liquid, or because *C. corticale* conidia require a solid surface to germinate (*Diplocarpon rosae* behaves similarly in water drops; Knight, 1976).

Measurement of water stress in sycamore

The Lamda porometer was most successful for measurement of water stress in individual sycamore trees. Other methods which were found unsatisfactory included:

a) The Shigometer. This delivers a pulsed electric current and the resistivity of tissues between the Shigometer electrodes is measured. The resistance of sapwood tissues could be affected by moisture deficiency stress. To test this needle probes fixed at 1 cm apart were inserted into the bark and then the sapwood of 2 matched pairs of trees at 30, 60 and 90 cm above soil level. One tree of each pair was stressed to the point of wilting and the other was a well-watered control. Results showed no differences, the Shigometer was evidently not sufficiently sensitive to detect water stress in sycamore.

The Shigometer is commonly used to detect decay in trees, and colonisation by *C. corticale* is likely to alter the resistance of sycamore tissue. However, in 3 trees readings from 1 cm below the point of inoculation were similar to readings from the opposite (uncolonised) side of the stem.

b) Leaf pressure bomb. Leaves were cut near the petiole base using a sharp razor blade. Single leaves were rolled carefully to prevent damage and placed in the bomb with the cut end exposed, within 5-10 min. of sampling. Pressure on the leaf was gradually increased until sap was exuded from the cut end. The pressure required is usually dependent on the leaf water potential. However, the technique was unsatisfactory for sycamore leaves because the sap is too viscous and only c. 20% of sampled leaves yielded sap even at 120 lb/in^2 (i.e. 8437 g/cm^2). Results for these leaves were unsatisfactory since the reading for a particular leaf

could not often be verified by reducing pressure until sap was no longer exuded, due to "plugging" of the sap.