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STUDIES ON THE PARASITISM OF THANATEPHORUS CUCUMERIS (FRANK) DONK.

by

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FEBRUARY 1969.

Availability of Thesis

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SUMMARY

Three crucifer stem strains and one root strain of T. cucumeris were studied. Culture media were found to influence the virulence of T. cucumeris strains. The virulence of each strain varied according to the method used to inoculate plants with it. Stem strains were virulent on crucifers under all conditions tested. Susceptibility of plants depended upon their stage of development.

On surfaces of crucifers, hyphae of stem strains followed closely the junctions of epidermal cells and produced infection cushions. However, on surfaces of non-crucifers, hyphae of stem strains either followed cell junctions or grew randomly with or without the formation of cushion-like structures. The root strain behaved similarly except that growth was slower. Stem strains penetrated stems by single hyphae into stomata of cucumber, pea, tomato and wheat, via natural wounds in lettuce and beneath infection cushions in turnip and radish. Sclerotia did not penetrate radish hypocotyls directly but produced infection cushions from which penetration occurred. Entry into roots of radish and wheat was mainly by fine infection pegs into root hairs and the piliferous layer.

The relationships between the pathogen and radish

hypocotyl surfaces were studied using light and electron microscopes. There was evidence of pores and areas of greater permeability at sites of infection cushion formation on radish surfaces which may be associated with diffusion of exudates. Hyphae penetrated radish hypocotyls through splits in the cuticle caused by epidermal cells being forced apart as the result of the rapid development of infection cushions in confined spaces between epidermal cells. There was no evidence for direct penetration of intact cuticle from cushions on radish and no signs of constriction of penetrating hyphae. Proliferation within radish hypocotyls was first intracellular forming lesions and then mainly intercellular through the cortex and again intracellular in the phloem without entry into xylem vessels. In roots, spread of pathogens was mainly intracellular by means of fine infection pegs without the formation of lesions.

Respiratory increases were mainly in lesions (5 - 7 times that of healthy tissues) with some increases (1.2 times) in adjacent tissues possibly due to ethylene from damaged tissues. Lesion centres were slightly alkaline and the edges were acidic. Water-soaked appearance of lesions was not due to an accumulation of water. No correlation was found between the production of cell-wall degrading enzymes <u>in vitro</u> and <u>in vivo</u> and the virulence

of T. cucumeris strains. Treatment with calcium chloride induced an increase in resistance of radish hypocotyls possibly due to the formation of calcium pectate. Treatment with ethylene increased radish respiration slightly (1.2 times) but had little effect on susceptibility to attacks by T. cucumeris.

STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma and contains no material previously published or written by another person, except when due reference is made in the text.

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INTRODUCTION

Rhizoctonia solani Kühn, the vegetative stage of the basidiomycete, *Thanatephorus cucumeris* (Frank) Donk, has interested mycologists and plant pathologists for over a century because of its wide host range and world-wide distribution as a soil-borne pathogen. As a pathogen, it has been reported on over 230 species of plants comprising 66 families (quoted by Kerr 1955) and including cultivated crops, ornamentals and weeds. Hence it is of enormous economic importance. It shows heterogeneity in form, cultural appearance and pathogenicity (Kernkamp *et al.* 1952, Flentje 1956). This may be due to the presence of a large number of races. There is evidence that some of these races are more specific for certain crops than others (Storey 1941, Kernkamp *et al.* 1952, Flentje 1957).

Although various aspects of *T. cucumeris* and the diseases it causes in higher plants have been intensively investigated both in Australia and elsewhere, there are still important gaps in our knowledge of both the fungus and the diseases and control methods are still unsatisfactory. For effective control of diseases caused by *T. cucumeris* various aspects of the pathogen, its hosts, the interactions between the host and the pathogen and the factors that influence such interactions must be taken

into account. An important gap in our understanding of plant diseases caused by T. cucumeris is in the field of physiology of parasitism.

The present study, which is concerned with this field, is presented in three main parts:-

I. The pathogenicity of T. cucumeris isolates,

II. The cytology of the host-pathogen relationship, and

III. The physiology of the host-pathogen relationship.

The three divisions are by no means distinct but merge into one another. It is hoped that this study combined with studies on other aspects of T. cucumeris will give a clearer picture of the Rhizoctonia disease situation and that more efficient control methods for these plant diseases can be developed.

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GENERAL SURVEY ON THE TAXONOMY AND ECOLOGY OF T. CUCUMERIS.

(i) Taxonomy

Rhizoctonia is a complex form-genus, probably consisting of numerous and often ill-defined species. The history of the concept of the genus has been reviewed by Saksena and Vaartaja (1961). The genus, erected by de Candolle in 1815, presents a difficult problem for specific classification because many isolates are not known to produce fruiting bodies. Confusion in the literature regarding the concept of species within the genus led to a taxonomic study by Dugger in 1915. Using vegetative mycelial characteristics, Dugger recognised two main species, R. solani Kühn and R. crocorum (Pers.) D.C. Since then, about 57 species of Rhizoctonia have been described based mainly on vegetative structures and to a lesser extent cultural characteristics and pathogenicity (Saksena and Vaartaja 1961).

Historical accounts of the nomenclature and taxonomy of the perfect stage of R. solani Kühn are given by Rogers (1943), Donk (1954), Flentje (1956), Olive (1957) and Talbot (1965). The perfect state of R. solani was named Pellicularia filamentosa by Rogers (1943). However, Talbot (1965) agrees with Donk's (1956) rejection of the binomial, Pellicularia filamentosa (Pat.) Rogers for the

perfect state of R. solani as nomen confusum and he accepts Donk's (1956) proposal that Thanatephorus cucumeris (Frank) Donk be the name given to the perfect state of R. solani.

The general characteristics of the vegetative state of T. cucumeris grown on agar have been described by Flentje (1956). Since the hyphal features of R. solani are common to the vegetative state of a number of fungi (Parmeter Jr. et al. 1967), it is important that the perfect state of the fungus be used for identification. Techniques for inducing R. solani to fruit have been described in detail (Flentje 1956, Stretton et al. 1964).

(ii) Existence of races and strains.

There appears to be an indefinite number of races or strains of R. solani Kühn distinguished by asexual morphological and physiological properties (Kernkamp et al. 1952, Flentje & Saksena 1957). Cultures obtained from infected plants differ greatly from each other and the population of R. solani in various soils appear to comprise a number of morphologically and pathologically distinct clones (Papavizas & Davey 1962b). Clones of the root strain differing in cultural characteristics have been isolated from the same locality and from different localities (de Beer, 1965). Under ordinary cultural conditions, cultures of R. solani are relatively stable

(Kernkamp et al. 1952, Flentje 1956, Maier 1959, Flentje & Stretton 1964, Pitt 1964b).

Pathogenic strains of T. cucumeris exist in nature as heterocaryons (Flentje & Stretton 1964). Stability in culture may be due to the multinucleate condition of the hyphae and the mechanism of conjugate nuclear division (Flentje & Stretton 1964). The same authors also suggested that the heterocaryotic conditions of the field isolates may have arisen either by anastomosis of different hyphae or by mutation of nuclei in individual hyphae. The vegetative cells of T. cucumeris contain 4 - 12 nuclei per cell. Uneven nuclear number among cells is due to frequent malfunction of the conjugate division (Flentje et al. 1963b). Production of secondary septa in older cells reduces nuclear number in the absence of nuclear division. Basidiospores are mainly uni-nucleate but a small percentage contains two nuclei due to malfunction during the sexual reproductive phase. Malfunctions during cell division, heterocaryon formation and anastomosis are some of the mechanisms of variations occuring in T. cucumeris (Flentje & Stretton 1964). This may also explain the large number of races and strains of the fungus.

Flentje and Saksena (1957) examined sixty-eight

isolates of T. cucumeris from England and Australia. They distinguished three strains, fifty-four isolates were stem-attacking strains, thirteen isolates were root-attacking strains and one a non-pathogen. Of the stem strains, the majority were specific in their attack on one or, rarely, two families of plants. The root-attacking strains showed no evidence of host specialization. Although from one locality, isolates of the same strain could easily be distinguished from the other strains in cultural appearance. Flentje and Saksena (1957) found that there were no correlations between cultural characters and pathogenicity in strains isolated from different localities.

(iii) Saprophytic and parasitic phases in soil.

R. solani has been shown to exist in soil as mycelium (Garrett 1956, Thornton 1956, Warcup & Talbot 1962) and in the form of sclerotia and resistant cells among organic debris (Boosalis & Scharen 1959, de Beer 1965) and on perenating organs of green plants (el Zarka 1965).

The saprophytic behaviour of R. solani is known in some detail. The fungus can exist as an active saprophyte in soil in the absence of its host by competitive colonization of dead soil organic matter (Garrett 1956). During substrate utilization, the saprophytic phase of

R. solani possesses two types of hyphae, the "runner" hyphae and the "feeder" branches (de Beer 1965). The runner hyphae, found in soil and around plant roots, serve to spread the fungus through the soil. The feeder branches are of larger diameter than the runner hyphae and are irregular in shape. The feeder branches are produced on and in the substrate and are thought to serve mainly as absorptive organs. Saprophytic growth in soil is most rapid at moderate moisture level (30% saturation reported by Blair in 1943 and 50% drained pore space reported by de Beer in 1965). At high moisture levels and in soil with discontinuous distribution of moisture, growth of R. solani is inhibited (de Beer 1965).

Blair (1942) showed that the spread of R. solani was faster radially than vertically in soil and that radial growth rate decreased with depth. In "bare patches" of wheat field, de Beer (1965) found that the greatest density of hyphae was in the top three inches although hyphae could reach a depth of 15 inches. A higher population of R. solani hyphae was found inside than outside "bare patches" (Kerr 1955, de Beer 1965).

The parasitic activities of the root strain are confined to the young tissues and result in the death of the root tips (Samuel & Garrett 1932). The stages of

the infection by the stem strain have been reported by Flentje (1957). The infection process and the effect of the host exudates on the pathogen will be dealt with in the main part of this dissertation.

The root lesion nematode, Pratylenchus negletus (Rensch) Chitwood and Oteita (Benedict & Mountain 1956, de Beer 1965) and the cereal eelworm, Heterodera avenae Wollenweber (Samuel & Garrett 1932, Fisher 1960) have been found in association with Rhizoctonia "bare patch" of cereals. These authors suggested that synergistic relationships between R. solani and the nematodes cause the "bare patches" of cereals.

Since hyphae of R. solani are able to grow freely through non-sterile soil (Blair 1943, Garrett 1956) and viable mycelium of R. solani has been isolated from soil (Warcup & Talbot 1962) and living roots (de Beer 1965), the saprophytic phase of its life cycle is present, when conditions are favourable, during periods of cropping as well as between crops. Therefore, the saprophytic and the parasitic phases of the life cycle of R. solani can exist at the same time. Under suitable conditions, the fungus can assume a parasitic status from a saprophytic one (Papavizas & Davey 1961). A close correlation has been found (Martinson 1963) between the pathogenicity of R. solani and its competitive saprophytic ability.

(iv) Persistence in the soil.

Since R. solani is a soil-borne fungus, knowledge of its longevity in soil is important in the formation of any control measures. R. solani has been described as a typical soil-inhabiting parasite (Garrett 1956). Its mycelium has been found on grass roots in a state of symptomless parasitism (Daniels 1963) and is common in natural grassland soil (Thornton 1956).

Although there is much support (Blair 1943, Papavizas & Davey 1961) for the views of Garrett (1950) that R. solani is capable of prolonged saprophytic life in the soil, very little is known of the conditions which affect the persistence of R. solani in soil. Mycelium of R. solani introduced into cereal fields in South Australia failed to persist from one season to the next (Kerr 1955, Flentje & Saksena 1957). Sclerotia of R. solani have been found to survive burial in soil for six months or longer (Palo 1926, Boosalis & Scharen 1959, Pitt 1964b). There is some evidence that certain isolates of R. solani may require a parasitic phase for survival. Thus Elmer (1942) found that Rhizoctonia from potato could persist from year to year as sclerotia and mycelium in the soils but if temperatures during the growing season were too high for sclerotial production and survival of soil-borne mycelium, the disease declined in the absence of a

susceptible crop immediately succeeding the potatoes. Sanford (1952) found that the pathogen disappeared from heavily infested soil in less than four months under non-susceptible cropping. Similarly, Pitt (1964b) showed that R. solani was not capable of prolonged saprophytic survival in bare soil even when crops previously grown in the soil were heavily infected and that parasitic survival of the fungus on susceptible crops contribute to the persistence of sharp "eye-spot" disease. Christou (1962) and Daniels (1963) also reported that a parasitic phase could be important in the persistence of certain strains of R. solani in soil.

The continuous return to the soil of infected plant tissues containing mycelium and sclerotia contribute to the survival and persistence of R. solani in soil (Boyle 1956, Boosalis & Scharen 1959). R. solani may be introduced into soil by seeds contaminated with mycelia or sclerotia (Baker 1947, Ashworth et al. 1962, Peeples & Bain 1966). Only a small proportion of the cells of sclerotia of isolates from wheat stem and potato germinate in response to a suitable stimulus leaving the remainder to respond to further stimuli at later dates and this may be of great importance in increasing their survival value (Pitt 1964b).

(v) Control measures.

Various methods have been suggested for the control of R. solani diseases. These include physical, chemical and biological methods or combination of these methods. The biological control of soil-borne plant pathogens has been given much attention in a recent international symposium (Baker & Synder 1965).

Taylor (1951) and Small (1927) found that Rhizoctonia could be controlled by soil sterilization by heat. Thomas (1927) found that soil treatment with mercurial compounds was effective in controlling Rhizoctonia diseases of cabbage and tomato seedlings. Weindling and Fawcett (1936) found that "damping-off" of citrus seedlings could be controlled by increasing the acidity of the soil since under these conditions R. solani was effectively parasitized by Trichoderma lignorum.

Since R. solani is soil-borne, much attention has been paid to the use of crop rotation as a means of control (Kernkamp et al. 1952). Various degrees of success in the control of the pathogen by crop rotation have been achieved. Williams and Walker (1966) in their studies of the Rhizoctonia disease of cabbage suggested that long rotations with non-crucifer crops could control the disease. They also found that "bottom-rot" of the

mature plant was satisfactorily controlled by a resistent variety. Crop rotation as a means of control of R. solani seems to be of localised importance since the pathogen has a wide host range and is composed of many parasitic strains.

There is abundant evidence that R. solani can be effectively suppressed by the addition of decomposable organic amendments to the soil (Blair 1943, Sanford 1952, Boosalis 1956, Kommedahl & Young 1956, Kendrick & Zentmyer 1957, Snyder et al. 1959, Davey & Papavizas 1960, Papavizas & Davey 1960). The importance of plant residues and organic amendments in the control of soil-borne plant pathogens have been reviewed by Patrick and Tousoun (1965). Garrett (1956) pointed out that it was likely that green manuring permitted the growth of rhizosphere micro-organisms in soil distant from the roots, resulting in a widened zone of competition around the plant. Since R. solani has an ectotrophic growth habit (Garrett 1956, de Beer 1965) and a saprophytic growth phase on the surfaces of the hosts prior to penetration (Flentje 1957), the enhanced rhizosphere zone would subject the hyphae of R. solani to direct competition with the rhizosphere microflora before reaching its hosts.

Similarly, nitrogenous amendments were found to reduce the activity, viability and mycelial density of the root strain of R. solani in unsterilized soil in South Australia (de Beer 1965, Chambers 1966). De Beer (1965) recorded a lower population of R. solani in heavier and more fertile soil than in sandy soil suggesting that increased soil fertility may be useful in controlling "bare patch" of cereals. He found that nitrogenous applications to soil under laboratory conditions reduced the growth and survival of R. solani. Chambers (1966) found that a single application of urea and ammonium sulphate (17 lb. nitrogen/acre) reduced "bare patch" in barley. However, contrary to the results of de Beer (1965) and Chambers (1966), Bloom and Couch (1960) found that disease severity of turf grass was greater at high than at low nitrogen levels. Introduction of plant residue into soil has also been found to stimulate the activity of R. solani in soil (Kommedahl & Young 1956, Snyder et al. 1959, Warcup & Talbot 1962). De Beer (1965) introduced wheat straw into soil inoculated with R. solani and found rapid colonization of the straw by the fungus within ten days. He concluded that the fungus could be a pioneer in the decomposition of the straw. An increase in the C/N ratio in unsterilized soil was found to decrease the

survival and saprophytic activity of R. solani (Papavizas & Davey 1961, Davey & Papavizas 1963). In contrast, the root strain of R. solani has been reported to be tolerant to high C/N ratio (de Beer 1965).

Reduction in the activity of R. solani after the addition to soil of plant residues or organic amendments has been attributed to a stimulation of competitive and antagonistic activity of saprophytic microflora (Blair 1943, Wood 1951, Wood & Tveit 1955, Chambers 1966). Wood (1951) found that in green house experiments "damping-off" of lettuce seedlings by R. solani was substantially controlled by selected antagonists in sterile soil. In unsterile soil, organisms highly antagonistic to R. solani in pure culture were generally ineffective in controlling the pathogen. Wood and Tveit (1955) in reviewing the literature on the use of antagonistic organisms in the control of plant diseases came to the general conclusion that many soil organisms were antagonistic to R. solani and that a few of these organisms were able to control the disease in soil in the absence of other saprophytes. A number of organisms antagonistic to R. solani have been isolated. Huber et al. (1966) found that 32% of Rhizoctonia hyphae became necrotic due to Bacillus subtilis which was found to lyse R. solani hyphae in soil (Olsen 1965) and on

agar (Naim & el Esawy 1965). B. subtilis is capable of producing a thermostable substance toxic to R. solani (Katznelson 1942). The incidence of "damping-off" in susceptible radish seedlings was lowered in soil containing B. subtilis (Olsen 1965). A strain of B. simplex is also antagonistic to R. solani (Cordon & Haenseler 1939). On agar, Aspergillus terreus and A. flavus were antagonistic to R. solani (Naim & el Esawy 1965). Trichoderma viride is antagonistic to R. solani in acid culture media whilst Chaetomium cochliorides and Penicillium clavariaeforme are effective in alkaline medium (Deshpande 1961). The presence of Trichoderma caused a decrease in seed decay and "damping-off" due to R. solani (Allen & Haenseler 1935). However, suppression of R. solani may be due as much to the general increase in microbial activity, after addition of amendments to soil, as to the actions of specific antagonists. This is because an increase in carbon dioxide concentration in the soil has a depressing effect on the activity of R. solani (Blair 1943, Durbin 1959, Papavizas & Davey 1962a).

In his review, Garrett (1965) concluded that the prospects for biological control of soil-borne plant pathogens was bright. However, he added that for effective disease control, nothing far short of

complete knowledge and comprehension of the biology and epidemiology of the disease and of the ecology of the crop plant would be required.

(vi) Distribution and importance in Australia.

In Australia R. solani has been reported to be widely distributed on potato (McAlpine 1912) to infect carrot and beets (McKnight 1945) and to cause "purple" or "bare-patch" of cereals (Samuel & Garrett 1932). "Bare-patch" is of major concern in South Australia (McKnight 1960) and may cause considerable losses in yield in New South Wales (Hynes 1933). Losses in tomato, lettuce, cabbage and cucurbit crops due to Rhizoctonia infection have also been reported (Saksena 1952). R. solani is also the causal organism of the lawn disease "brown-patch", common in South Australia (Kerr 1955).

In South Australia, a number of strains of T. cucumeris have been isolated from wheat fields (de Beer 1965) and the causal organism of "bare-patch" was a root-attacking strain (Kerr 1955). "Bare-patch" of wheat in South Australia occurs mainly on sandy, alkaline soil with an annual rainfall of 12-18 inches (Kerr 1955). The disease is characterised by severe

stunting due to extensive root rotting (Samuel & Garrett 1932). Besides causing "bare-patch", the stem strains may cause pre-emergence death of cereal seedlings and are responsible in causing lesions on the coleoptile known as "sharp eyespot". The latter disease has been recorded in South Australia (Butler 1961) and is probably of minor economic importance.

PART I: PATHOGENICITY OF *T. CUCUMERIS* ISOLATES.

Introduction

The symptoms exhibited by plants infected with *T. cucumeris* are varied. It is known to attack seedlings as well as roots, stems, fruits and tubers of mature plants. In seedlings, the pathogen causes both pre- and post- emergence "damping-off" and "wire-stem" of broad bean (Wright 1944). In mature plants, it is the causal organism of "bottom-" and "head-rot" of cabbage (Wellman 1932), "sore-shin" of broad-bean (Wright 1944), root and crown rots of alfalfa (Chi & Childers 1965), the "sharp-eye" disease of cereals (Pitt 1964a;b) and "black-scarf" of potato (el Zarka 1965). It has been reported (Cox 1966) that the pathogen attacks young tomato seedlings shortly after emergence and may remain parasitic throughout the life of the crop. Similarly, the pathogen can affect practically all parts of potato plants during their development (el Zarka 1965). Affected plants usually have leaves of reduced size and the underground portions of stems develop brown rotted areas of the cortex known as "cankers". Tubers may develop cracks, known as "scabs", or they may develop a wet rot.

It seems likely that the extent of the host range of T. cucumeris is equivocal because the host range may possibly be altered by time, kind of plants used, varietal resistance or susceptibility, by a number of soil micro-ecological factors, inoculum potential and the physiological properties of the strain of the pathogen. In the following experiments, an attempt was made to determine the effects of substrate composition and conditions of inoculation on the pathogenicity of four strains of T. cucumeris on a number of host plants from different families. Inoculations were done before and after germination of the seeds to determine the importance of T. cucumeris in causing pre-emergence and post-emergence "damping-off" of the various plants.

EXPERIMENTAL

Test plants and fungal isolates.

Table 1 *Thanatephorus cucumeris* isolates and the test plants used in the present investigation.

T. cucumeris Isolate No.	Origin	Pathogenicity	Ref.
48	Soil, Waite Inst., S. Aust.	Crucifer stem	Flentje *et al.* (1963a)
68	Lesion, cabbage stem, Hitchin, England		
69	Lesion, cabbage stem, Clare, S. Aust.		
R10	Soil, Moonta, S. Aust.	wheat root (unspecialised)	de Beer (1965)

Host plants

Common name	Family	Species
Cucumber (Long green)	Cucurbitaceae	*Cucumis sativus* L.
Lettuce (Imperial triumph)	Compositae	*Lactuca sativa* L.
Pea (Green feast)	Leguminosae	*Pisum sativum* L.
Radish (Long icicle)	Cruciferae	*Raphanus sativus* L.
Tomato (Dwarf red)	Solanaceae	*Lycopersicum esculentum* Mill.
Turnip (White stone)	Cruciferae	*Brassica campestris* L.
Wheat (Gabo)	Graminae	*Triticum sativum* L.

Stock cultures of T. cucumeris were maintained on RM2 or PMDA media (see Appendix). Cultural appearance on agar is illustrated in Fig. 1.

Effect of nutrient status on the pathogenicity of T. cucumeris isolates.

Method.

Two different media, potato dextrose agar (PDA) and Rhizoctonia agar medium (RM2) with different isolates of T. cucumeris were used in the experiments. The pathogen was grown on each of the media in 9 cm. diameter petri dishes at 26°C. When the mycelium reached the edges of the petri dishes, portions were used to inoculate plates containing fresh medium of the same composition. Five consecutive transfers were made on each medium before the cultures were used as inoculum for pathogenicity tests. Ten day old radish seedlings were inoculated using the glass slide method (see Pathogenicity tests).

Results.

It was found that after one and a half days incubation, isolates of T. cucumeris grown on RM2 medium had caused significant damage to the seedlings (table 2). Isolate 69 had caused total collapse of all the seedlings. Seedlings inoculated with isolates grown on PDA medium

Fig. 1: Five day old cultures of T. cucumeris grown on RM2 agar medium at 26°C.

(1) Isolate 48

(2) Isolate 68

(3) Isolate 69

(4) Isolate R10

Fig. 1

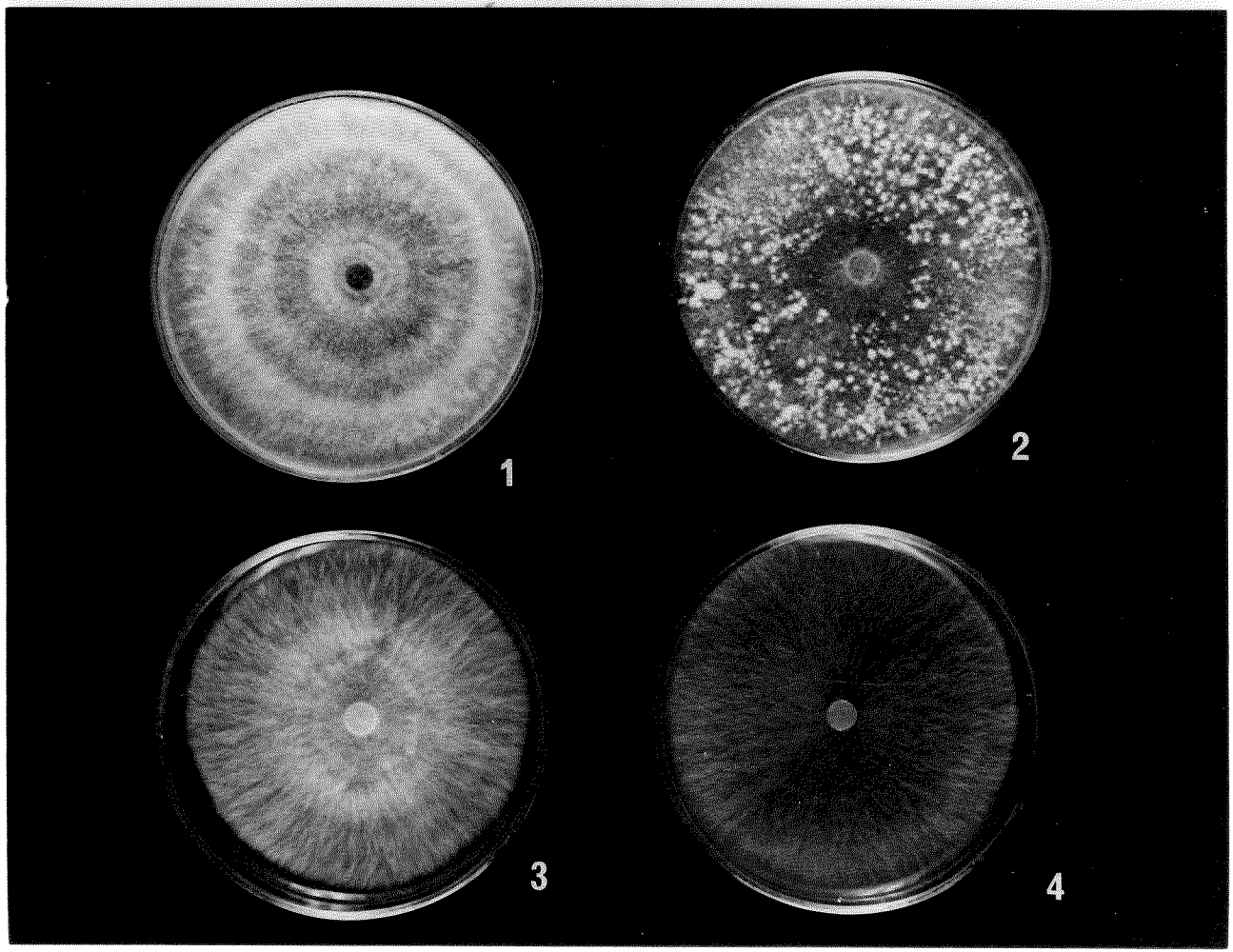

Table 2: Disease rating of infected radish hypocotyls after inoculation for one and a half days with T. cucumeris isolates grown on RM2 medium.

Fungal isolates	Disease rating*									Disease Index**
	0	1	2	3	4	5	6	7	8	
48	-	-	-	-	-	-	1	8	3	12/12
68	1	-	-	1	3	2	4	1	-	11/12
69	-	-	-	-	-	-	-	-	12	12/12
R10	3	1	3	-	4	-	1	-	-	9/12

Table 3: Disease rating of infected radish hypocotyls after inoculation with T. cucumeris isolates grown on PDA and incubated for one week.

Fungal isolates	Disease rating*									Disease Index**
	0	1	2	3	4	5	6	7	8	
48	-	-	-	2	-	-	4	2	4	12/12
68	3	4	2	-	-	1	1	-	-	8/11
69	-	3	-	4	3	-	1	-	1	12/12
R10	6	5	1	-	-	-	-	-	-	6/12

* See Table 13.

** $\frac{\text{Number of infected plants}}{\text{Number of inoculated plants}}$

had to be incubated for a week before significant damage was seen on the hypocotyls (table 3). Subsequent pathogenicity tests were made with T. cucumeris isolates grown on RM2 medium.

Pathogenicity tests.

(A) Under sterile conditions.

(i) Using agar plates:

This method is a modification of the one used by Garza-Chapa and Anderson (1966). Petri-dishes, 9 cm. diameter, containing RM2 nutrient medium, 3 mm. deep were inoculated in the centre with a 7 mm. diameter agar plug bearing actively growing mycelium of T. cucumeris. The mycelium reached the edges of the plates after incubation for about five days at 26°C. Seeds of test plants were surface-sterilized with 0.005% aqueous mercuric chloride for five minutes, rinsed five times with sterilized water and then soaked for 2 hours in sterile water. Twenty seeds were evenly spaced on top of the fungal colony in each plate. The plates were incubated at 26°C for three days. Seeds on water agar plates were used as controls. Three replicates per treatment were used.

Results

Details are tabulated in tables 4, 5, 6, 7, 8 and 9.

(a) effect on germination (table 4).

Of the three stem strains, isolate 69 had the greatest effect on decreasing the germination of seeds. The germination of cucumber and turnip seeds was decreased most effectively by isolate 69. The depression of germination of seeds by the root strain, R10, was greatest in turnip and lettuce. Of the seven test plants used, the germination of turnip seeds was decreased most significantly when inoculated with the stem strains, 68 and 69. Little or no effect on the germination rates of lettuce and radish seeds by the stem strains was noted. The root strain, R10, had little or no effect on the germination rates of radish and wheat seeds.

(b) effect on seedlings (table 5).

Of the three stem strains, isolate 69 was the most virulent. Wheat seedlings were not infected by the stem strains. No infection of tomato seedlings by isolate 48 and 68 was observed. The root strain, R10, was more pathogenic than any of the stem strains. It attacked 60-100% of the seedlings of the test plants. Lettuce, radish and turnip were the most susceptible hosts. Almost all the seedlings of these three species were attacked by both the stem and the root strains.

(c) effect on shoot production (table 6).

Shoot production was inhibited in cucumber and pea by all isolates. Isolates 48 and 69 reduced shoot production of turnip. Shoot production by other plants was not affected.

(d) effect on the length of the main root (table 7).

With the exception of wheat, the plants tested showed a marked inhibition of root growth by the stem strains of the pathogen. The root strain, R10, markedly decreasedthe root length of all the plants tested.

(e) effect on the length of the shoot (table 8).

With the exceptions of tomato and wheat, shoot lengths of the plants inoculated with the stem strains were severely reduced. In the case of tomato, an increase in shoot length occurred when the plants were inoculated with isolates 48 and 69. The root strain, R10, markedly decreased the shoot length of all the plants tested.

(f) pathogenicity on seedlings (table 9).

The stem strains were non-pathogenic to wheat, slightly pathogenic to tomato, mildly pathogenic to cucumber, lettuce and pea and highly pathogenic to radish

and turnip. With cucumber, lettuce and pea, some of the roots were colonized at the tips and on the shoots, discoloured spots indicated sites of attempted penetration by the pathogen. With radish and turnip, extensive damage was seen in both the shoots and the roots. The root strain was pathogenic to the roots of all the plants tested but non-pathogenic to the shoots with the exception of lettuce which had discoloured spots on the shoots.

Table 4: Percentage germination* of seeds inoculated with *T. cucumeris* isolates using the agar plate method.

Test plants	Fungal isolates				
	48	68	69	R10	CONTROL**
Cucumber	85	92	63	81	87
Lettuce	102	98	102	32	98
Pea	91	87	78	87	92
Radish	95	98	93	102	95
Tomato	113	106	102	94	84
Turnip	89	70	32	77	95
Wheat	95	96	89	96	95

* $\frac{\text{Number of inoculated seeds that germinated}}{\text{Number of uninoculated seeds that germinated}} \times 100$

** $\frac{\text{Number of uninoculated seeds that germinated}}{\text{Number of uninoculated seeds sown}} \times 100$

Table 5: Disease index* of seedlings inoculated, before germination, with T. cucumeris isolates using the agar plate method.

Test plants	Fungal isolates			
	48	68	69	R10
Cucumber	23	29	70	88
Lettuce	100	100	100	100
Pea	22	2	42	58
Radish	100	100	100	100
Tomato	0	0	21	71
Turnip	100	97	100	100
Wheat	0	0	0	100

* $\frac{\text{Number of seedlings attacked by the pathogen}}{\text{Number of seedlings present}} \times 100$

Table 6: Shoot production index* of germinated seeds which were inoculated, before germination, with T. cucumeris isolates using the agar plate method.

Test plants	Fungal isolates				Control
	48	68	69	R10	
Cucumber	1/44	1/48	2/33	0/42	20/52
Lettuce	60/60	58/58	60/60	19/19	59/59
Pea	2/50	11/48	8/43	0/48	15/55
Radish	48/55	56/56	50/53	58/58	57/57
Tomato	47/53	44/50	44/48	41/44	39/47
Turnip	12/39	20/31	2/14	33/34	35/44
Wheat	54/54	51/55	50/51	54/55	57/57

Table 7: Percentage decreases in the length of the main roots of seedlings inoculated, before germination, with T. cucumeris isolates using the agar plate method.

Test plants	Fungal isolates			
	48	68	69	R10
Cucumber	62	46	78	78
Lettuce	31	56	69	-
Pea	32	24	24	44
Radish	77	77	-	88
Tomato	53	26	42	79
Turnip	-	77	-	-
Wheat	14	13	14	78

* Numerator = number of seedlings with shoot.
Denominator = number of seeds that germinated.
\- = measurements not done (specimen rotted or insufficient specimen).

Table 8: Relative shoot length* of seedlings inoculated, before germination, with T. cucumeris isolates using the agar plate method.

Test plants	Fungal isolates			
	48	68	69	R10
Cucumber	-	-	-	-
Lettuce	20	33	37	10
Pea	60	80	60	-
Radish	12	16	20	36
Tomato	171	100	129	29
Turnip	-	39	-	13
Wheat	95	84	100	16

* $\frac{\text{Average length of shoots of inoculated seedlings}}{\text{Average length of shoots of uninoculated seedlings}} \times 100$

- = measurements not done, specimen rotted away or not enough specimens.

Table 9: Disease symptoms of seedlings inoculated, before germination, with *T. cucumeris* isolates using the agar plate method.

Fungal isolate	48	68	69	R10
Cucumber	A number of germinated seeds had their radicles colonized and killed by the pathogen. A few of the seedlings with elongated radicles had their root caps and root tips destroyed by the fungus. The rest of the seedlings were healthy. Side roots were not attacked.	A few seedlings were killed on germination. Seedlings with elongated radicles had their root caps attacked by the fungus. Side roots were healthy.	Almost all the seedlings were attacked. Most of the roots were stunted. A few seedlings had side roots. All the root tips were destroyed.	Most seedlings were attacked. The lower halves of roots had rotted away leaving stumps. About half the plants had healthy side roots.
Lettuce	All seeds germinated. Seedlings were stunted. The hypocotyls and roots had small yellowish spots due to attempted penetration by the fungus. Most of the root tips were colonized.	Similar to that described for isolate 48.	All the seeds had germinated. Most of the roots had rotted away leaving stumps of brownish tissues. Small yellowish spots were present on hypocotyls due to attempted penetration by the fungus.	Less than half the seeds had germinated. Seedlings had their roots rotted away. Hypocotyls had yellowish spots indicating sites of fungal attack.
Pea	Most of the seeds germinated and their cotyledons were attacked by the fungus. A few root tips were attacked. Most of the radicles were stunted.	A few plants had parts of their cotyledons attacked by the fungus. Roots were healthy.	Radicles were stunted. About half the roots had their tips discoloured. The testa and cotyledons were attacked.	More than half of the germinated seeds had infected root tips. A few roots were destroyed. The pathogen attacked the testa and the cotyledons.
Radish	All seedlings were attacked by the fungus at the roots and the hypocotyls. Some seedlings were killed. In most cases the roots had rotted away.	Seedlings were stunted. Their roots were colonized. A few seedlings had both the roots and hypocotyls destroyed.	Hypocotyls were attacked and the roots rotted away. More than half the seedlings were killed.	Plants were stunted. Hypocotyls were healthy but roots were colonized by the fungus. A few cotyledons were attacked.
Tomato	Healthy.	Healthy.	Seedlings were stunted. Hypocotyls were healthy. A few seedlings had their root tips destroyed.	Seedlings were stunted. Hypocotyls were healthy. Most of the root tips were destroyed.
Turnip	All the seedlings were killed.	Most of the seedlings were killed. Surviving seedlings had healthy hypocotyls and rotted roots. Seedlings were stunted.	Seedlings were killed after germination.	Hypocotyls were stunted but healthy. Most of the roots had rotted away.
Wheat	Healthy	Healthy	Healthy.	Seedlings were stunted. Shoots were not attacked. Lower halves of roots rotted away leaving stumps.

(ii) Using large test-tubes (inoculation after germination).

This method is illustrated in Fig. 2. Large test tubes (20 cm. long and 3.0 or 3.5 cm.* in diameter), each containing a 11 cm. diameter filter paper rolled into a cylinder, were fitted with metal caps. The filter paper was moistened with 10 or 15 ml.* of RM2 nutrient solution and then autoclaved at 15 lb/in^2 for 20 minutes. After cooling, the tubes were inoculated with a 7 mm. diameter agar plug bearing actively growing mycelium of an isolate of T. cucumeris. The pathogen was incubated at 26°C. for a week by which time, it had covered the filter paper. Two day old sterile seedlings of test plants were placed in the test tubes (two seedlings per tube) and held upright between the filter papers and the walls of the tubes. The lower three-quarters of the tubes were covered with brown paper. The tubes were then incubated in a growth cabinet.** Uninoculated plants were used as controls. Each treatment was done in triplicate. The plants were examined one week after they were placed in the test tubes.

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* The larger of the two volumes was placed in the larger tubes to which large seeds, e.g. peas, were added.

** Day temp. 23°C, night temp. 20°C, day length 15 hr.

Results:

Details are set out in tables 10, 11 and 12.

(a) effect on shoot length (table 10).

When inoculated with the stem strains, 48 and 68, cucumber shoots were reduced in length while wheat shoots were increased in length by about 70%, when inoculated with any of the stem strains. Other inoculated plants which showed an increase in shoot length compared with controls were pea (inoculated with isolates 48 and 68) radish (inoculated with isolate 69) and tomato (inoculated with isolate 48). Little or no change in shoot length was seen when lettuce was inoculated with any one of the stem strains. When inoculated with the root strain, R10, significant decreases in shoot lengths were seen in cucumber, lettuce and turnip. There was little or no change in shoot lengths of other test plants.

(b) effect on root length (table 11).

In all cases, except wheat and radish (inoculated with isolate 69), when roots were inoculated with the stem strains, significant decreases in root lengths of the test plants were observed. The root strain, R10, caused significant decreases in root lengths of cucumber, lettuce and radish and an increase in root length of pea.

Little or no effect was seen in the roots of other plants when inoculated with the root strain.

(c) pathogenicity on seedlings (table 12).

The roots of all test plants were attacked when inoculated with either stem or root strains of the pathogen. In general, the root strain caused more damage to the roots of test plants than the stem strains. The main area of attack was at the root tip (Fig. 2 and 3). The most susceptible plants were turnip and radish. The aerial parts of the plants, with the exception of turnip and lettuce, were healthy. In the case of lettuce, yellow spots on the shoots indicated the sites where the pathogen attempted penetration into the host. With turnip, the pathogen had killed the entire plant.

Fig. 2: Method of inoculation of radish seedlings in test tubes using T. cucumeris isolates (L-R) control, inoculated with isolates R10, 48, 68, 69.
The pathogen attacked the root tips and suppressed the production of lateral roots.

Fig. 2

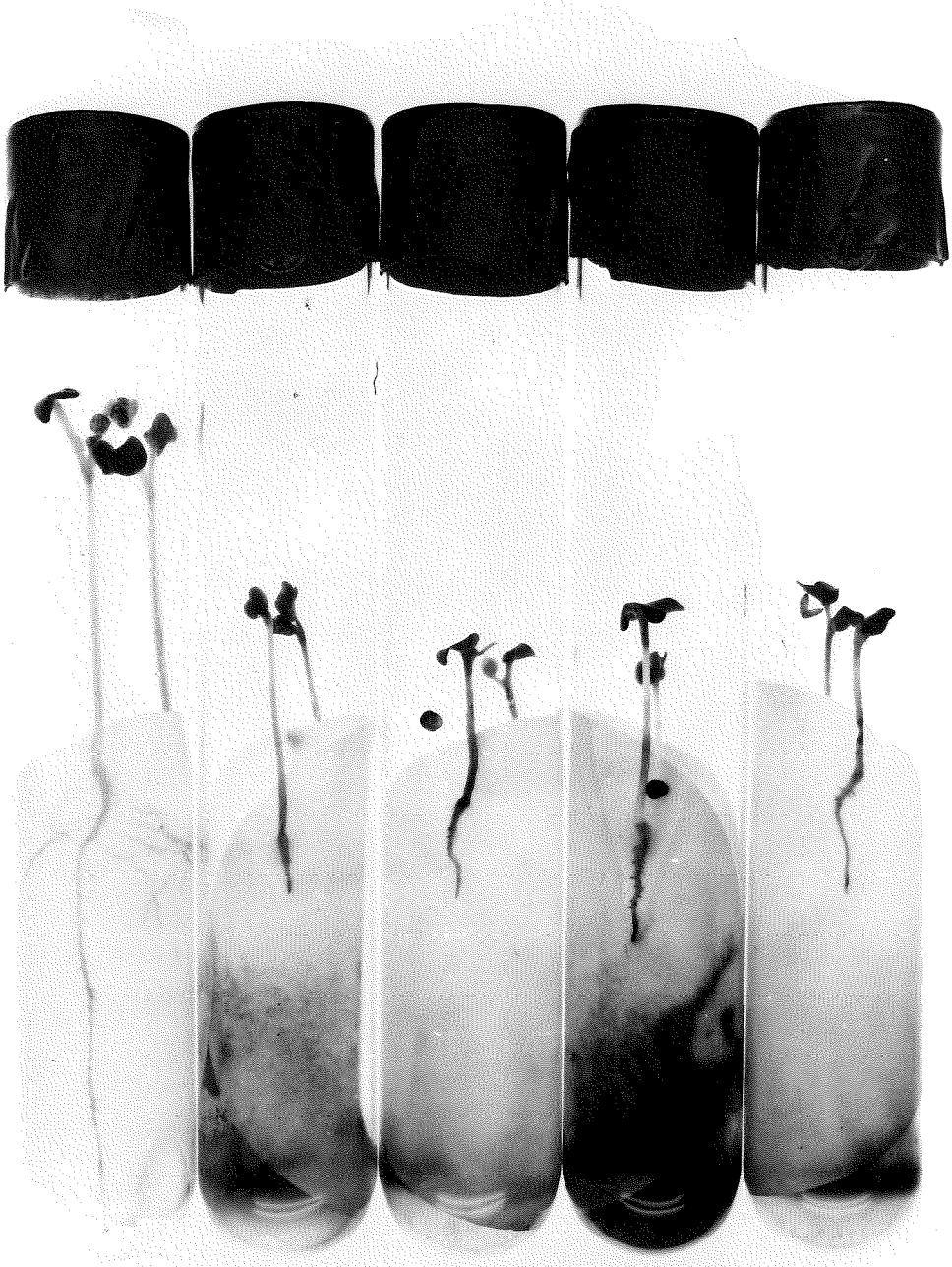


Fig. 3: Wheat seedlings inoculated with T. cucumeris isolates in large test tubes.
(L-R) Seedlings inoculated with sterile agar (control), isolates R10, 48, 68, and 69. The pathogen attacked mainly the root tips.

Fig. 3

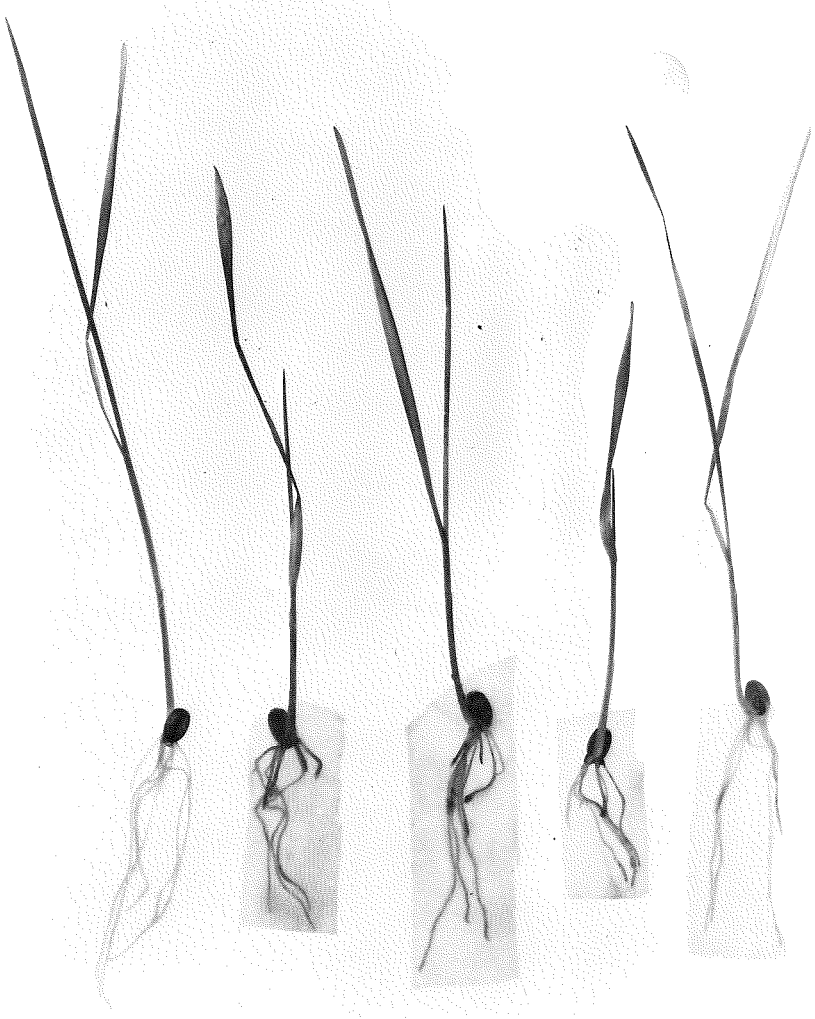


Table 10: Relative* length of the shoots of seedlings inoculated, after germination, with T. cucumeris isolates in large test tubes.

Host	Fungal isolates			
	48	68	69	R10
Cucumber	79	78	91	48
Lettuce	103	103	91	50
Pea	160	120	0	113
Radish	96	117	142	92
Tomato	120	110	90	95
Turnip	-	-	-	59
Wheat	170	175	172	88

Table 11: Relative* length of the roots of seedlings inoculated, after germination, with T. cucumeris isolates in large test tubes.

Host	Fungal isolates			
	48	68	69	R10
Cucumber	38	62	49	40
Lettuce	62	67	67	50
Pea	68	71	58	148
Radish	66	91	109	79
Tomato	67	73	60	87
Turnip	-	-	-	111
Wheat	142	136	144	102

* $\frac{\text{Average length of shoots or roots of inoculated seedlings}}{\text{Average length of shoots or roots of control seedlings}} \times 100$

\- Measurements not taken, seedlings were killed.

Table 12: Disease symptoms of seedlings of various plants inoculated, after germination, with *T. cucumeris* isolates inside large test tubes.

Fungal isolate	48	68	69	R10
Cucumber	The root tips of the main roots and the lateral roots were attacked. The aerial parts and the numerous adventitious roots were healthy. The plants were stunted.	Similar to that described for isolate 48.	The root tip of only one plant was destroyed. The rest were healthy. There were numerous lateral and adventitious roots. Aerial parts were healthy.	Five out of six plants had their roots destroyed. Aerial parts and adventitious roots were healthy. Two plants were severely stunted.
Lettuce	Cushion-like structures, without penetration, were formed on the surface of the roots. A few necrotic spots, bearing small hyphal clumps, were seen on the hypocotyls. Otherwise, the seedlings were healthy.	The roots of two plants had rotted away leaving brownish stumps. Lesions were seen on other roots. Lesions and yellow necrotic spots, due to fungal penetration, were seen on the hypocotyls.	The root tips and the bases of the hypocotyls were attacked. Yellow necrotic spots and lesions on the hypocotyls were sites of fungal penetration.	The lower parts of the roots had rotted away. The tips of a number of young lateral roots had been invaded. Fungal attacks caused necrotic lesions on the hypocotyls.
Pea	The root tips and the zones of elongation of the roots of all seedlings were destroyed. The tips of the lateral roots were also attacked. The aerial parts were healthy.	The lower parts of all roots were destroyed. Some of the root tips of the lateral roots were attacked. The shoots were healthy.	Similar to that described for isolate 48.	The lower two-thirds of the main roots were discoloured due to fungal attack of the root surface and the root caps. The tips of the lateral roots were discoloured. The shoots were healthy.
Radish	The lower parts of the main root and the lateral root tips were attacked. The hypocotyls were healthy. No sclerotia were formed on the roots.	Similar to that described for isolate 48 except that a few sclerotia were formed on the roots.	The root tips and the regions of elongation of the roots were attacked. The roots had a few sclerotia. The hypocotyls were healthy.	The roots were attacked and the tips had rotted away. A number of sclerotia were formed on the roots. The hypocotyls were healthy.
Tomato	The root tips were destroyed. Parts of the roots were colonized. The aerial parts were healthy.	The root caps were colonized. The other parts of the seedlings were healthy.	Four plants had their root tips destroyed. The rest were healthy.	Three plants had their root tips destroyed. The rest were healthy.
Turnip	All seedlings were killed.	All seedlings were killed.	All seedlings were killed.	The root tips were colonized. Small lesions indicated sites of fungal attack. The shoots were healthy.
Wheat	The root tips, the zones of elongation and the bases of the roots were attacked. The shoots were healthy.	Similar to that described for isolate 48.	Similar to that described for isolate 48.	The root tips had rotted away. Various parts of the roots were attacked. Lesions up to 1 mm. in diameter were seen. Root bases and the shoots were healthy.

(B) Under semi-sterile conditions.

(i) Using glass slides.

Seedlings were grown in soil (U.C. mixture, see Appendix) in a growth cabinet (day temp. 23°C, night temp. 20°C, day length 15 hr.). Ten day old seedlings were washed free of soil. Uniform seedlings were chosen. Two seedlings were held in position with rubber bands on one side of each sterile 3" x 1" microscope slide. Each pair of seedlings was inoculated by placing between them a strip of agar bearing actively growing mycelium of an isolate of T. cucumeris (Fig. 4). Inoculated seedlings were transferred to humid chambers and returned to the growth cabinet. Controls were similarly done except that sterile agar strips were used. Each treatment consisted of six replicates.

Infected plants were stained with 0.1% trypan blue in 6% acetic acid for 2 minutes. Excess stain was removed with tap water. Superficial hyphae were removed under a dissecting microscope. Plants were restained with trypan blue. Removal of superficial hyphae was important because hyphae on the host surface may give a false impression of the amount of tissue invaded by the pathogen. The cuticle of the host plant

does not stain, but the mycelium, together with areas invaded by the pathogen, stain deep blue. Disease rating of each infected plant was scored using the system in table 13.

(ii) Glass plate method.

This is a modification of the glass slide method. 8-12 day old seedlings were spaced on a glass plate (12 plants per plate) measuring 15.5 cm. x 11.5 cm. The seedlings were held in position by rubber bands. Inoculum, on agar strips, was placed between and in contact with shoots of the test plants. Controls were done with sterile agar strips. Inoculated plants were placed in humid chambers and incubated as in the glass slide method.

Results:

All test plants were susceptible to pathogenic attack by the stem and root strains of T. cucumeris (table 14) but the degree of susceptibility differed from host to host. The most susceptible plants were turnip and radish seedlings which showed significant damage by the stem strains after an incubation period of about two days. Extensive lesions or totally collapsed stems were seen on radish and turnip seedlings when inoculated with the stem strains for ten days.

Fig. 4: Two week old radish seedlings which had been in contact for a week with inoculum of T. cucumeris isolates grown on PDA medium. (L-R): Seedlings inoculated with isolates 48, 68, 69, R10 and sterile agar (control).

Note the extensive rotting of some hypocotyls with no apparent symptoms on the cotyledons and leaves.

Fig. 4

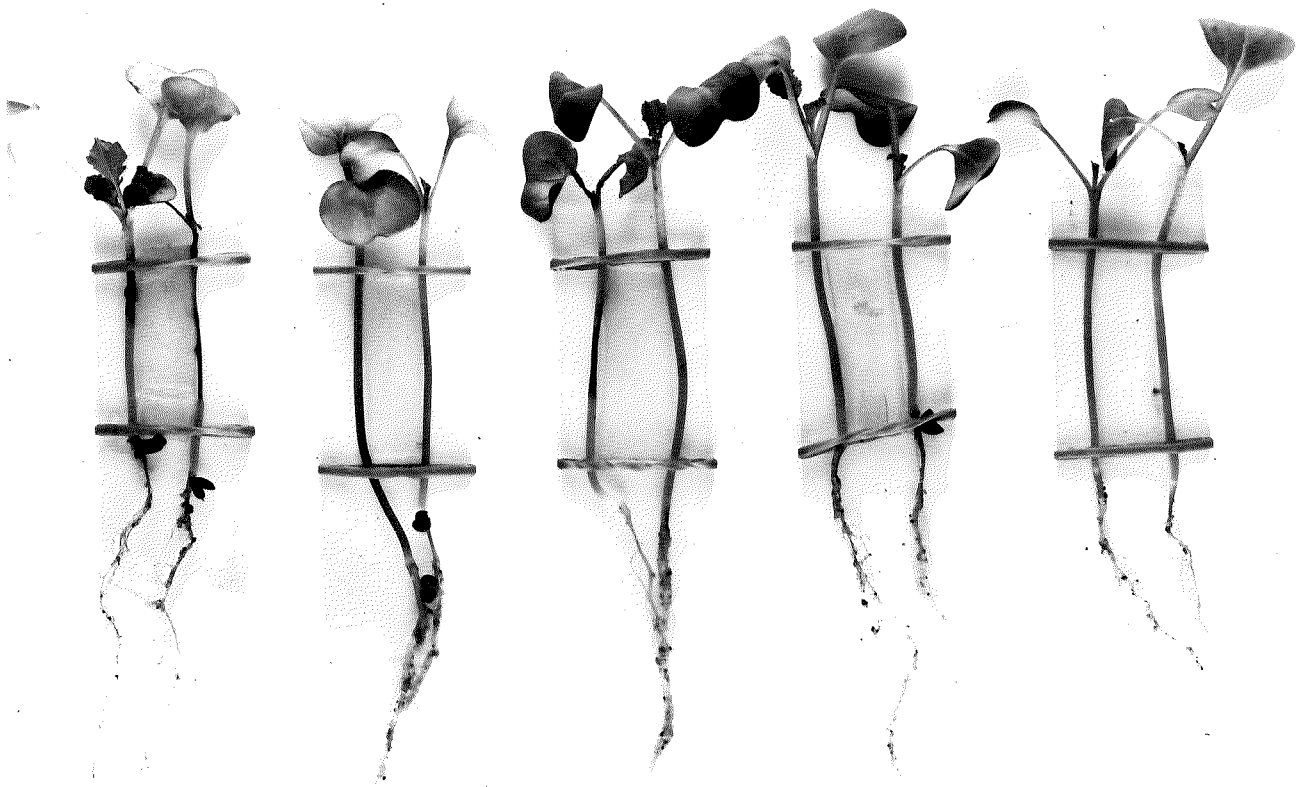


Table 13: Disease rating used for infected plants after staining with trypan blue.

0	=	not infected
1	=	1 or 2 small lesions
2	=	3 to 5 small lesions
3	=	more than 5 small lesions or up to 2 mm. of collapsed tissue*
4	=	3 - 5 mm. of collapsed tissue
5	=	6 - 10 mm. of collapsed tissue
6	=	11 - 20 mm. of collapsed tissue
7	=	more than 20 mm. of collapsed tissue
8	=	total collapse of test plant.

* measurements were done longitudinally.

Less damage was caused on the stems of these two plants when inoculated with the root strain for the same period. The most resistent plant was wheat which was attacked at the coleoptile and occasionally one or two small lesions developed on leaf bases. Pea and tomato were moderately resistent and most of the plants had small lesions. Cucumber and lettuce were fairly susceptible and degradation of tissue was extensive in a number of plants. In general, when plants were inoculated with the root strain they were less severely diseased than when inoculated with the stem strains. Exceptions were lettuce and tomato which were extensively rotted when inoculated with the root strain.

(C) Under non-sterile conditions.

(i) Pre-germination inoculation.

A quarter of an RM2 agar plate bearing mycelium of an isolate of T. cucumeris was macerated with 15 ml. of sterile water in a 25 ml. glass homogenizer (M.S.E. 'Masticator'). The dispersed mycelium was used to inoculate 200 ml. of RM2 liquid medium in a 500 ml. Erlenmeyer flask. Inoculated flasks were incubated at 26°C on a rotary shaker for five days. The fungal mycelium was harvested using a Buchner funnel. Fungal mat from each flask was washed thrice with distilled

Table 14: Disease rating of infected shoots after inoculation with *T. cucumeris* isolates grown on RM2 medium.

Test plants	Age when inoculated*	Fungal Isolate	Time after inoculation	Disease rating **									Disease Index***
				0	1	2	3	4	5	6	7	8	
Cucumber	8 days	48	10 days	-	-	-	-	-	-	3	5	4	12/12
		68		-	-	-	6	4	2	-	-	-	12/12
		69		-	-	-	5	1	3	3	-	-	12/12
		R10		-	-	-	7	3	2	-	-	-	12/12
Lettuce	9 days	48	10 days	-	-	-	3	1	3	-	5	-	12/12
		68		-	-	-	4	4	4	-	-	-	12/12
		69		-	-	-	5	1	3	-	1	2	12/12
		R10		-	-	-	-	1	3	2	-	6	12/12
Pea	9 days	48	7 days	-	-	-	12	-	-	-	-	-	12/12
		68		-	-	-	12	-	-	-	-	-	12/12
		69		-	-	-	7	1	3	1	-	-	12/12
		R10		3	1	1	7	-	-	-	-	-	9/12
Radish	10 days	48	1½ days	-	-	-	-	-	-	1	8	3	12/12
		68		1	-	-	1	3	2	4	1	-	11/12
		69		-	-	-	-	-	-	-	-	12	12/12
		R10		3	1	3	-	4	-	1	-	-	9/12
Tomato	12 days	48	10 days	3	2	2	3	1	1	-	-	-	9/12
		68		-	3	1	8	-	-	-	-	-	12/12
		69		-	-	2	10	-	-	-	-	-	12/12
		R10		-	-	-	-	-	1	3	3	5	12/12
Turnip	11 days	48	2 days	-	-	-	-	-	-	-	-	12	12/12
		68		-	-	-	-	-	2	3	3	4	12/12
		69		-	-	-	-	-	-	-	-	12	12/12
		R10		4	1	1	5	-	1	-	-	-	8/12
Wheat	11 days	48	7 days	9	-	3	-	-	-	-	-	-	3/12
		68		10	2	-	-	-	-	-	-	-	2/12
		69		10	2	-	-	-	-	-	-	-	2/12
		R10		8	4	-	-	-	-	-	-	-	4/12

* age from the day of sowing

** see Table 13.

*** $\frac{\text{Number of infected plants}}{\text{Number of inoculated plants}}$

water and dispersed in 100 ml. of water using an homogenizer. Soil (U.C. mixture) was inoculated by thoroughly mixing 200 ml. of dispersed mycelium with 100 g. of soil.

Plastic pots, 12.5 cm. diameter, were three-quarters filled with U.C. mixture, moistened thoroughly with tap water and then covered to a depth of half an inch with inoculated soil. Ten seeds per pot were spaced on top of the soil and then covered with an inch-thick layer of inoculated soil. Controls were similarly done but with uninoculated soil. Each treatment consisted of four replicates. Planted seeds were incubated for a week in a glass house.

Results.

There were no significant differences between the germination rates of the test plants, inoculated with T. cucumeris isolates, and the controls with the exception of turnip when inoculated with the stem strain 69 (table 15). Of the plants that emerged, only radish and turnip seedlings were attacked by the stem strains of the pathogen. Radish was the most susceptible plant. The stem strain, isolate 48, was the most virulent. No symptoms were seen when cucumber, lettuce, pea, tomato and wheat were inoculated with the stem strains of the

Table 15: Disease index* of seedlings grown for one week in soil inoculated with T. cucumeris isolates.

* Numerator, number of seeds attacked.
Denominator, number of seedlings emerged.

Fungal isolate	Test plants	Replicates* 1	2	3	4	Control**
	Cucumber	0/8	0/6	0/8	0/9	9
	Lettuce	0/10	0/9	0/9	0/9	10
	Pea	0/9	0/10	0/10	0/10	10
48	Radish	5/8	1/7	6/10	5/8	9
	Tomato	0/8	0/5	0/9	0/10	10
	Turnip	6/8	4/5	6/8	4/7	7
	Wheat	0/7	0/7	0/8	0/9	9
	Cucumber	0/8	0/8	0/7	0/9	9
	Lettuce	0/7	0/7	0/8	0/10	8
	Pea	0/9	0/10	0/8	0/8	8
68	Radish	3/9	0/10	4/10	0/8	10
	Tomato	0/8	0/7	0/8	0/8	8
	Turnip	2/6	0/7	0/6	0/4	8
	Wheat	0/10	0/8	0/9	0/10	8
	Cucumber	0/7	0/9	0/6	0/8	7
	Lettuce	0/8	0/7	0/7	0/6	9
	Pea	0/7	0/9	0/8	0/7	9
69	Radish	3/6	3/8	3/5	2/9	10
	Tomato	0/9	0/7	0/10	0/9	8
	Turnip	0/3	0/3	2/4	1/5	8
	Wheat	0/7	0/8	0/10	0/8	9
	Cucumber	0/10	0/10	0/10	0/10	10
	Lettuce	0/9	0/10	0/10	0/10	10
	Pea	0/10	2/9	1/9	0/9	10
R10	Radish	0/9	2/9	0/10	0/9	10
	Tomato	0/8	0/9	0/9	0/10	9
	Turnip	1/9	2/7	1/8	0/8	9
	Wheat	0/10	0/10	0/9	0/8	9

* Ten seeds per replicate were sown.

** Number of plants emerged from ten planted seeds.

pathogen. Only pea, radish and turnip seedlings were mildly attacked by the root strain.

(ii) Post-germination inoculation.

Erlenmeyer flasks, 500 ml., each containing 150 ml. of vermiculite moistened with 150 ml. of RM2 nutrient solution, were autoclaved at a pressure of 15 lb. per sq. inch for 20 minutes. After cooling each flask was inoculated with five 7 mm. agar discs bearing an isolate of T. cucumeris. Inoculated flasks were incubated at 26°C and shaken once daily to ensure uniformity of fungal growth. The cultures were harvested after two weeks growth and mixed with soil (U.C. mixture) in the proportion of one part of infested vermiculite to ten parts of soil.

Plastic pots, 12.5 cm. in diameter, were three-quarters filled with U.C. mixture. The pots were then filled to the top with inoculated soil which was thoroughly moistened with tap water. Pots were incubated for one or two days before ten newly germinated seeds per pot were planted in the soil. Controls consisted of newly germinated seeds placed in soil mixed with uninoculated vermiculite, moistened with RM2 nutrient solution. Each treatment was done in triplicate.

Results

(i) Pre-emergence damping-off (table 16).

Excellent emergence of seedlings occurred when cucumber, lettuce, pea, tomato and wheat were inoculated, after germination, with the stem and root strains of T. cucumeris. None of the seedlings of radish and turnip emerged when inoculated with isolate 48. Only one radish seedling and eleven turnip seedlings emerged when inoculated with isolate 69.

(ii) Pathogenic attack on emerged seedlings (table 17).

The roots and shoots of cucumber, tomato and wheat seedlings, inoculated with the stem or root strains of T. cucumeris, were healthy. A few lettuce plants were attacked by isolate 69. These plants showed a few lesions on their stems. No symptoms were seen when lettuce was inoculated with isolates 48 and 68. When inoculated with the root strain, a few lettuce seedlings were attacked. Peas were moderately susceptible to the stem strains. Lesions were formed on a number of plants at the sites of attachment of the cotyledons (Fig. 5). Cortical tissues were destroyed leaving the thin vascular tissues joining the roots and the shoots. When inoculated with the root strain, pea seedlings showed similar symptoms to those caused by the stem

Table 16: Number of seedlings* emerged one week after planting in soil inoculated with *T. cucumeris* isolates.

	48	68	69	R10	Control
Cucumber	30	30	30	30	30
Lettuce	29	30	30	29	30
Pea	25	29	27	30	30
Radish	0	29 (23)	1	30	30
Tomato	30	30	29	29	30
Turnip	0	30 (14)	11 (0)	30	30
Wheat	29	29	29	29	30

* Three replicates of ten germinated seeds were sown.

( ) = number of plants surviving three weeks after sowing.

strains. Symptoms were seen only in a few plants. All pea seedlings showing disease symptoms were stunted.

Turnip and radish seedlings were most susceptible to pathogenic attack by the stem strains. None of the turnip seedlings emerged when inoculated with isolate 48. All the turnip seedlings that emerged were killed by isolate 69. Turnip seedlings were attacked by isolate 68 at the base of the shoot causing the collapse of the cortex of that region (Fig. 6). In the case of radish, none of the seedlings emerged when inoculated with isolate 48. Only one seedling emerged when inoculated with isolate 69. This plant had all of its hypocotyl destroyed resulting in a darkish wire-like structure. Seedlings that emerged from soil inoculated with isolate 68 were either killed or were severely damaged by the pathogen at the bases of their hypocotyls (Fig. 7). The root strain was slightly pathogenic to turnip and radish seedlings and caused lesions at the bases of the hypocotyls of a few plants.

Table 17: Disease symptoms of seedlings*, three weeks after sowing, in soil inoculated with T. cucumeris isolates.

* Number of seedlings surviving after three weeks, is given in Table 16.

Fungal isolate	48	68	69	R10
Cucumber	Healthy.	Healthy.	Healthy.	Healthy.
Lettuce	Healthy.	Healthy.	Three plants had a lesion each on the shoots. The rest were healthy.	Two plants had small lesions on their roots. One plant had a lesion on the hypocotyl.
Pea	Seven plants were stunted. These had root lesions. The rest were healthy.	Four plants were stunted. Lesions were formed at or near the sites of cotyledon attachment.	Six plants were stunted. Lesions formed at or near the sites of cotyledon attachment.	One plant was stunted. This plant and another plant had a lesion each at or near the attachment of cotyledons.
Radish	All plants had died before emergence.	All plants were attacked at the base of the hypocotyls. Roots were healthy.	Only one plant survived. The whole of the cortex had rotted away leaving a wire-like stem. Leaves showed no symptoms.	Three plants had well defined lesions at the bases of their hypocotyls. The rest were healthy.
Tomato	Healthy.	Healthy.	Healthy.	Healthy.
Turnip	All plants had died before emergence.	Seedlings had extensive lesions at the bases of their hypocotyls.	All plants were killed after emergence.	Four seedlings had lesions at the bases of their hypocotyls. The rest were healthy.
Wheat	Healthy.	Healthy.	Healthy.	Healthy.

Fig. 5: Pea seedlings sown in soil and inoculated with *T. cucumeris* isolates for three weeks.

(L-R): Control, inoculated with isolates 48, 68, 69 and R10.
Lesions formed at the sites of cotyledon attachment caused girdlings of the plants.
Arrows indicate lesion sites.

Fig. 5

Fig. 6: Turnip seedlings sown in soil inoculated with *T. cucumeris* isolates for three weeks.

Left: control.

Right: seedlings attacked by isolate 68. Seeds inoculated with isolate 48 and 69 either failed to emerge or all the seedlings were killed after emergence.

Arrow shows diseased area of hypocotyl.

Fig. 6

Fig. 7: Radish seedlings sown in soil inoculated with T. cucumeris isolates for three weeks.

(L-R) Control, inoculated with isolate 68, 69, R10. Seeds inoculated with isolate 48 failed to emerge.

Arrows show diseased areas of hypocotyls.

Fig. 7

Discussion.

The host range of T. cucumeris is extremely wide (Kernkamp et al. 1952) and it is not surprising to find the test plants used in the present study are among those listed as susceptible to pathogenic attack by T. cucumeris. The stem and root strains of T. cucumeris tested for virulence on seven test plants ranged from avirulent to highly virulent depending on the plant-pathogen combination and the conditions under which the experiments were performed. Some of the plants which were resistent under non-sterile conditions were susceptible when in contact with the pathogen under sterile conditions. A number of investigators (Le Clerg 1934, Maier 1959, Maier & Staffeldt 1960, Daniels 1963, Shatla & Sinclair 1965, Pitt 1964a) in their studies of the parasitism of T. cucumeris also found great variability in the pathogenicity which ranged from non-pathogenic to highly pathogenic. Similar results have been obtained using single basidiospore isolates (Papavizas & Ayers 1965).

Houston (1945) claimed that correlations could be found between cultural types and pathogenicity of R. solani. In the present study, although the four strains of T. cucumeris could be distinguished morphologically (Fig. 1), no correlation was found

between cultural types and pathogenicity. Similar results were obtained by Flentje and Saksena (1957) who studied sixty-eight isolates of Pellicularia filamentosa (T. cucumeris) and el Zarka (1965) who studied fifteen isolates of R. solani from potato.

Under sterile conditions, germination of the seeds of the seven test plants when inoculated with the stem and root strains of T. cucumeris were either slightly stimulated or inhibited by up to 70 per cent. The most susceptible seeds were turnip. R10 and 69 were the most virulent fungal strains. When germinated seeds were inoculated with the stem and root strains of T. cucumeris under sterile conditions, all the plants were susceptible to fungal attack mainly at the root tips. The most susceptible seedlings were turnip and radish.

When germinated seeds were inoculated under sterile conditions using large test tubes, a number of combinations of plant-fungal strain resulted in the stimulation of growth of the host shoot and/or the host main root. Stimulation of root growth was pronounced in the case of wheat seedlings when inoculated with all strains of the pathogen and pea seedlings when inoculated with the root strain, R10. Stimulation could be due to the presence of growth factors in the fungal metabolites since the

fungal cultures had grown for a week before the introduction of germinated seeds. Fungi are known to produce auxins (Gruen 1959) and R. solani has been shown to do so (Dodman et al. 1966). Stimulation of shoot growth may also be due to absorption of the fungal metabolite or to a disturbance of the host growth regulators (Sequeira 1963) or both.

Under semi-sterile conditions, all the seven test plants were susceptible to pathogenic attack by the stem and root strains of the pathogen although the range of susceptibility varied from near resistance to complete susceptibility. The most susceptible plants were radish and turnip. A number of authors (Le Clerg 1941, Storey 1941, Blair 1942, Kernkamp et al. 1952, Flentje 1957, Flentje & Saksena 1957) have reported that T. cucumeris consists of a number of pathogenic strains, some limited to hosts of one or two families of plants while others less specialised and capable of attacking a greater range of host plants. The three stem strains used in this study have been reported to be specific to host plants of one family, the Cruciferae (Flentje et al. 1963a) and the root strain as being unspecialised (de Beer 1965).

Under non-sterile conditions, when inoculated with the stem strains, most of the radish and turnip seeds

either failed to emerge above the soil level or were severely infected after emergence. Other plants were either uninfected or lightly to moderately infected. Only in turnip and radish seedlings were damping-off seen. As such, specificity of the stem strains to members of the Cruciferae could only be demonstrated in the case of damping-off of seedlings under non-sterile conditions. In the case of the root strain, no specificity for any one type of test plants was evident. The damage caused to the seedlings by the root strain was slight.

It can be seen that turnip and radish were highly susceptible to pathogenic attack by T. cucumeris under all the conditions tested. The resistance of the other test plants varied according to the experimental conditions. This is not surprising since it is known that other soil micro-organisms may exert a great effect on host-pathogen relations. This is especially true of soil-borne fungal diseases as pointed out in a number of reviews (Garrett 1956, Parkinson & Waid 1960, Park 1963, Rovira 1965a;b). Cases are known in which the presence of a rhizosphere microflora may prevent infection of test plants. Müller-Kögler (1938) found that Ophiobolus graminis could penetrate the roots of many Angiosperms growing in sterile soil but these plants

were immuned when grown in natural soil. Garrett (1939) was able to infect roots of wheat seedlings, using ascospores of O. graminis in sterile sand but not in unsterilized soil. Brooks (1965) confirmed Garrett's observation but also noted that ascospores readily infect the proximal parts of the roots produced by wheat seeds germinating on the surface of moist non-sterile soil. The root surface microflora, on the proximal parts of the roots between the seed and the soil had not developed fully until two weeks after germination.

The ability of the stem and root strain of T. cucumeris to infect otherwise resistent host plants under semi-sterile and sterile conditions may indicate the potential of the pathogen. Under these two conditions, the amount of inoculum in contact with the test plants was high and probably greater than the critical inoculum potential required for infection. Inoculum potential has been defined by Garrett (1956) as the "energy of growth of a fungal parasite available for infection of a host at the surface of the host organ to be infected". Garrett (1956) also stated that the inoculum potential could be increased by increased nutritional status of the infecting fungus. Shephard and Wood (1963) studied the effect of environment and nutrition of pathogen and host in the damping-off of

lettuce and cauliflower seedlings by R. solani. They found that the nutrition of the fungus, its vigour and the quantity of inoculum were important in establishing infection. Weinhold and Bowman (1967) found that decreasing the concentration of nutrients in culture media resulted in reduced virulence of R. solani mycelium. They suggested that a certain quantity of utilizable nutrients, greater than that required for vegetative growth, must be present within the mycelium for R. solani to function as a pathogen. The present author compared the pathogenicity of T. cucumeris isolates grown on PDA and RM2 nutrient agar using radish seedlings as test plants. Cultures grown on RM2 medium caused extensive rotting of radish plants in one and a half days, whereas those grown on PDA medium took a week to produce comparable rotting. This indicated that the nutrients present in the inoculum could be an important factor affecting disease severity.

The root strain of T. cucumeris has been reported to be the causal organism of the "bare-patch" disease of cereals in South Australia (Kerr 1955, de Beer 1965). The root strain, R10, used in the present investigations was isolated from "bare-patch" of wheat by de Beer (1965). He found that the root strain occurred consistently in

"bare-patch" of cereal with high populations of the nematode, Pratylenchus neglectus. He suggested that "bare-patch" of cereal could be the result of a synergistic relationship between the nematode and R. solani. No experimental evidence was presented by him to show the relative importance of the nematode in causing "bare-patch" of cereals. In the present investigations, the absence of disease symptoms when wheat was inoculated with the root strain under non-sterile conditions supported the evidence of such a synergistic relationship and suggests that local injuries of the root caused by the nematodes may predispose seedlings to pathogenic attack by R. solani. A similar synergistic relationship had been suggested by Benedict and Mountain (1956) in their study of Rhizoctonia root rot of Winter wheat in Canada.

PART II: THE CYTOLOGY OF THE HOST-PATHOGEN RELATIONSHIP.

Introduction.

The relationship between fungal pathogens and their hosts at the cellular and sub-cellular levels is poorly understood. Knowledge of these aspects of pathogenicity is essential if we are to exploit fully the possibility of disease control through host resistance. This is especially true of plant diseases caused by T. cucumeris because the diseases take a variety of forms. This variety is probably due to the species consisting of a large number of strains exhibiting an exceedingly broad variety of physiological and biochemical properties (Kernkamp et al. 1952). The present author has shown that T. cucumeris is capable of parasitising all of the hosts used in this study and that some of these hosts are susceptible to the pathogen under all conditions used for the pathogenicity tests. However, other host species were resistant to attack by T. cucumeris but only under non-sterile conditions. The mechanism of resistance or susceptibility of plants to T. cucumeris is unknown.

The interaction of a host and a pathogen has been conveniently divided into three inter-dependent parts (Brown 1936). This consists of (i) the growth of the pathogen prior to penetration (ii) penetration and

(iii) the stages after penetration. The key to the resistance or susceptibility of a host to its pathogen may be found in any of the stages of pathogenesis. In the present investigations, Brown's (1936) scheme of divisions of the host-pathogen relationship into different stages is used. A number of studies have been done on the cytology of the host-pathogen relationship of plant diseases caused by T. cucumeris. However, most of the detailed studies were confined to one or two stages of the host-pathogen relationship. Studies concerning the pre-penetration stages of plant diseases caused by T. cucumeris are limited to a few combinations of host plants and pathogenic fungal strains. Since T. cucumeris has a wide host range, comparison of its behaviour on the surfaces and the modes of penetration of different plants will provide useful information concerning its pathogenic habits.

Of the seven test plants used, turnip and radish seedlings are the most susceptible to fungal attack by T. cucumeris isolates. Although it is known that host penetration can be accomplished beneath the infection cushions of T. cucumeris on crucifer host plants, the mechanism of penetration in relation to the infection cushion has not been examined critically. To understand the relationship between the infection cushions and host

penetration various aspects of the fungal cushions, the host hypocotyl surface and the interactions between the pathogen and the host were examined.

No detailed studies have been made of the colonization of hypocotyls and the roots of radish seedlings by T. cucumeris. Similarly, the spread of the pathogen within wheat roots has not been reported in detail. In this investigation, the modes of entry and proliferation of T. cucumeris within radish hypocotyls and the roots of radish and wheat were studied. It is hoped that this study will enable us to understand more fully the behaviour of T. cucumeris within its hosts.

(i) Growth of the pathogen prior to penetration.

Most of the early work in this field was done with fungi that infect the aerial parts of plants and excellent reviews are available (Flentje 1959, Dickinson 1960, Wood 1960; 1967, Akai et al. 1967, Goodman et al. 1967, Shaw 1967). The ecological factors affecting the distribution, survival and behaviour of the pathogen prior to contact with the host can be very important in determining the success or failure of the pathogen. These factors have been discussed in the general review of literature at the beginning of this thesis.

It is known that aerial fungal pathogens usually reach their hosts passively, but soil-borne pathogens have to grow through soil to reach their hosts. Under suitable conditions, on the host surface pathogens may undergo a series of activities including spore germination, hyphal growth and formation of appressoria or other infection structures before penetrating the host. Two opposing theories have been proposed to explain the behaviour of the pathogen on the host surface prior to the infection of the host, the contact and the chemical stimuli theories.

Brown and Harvey (1927) using spores of Botrytis cinerea and epidermal strips of Allium cepa found that the pathogen could penetrate either side of epidermal strips even after they had been washed thoroughly. This and other evidence, for example, the ability of certain fungi to penetrate inert membranes, led Brown to propose the theory that penetration is the result of a physical contact stimulus. The significance of the host surfaces as a major factor in the development of obligate parasites was illustrated in a series of publications by Dickinson (see Dickinson 1960). Using artificial membranes Dickinson was able to follow the development of infection structures similar to those occuring in the normal course of infection. He

demonstrated that thigmotropism is important in inducing a fungal pathogen to adhere to the host and that a contact stimulus is involved in the formation of the appressorium. Recently, it has been shown (Maheshwari et al. 1967) that the properties of the host surface are important in the establishment of rust colonies and that infection cannot be established if the epidermis is removed before inoculation. The same authors also found that contact of the germ tube with isolated cuticle was sufficient to evoke the sequential differentiation of appressorium, vesicle and infection hyphae. However, Chakravarti (1966) attempted to infect wheat seedlings by introducing urediospores of Puccinia graminis on wheat mesophyll cells beneath the epidermis and found that germ tubes could not infect the host directly. Infection occurred only from hyphae produced by appressoria which were formed by the germ tubes upon contact with the mesophyll.

Flentje (1957) working with T. cucumeris reported that the pathogen did not form appressoria on inert surfaces. This indicated that the response to contact stimuli exhibited by obligate aerial pathogens was not necessarily exhibited by soil-borne pathogens. A close correlation had been demonstrated between susceptibility of the host plant and the chemical stimulation of

T. cucumeris (Kerr 1956, Kerr & Flentje 1957, Flentje 1957, Flentje et al. 1963a, Husain & McKeen 1963, Dodman 1965).

The whole of the plant including the root is covered with a thin cuticle (Scott 1965). Since the fungal pathogen first came into contact with the cuticle, the pathogenesis of the fungal pathogen may be intimately related to the chemical composition of the plant cuticle. The literature dealing with plant surfaces is very extensive and various aspects of the subject had been reviewed as follows: waxes (Kreger 1948), angiosperm cuticle (Priestley 1943), cuticle in relation to the use of pesticides, growth regulators and nutrients (Van Overbeck 1956, Currier & Dybing 1959, Wittwer & Teubner 1959, Mitchell et al. 1960, Crafts & Foy 1962, Franke 1967) and cuticle in relation to plant diseases (Martin 1964, Wood 1967). Plant surfaces are highly complex, having protuberances of many forms, wax crystals of innumerable types, cutin layers of varying amounts and composition, including wax platelets of crystalline nature, pectic and cellulosic components. One of the most important features of plant cuticle is its extreme variability. This involves chemical variability, variability in distribution over the plant surface, in continuity and degree of perforation, in thickness on

various parts of a single leaf and in chemical and physical properties as between different plants and plants from different environments. As a result of this variability the rate of movement of organic substances and even of water may not be the same when they are escaping from as when they are entering the leaf.

The importance of the cuticle as a barrier to fungal invasion is still debatable. Its complexity and variability make it extremely difficult for general experimental interpretations. Roberts et al. (1961) found that young lime leaves with little cutin were susceptible to Gleosporium but hardened leaves bearing appreciable deposits of cutin were resistant. On the other hand, Euonymus leaves were susceptible to mildew attack despite large increases in cutin formation. The degree of resistance of apple and rose leaves to their mildews was not correlated with the levels of depositions of waxes or cutin. Moreover, leaves of susceptible and resistant apple varieties taken at an early stage in development show no differences in cuticle components. It is known that the cuticle swells when absorbing water and this may increase its permeability to solutes in water. Thus the water content of the epidermal cells may regulate the permeability of the cuticle. This is of significant importance in infection since it is known

that plant exudates can affect the behaviour of plant pathogens on host surfaces.

It is known that before penetrating their host plants, fungal pathogens usually exhibit ectotropic growth on the surface of the host. Ectotropic growth of hyphae of T. cucumeris on the surface of the host (Garrett 1956, Flentje 1957) and non-host plants (Thornton 1956, Warcup 1957, Warcup & Talbot 1962) has been reported. The differences in growth habits on the plant surfaces could be important in determining the resistance or susceptibility of the host to fungal attack and may be useful in devising the type of control measures.

(ii) Penetration into the host.

The mechanism of penetration into hosts by fungal pathogens is complex and this has been reviewed by Akai (1959), Flentje (1959), Dickinson (1960) and Wood (1960, 1967). The bulk of the literature deals with the entry of air-borne pathogens into leaves. According to Wood (1967), there are three main ways in which fungal pathogens enter their hosts. Penetration through the intact host surface is by an infection peg from an adhesive structure such as an appressorium. Penetration through the stomata may or may not be preceded by the formation of an appressorium. Some soil-borne plant

pathogens and facultative parasites enter their hosts through wounds.

Penetration by T. cucumeris into host plants can occur through stomata (Ullstrup 1936, Shurtleff 1953) through wounds (Boosalis 1950, Wyllie 1959, Khadga et al. 1963) by single hyphae (Khadga et al. 1963) and beneath infection cushions. Dodman et al. (1968a) examined the modes of penetration of 53 isolates of R. solani from different hosts and localities using bean and radish as test plants. They found that 33 isolates penetrated from dome-shaped cushions while the other 20 penetrated from lobate appressoria. Isolates that form lobate appressoria also consistently penetrate through stomata. The same authors found that isolates from stems or roots penetrate from dome-shaped infection cushions whereas isolates from foliage commonly penetrate from lobate appressoria and through stomata.

The mode of penetration of intact host surface from infection cushion of T. cucumeris is of special interest since a large number of the fungal pathogens penetrate their hosts directly by means of simple appressoria. Abdel Salem (1933) working with lettuce, Flentje (1957) with crucifer and compositae, Christou (1962) with bean, Gonzalez and Owen (1963) with tomato fruit, Khadga et al. (1963) with cotton suggested that each infection cushion

of R. solani consists of a large number of appressoria and that each appressorium is pressed against the cuticle and produces a slender infection peg which then penetrates the cuticle and the epidermal cell wall and assumes its normal diameter on emergence into the cell lumen. However, Boosalis (1950) investigating the parasitism of R. solani on soybean and Dodman (1965) on radish could not detect fine penetration pegs beneath the cushions at the sites of penetration. Similarly, Chi and Childers (1964) in their study of the penetration of alfalfa and red clover reported that penetration of the host was beneath the infection cushion but they did not report the presence of infection pegs and their photographs did not show them to be present. Dodman et al. (1968b) examined the modes of penetration of several isolates of R. solani on bean and radish. They found that on bean hypocotyls, numerous fine infection pegs, developed at the bases of the cushions, penetrated the cuticle. On radish hypocotyls, they suggested that initial penetration occurs at the centre of the cushion by hyphal tips forcing their way between epidermal walls without any formation of constricted infection pegs. No concrete explanation was offered to account for host penetration without the formation of infection pegs.

From the above account, it is clear that there are

two methods of penetration of intact host surface from infection cushions; one in which the cushion acts as multiple appressoria with fine infection pegs and the other in which it acts in an unknown manner and allows the entry of hyphae without constriction of their diameters.

(iii) Stages after penetration.

This part of the pathogenesis of plant pathogens has attracted the most attention from plant pathologists due probably to the development, at this stage, of characteristic symptoms of the disease. The importance of this stage of pathogenesis is widely accepted since the effect of the pathogen on the host is greatest. Studies on a number of host plants and fungal strains combinations are available with diseases caused by T. cucumeris. Taking into consideration the large variety of plant diseases caused by T. cucumeris, many more combinations of host plants and fungal strains must be studied before any definite conclusions can be made about the behaviour of the pathogen within its host.

Abdel-Salem (1933) studied the infection of a lettuce and a tomato strain of R. solani on lettuce. He found that the hyphae of the lettuce strain were intercellular and only very occasionally intracellular.

There was no disorganization of tissue in advance of the hyphae. In contrast, the hyphae of the tomato strain were mainly intracellular and lethal action was detected in advance of hyphal penetration, in the presence of collapsed disorganized tissue. The hyphae within the tissue in both cases were hyaline. The pathogen colonized the cortex, phloem and vessels of the seedlings. Flentje (1957) working with crucifer, composite and solanaceous stem strains of Pellicularia filamentosa and their respective hosts, found that after entry, the hyphae rapidly spread through the cortex both inter- and intra-cellularly. Infection was confined to the cortex. The root strain spread through the cortex mainly intracellularly. Collapse and breakdown of host cell contents occurred at or just behind the advancing hyphal tips of both the stem and root strains of the pathogen. Christou (1962) investigating the host-parasite relationship of R. solani in bean seedlings reported that after entry into the host, hyphae rapidly invaded the epidermal cells and the outer layers of cortical cells, mostly intracellularly. From these heavily infected cells, the remainder of the cortex was colonised mainly intercellularly. Invading hyphae were usually stopped at a depth of 1-4 cell layers from the epidermis and there was no invasion of the endodermis, vascular

elements and the pith. At completion of infection, sclerotia of various sizes developed in the infected tissue. A root strain from another host when tested on bean seedlings invaded only a few epidermal and cortical cells around the infection site, mostly intracellularly.

Chi and Childer (1964) reported that after entry into alfalfa, hyphae of Pellicularia filamentosa colonised the whole of the cortex, the stele and the pith. Colonization was both inter- and intra-cellular. Invaded cells sometimes collapsed and the tissues became compressed. Sclerotial tissues were found in the cortex and the pith but not in the stelar tissue. Khadga et al. (1963) studied the infection of cotton seedlings by R. solani and found that after entry into the host, hyphae tended to grow in the space in between the epidermis and the cortex causing the separation of the epidermis from the cortex and eventual distruction of the epidermis at the site of entry. The hyphae were intercellular in the first three or four outer layers of cortical cells and their growth inwards was mostly intracellular. Often, the tissue in advance of the invading hyphae showed granulation. Phloem, cambium and pith but not xylem tissues were attacked.

Materials and Methods.

A. Inoculation method for studying the mechanism of penetration.

The glass slide method employed for testing the pathogenicity of T. cucumeris isolates was found to be unsuitable for studying the mechanism of penetration beneath the cushions since superficial hyphae obscured the direct viewing of the infection cushions. Also, when infection cushions are numerous it is difficult to determine from which cushion penetration has occurred. A number of methods were investigated and a modification of the glass slide method was found suitable. Ten day old radish seedlings grown in U.C. mixture in a growth cabinet were used. The radish seedlings were strapped onto glass slides, one per slide, and inoculated with a 2 mm cube of agar bearing actively growing mycelium of a stem strain of T. cucumeris by placing the inoculum directly on the hypocotyl (Fig. 8a). After about ten hours incubation under moist conditions in a growth cabinet, infection cushions at different stages of development were seen (Fig. 8b).

B. Electron microscopy.

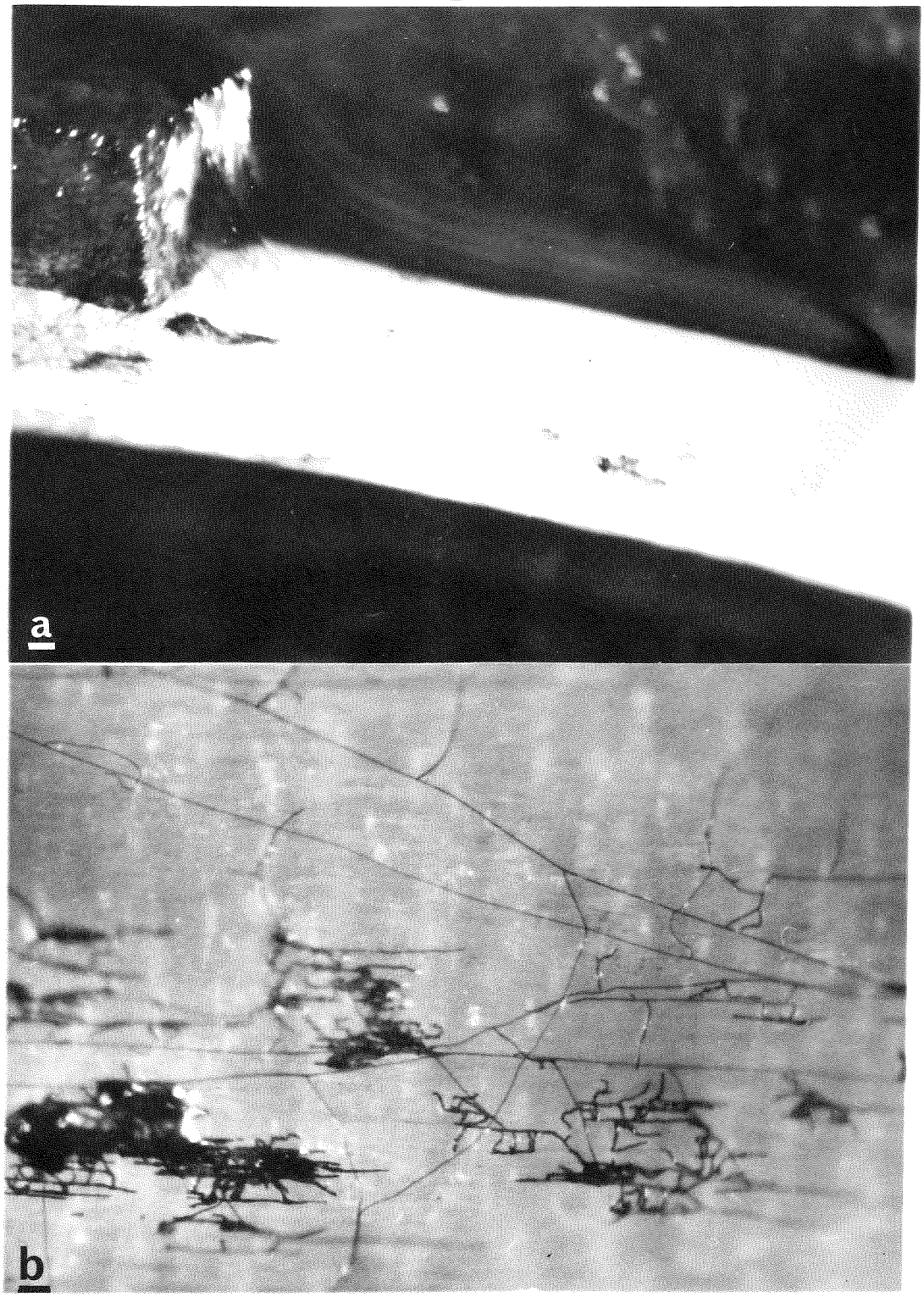
The general techniques used in electron microscopy were those recommended in the book "Techniques for

Fig. 8: Inoculation method used for studying the fungal cushion morphology and the mechanism of host penetration beneath the infection cushions. The specimen was stained with 0.1% trypan blue in 6% acetic acid and photographed using reflected light.

(a) Inoculum shown at left top corner of photograph. (x100.)

(b) An enlargement of the fungal organization on the host surface as shown in (a). (x200.)

Fig. 8



Electron Microscopy" edited by Kay (1965).

(1) Surface replicas.

Radish seedlings of various ages were grown in U.C. mixture in a growth cabinet. Care was taken not to disturb any part of the surface of the hypocotyl to be used for examination. Small longitudinal pieces of epidermis were carefully removed from the hypocotyl using a sharp scalpel under a dissecting microscope. Each epidermal strip was arranged with the cuticular surface upwards at right angles to the length of the glass slides. The ends were then secured with two lengths of adhesive tape. Replicas of the host surfaces were prepared before and after removal of the host surface waxes. The waxes were either removed by dipping epidermal pieces into chloroform or by passing them through increasing concentrations of ethyl alcohol to xylene and then through decreasing concentrations of alcohol back to water. Replicas were also prepared of T. cucumeris hyphae growing on the surface of radish hypocotyls. The following replica techniques were used.

(a) Double stage replica (negative replication).

A small amount of methyl acetate (A.R. grade) was applied onto the surface to be replicated. A piece of

acetyl cellulose replicating film was immediately placed over the specimen from one end so that no bubbles were caught underneath. When the solvent had evaporated completely the acetyl cellulose film was removed from the specimen using a pair of forceps. The film was then fastened with adhesive tape to a glass slide, placed in a vacuum evaporator, shadow-casted longitudinally at an angle of 23° with half a pellet of platinum-carbon at a pressure of 6×10^{-5} mm Hg and backed by a light carbon film from a vertical source. The replica was cut into sizes suitable for mounting on grids. Pieces were transferred, with evaporated film downwards, to a warm glass slide smeared with a thin layer of liquid wax. Replicas were held in position by cooling the wax. The acetyl cellulose was removed with methyl acetate and then the wax was removed with xylol. Replicas were picked up with new specimen grids.

(b) Single stage replica (true replication).

The specimen was shadow-casted longitudinally with platinum-carbon and backed with a thin carbon film in the same way as for the double stage replica. Replicas were removed using one of the following two methods.

(1) Collodion backing: Repeated application of 2% collodion in amyl acetate resulted in a firm film over

the replica which was then stripped off the specimen. The procedure for separation of the replica from the collodion was similar to that described for the double stage replica except that amyl acetate was used.

(2) Plastic backing (Juniper & Bradley 1958): The replica was first backed with Formvar by application of 2% formvar in chloroform and a second backing was applied with Bedacryl 122X (I.C.I.) at a concentration of 5-7% in benzene. When dried, the combined plastic-replica film was stripped from the specimen using adhesive tape. The Bedacryl was removed with acetone. The replica was then supported on grids before the formvar backing was removed with chloroform.

(ii) Ultra-thin sections.

Half millimeter portions of infected tissue were fixed by one of the following treatments:

(a) Caulfield's buffered osmium tetroxide fixative with sucrose (see Appendix) for one and a half hours at 0-5°C.

(b) Millonig's phosphate buffered osmium tetroxide fixative (see Appendix) for one and half hours at 0-5°C.

(c) Luft's buffered permanganate fixative (see Appendix) for one hour at 0-5°C.

(d) Phosphate buffered paraformaldehyde fixative (see Appendix) for two hours at 0-5°C and then post fixed in Caulfield's buffered osmium tetroxide fixative with sucrose for one and a half hours at 0-5°C.

Fixed materials were washed briefly (approx. ten minutes) in the appropriate buffer, dehydrated with ethanol series according to Glauert (1965), passed through propylene oxide and embedded in Araldite (CIBA Ltd). Sections were cut with glass knives on a "Si-Ro-Flex" ultramicrotome and floated on water. Grey to silver sections were stretched with chloroform, picked up on collodion and carbon-coated grids and stained with Reynold's lead citrate (see Appendix) for 15 minutes.

Specimens were examined using a Phillip EM 100B electron microscope at 80 kV for replicas and 60 kV for sections. Photo-micrographs were taken on Ilford fine grain positive film.

(iii) Lead infiltration into epidermal cell wall at the sites of fungal cushion formation.

Ten millimeter lengths of radish hypocotyls each bearing a few small infection cushions, were pre-fixed in 3% glutaraldehyde in 0.1M phosphate buffer, pH 7.1 for two hours, washed with the buffer, transferred to

Reynold's lead citrate solution (see Appendix) for 15 minutes and washed with the buffer. The ends were removed and portions of 2 mm length bearing cushions from which penetration had not taken place, were fixed in osmium tetroxide, embedded in araldite and sectioned as above. Sections were examined unstained. Some of the sections were shadowed with carbon at an angle of 23°.

C. Light microscopy.

(i) The behaviour of the pathogen on host surfaces.

Cucumber, lettuce, pea, radish, tomato, turnip and wheat seedlings, 7-10 day old, were inoculated with T. cucumeris isolates using the glass slide method (see Part I). The number of seedlings was adjusted so that enough were available for periodical removal for examination up to a period of three days after inoculation. Specimens were examined, first unstained and then stained with 0.1% trypan blue in 6% acetic acid, using a dissecting microscope at magnifications up to X 100. Infection cushions or fungal aggregates on the surface of the plants were gently dislodged using a needle to determine whether or not penetration had occurred beneath.

(ii) Inoculation with sclerotia.

Sclerotial cells are very similar to the morphology

of the cells of the fungal cushions and there is little difference between the morphology of sclerotia formed on the host surface and infection cushions. To determine whether or not sclerotia could act as infection cushions, radish hypocotyls were inoculated by placing sclerotia of a stem strain of T. cucumeris onto the hypocotyls, incubated for two days under moist conditions and then examined using a dissecting microscope.

(iii) Preparation of sections from wax-embedded tissue.

Portions of fresh hypocotyl, 2-5 mm in length, were fixed in Newcomer's fluid (see Appendix) for 2 hours. Fixed tissue were dehydrated, cleared in tertiary butyl alcohol and embedded in wax using the method of Johansen (1940). Longitudinal and transverse sections, 10-15µ thick, were cut using a rotary microtome. Sections were attached to glass slides using 2% serum albumin, dewaxed with xylol and hydrated (Johansen 1940). The staining technique is a modification of the periodic acid-Schiff's method described by Jensen (1962). Sections were placed first in 2% periodic acid for 15 minutes and then in Feulgen's reagent (see Appendix) for 30 minutes. Excess Feulgen's reagent was removed by washing in running tap water for two minutes. Sections were dehydrated by being passed through a graded alcohol

series, counter-stained with 1% alcoholic fast green for ten seconds, differentiated in clove oil, cleared in xylol and finally mounted in euparol.

Sections were examined microscopically. Photomicrographs were taken with a Leica camera using Adox KB 17 film which was developed with Adox Neofin blue.

(iv) Examination of radish hypocotyls which were injured prior to inoculation.

Ten day old radish seedlings, grown in U.C. mixture in the growth cabinet, were strapped to glass slides using rubber bands. Punctures, 1cm apart, were made along the hypocotyls with the tip of a sterile needle under a dissecting microscope. Residual epidermis over the punctures was removed. The hypocotyls were inoculated by placing a 2 mm cube of agar bearing a stem isolate of T. cucumeris in between punctures. Inoculated plants were incubated in a growth cabinet under moist conditions and examined at regular intervals. Any infection cushions that were formed at the edge of the punctures were removed. Two days after inoculation, the seedlings were stained with 0.1% trypan blue in 6% acetic acid and examined under the dissecting microscope. Portions of the hypocotyls containing hyphae within the

punctures were fixed in Newcomer's fluid and wax-embedded sections were prepared as above.

(v) Examination of host tissues which were not injured prior to inoculation.

(a) Hypocotyl. Ten day old radish seedlings, grown in U.C. mixture, were inoculated with a stem strain of T. cucumeris using the glass slide method. Free hand sections were prepared from material showing different stages of disease development for preliminary examination. For detailed examination 2 mm portions of diseased materials were fixed in Newcomer's fluid (see Appendix) and wax-embedded sections prepared as above.

(b) Root. Wheat and radish roots were inoculated with a stem or root strain of T. cucumeris using the test tube method (see Part I). Histological examinations were done with wax-embedded sections.

Results.

A. The growth of T. cucumeris on the surfaces of test plants and the modes of host penetration.

Cucumber:

Hyphae of all strains T. cucumeris tested grew randomly over the host surface. Cushion-like structures were formed. There was infection beneath a few cushions and penetration into the host was by way of stomata. Penetration via natural wound caused by adventitious roots was not seen.

Lettuce:

Mycelial growth of all strains on the host surface was random. Cushion-like structures were formed and penetration into the host beneath a few cushions was seen. Entry into the host via natural wounds caused by adventitious roots was seen when the host plants were inoculated with isolates 68, 69 and R10 but not with isolate 48. Penetration into the host via stomata was not seen.

Pea:

Growth of mycelium of all strains on the host surface was random. There were a number of cushion-like structures which were loosely attached and no penetration

beneath these cushions into the host was seen. Penetration into the host via stomata was frequent when inoculated with the stem strains. No such penetration was seen in the case of the root strain, R10.

Tomato:

Mycelium growth of all strains on the surface was sparse and the distribution of fungal hyphae was random. No cushion-like structures were formed. Hyphae were seen to coil around some of the hairs of the stems. A few attempts at penetration into the host via stomata were seen when the plants were inoculated with the stem strains but not the root strain.

Turnip:

All strains produced two types of hyphae on the surface of the host. Loose hyphae which grew in a random manner and hyphae that adhered closely to the host surface. The latter followed the lines of junction between the epidermal cells mainly along the longitudinal axis of the seedlings. Lateral branches of limited growth usually curled upon themselves or other hyphae forming an infection cushion. Infection was seen beneath cushions produced by the stem strains one and a half days after host pathogen contact. A few cushion-like structures were seen when turnip seedlings were

inoculated with the root strain, R10. No infection was seen beneath these cushions.

Wheat:

Most of the mycelium produced by all strains formed a loose mat over the outer-most leaf base. Hyphae were seen to coil around the epidermal hairs and to follow the junctions of epidermal cells (Fig. 9a). There were loose fungal aggregates. No host penetration was seen beneath these fungal aggregates. Cushion-like structures were formed on the ribs of the coleoptile and penetration into the stomata beneath these cushions were detected (Fig. 9c). Single hyphae were also seen to enter stomata (Fig. 9a) and the distribution of infection cushions closely followed the distribution of stomata on the ribs of the coleoptile (Fig. 9b).

Radish:

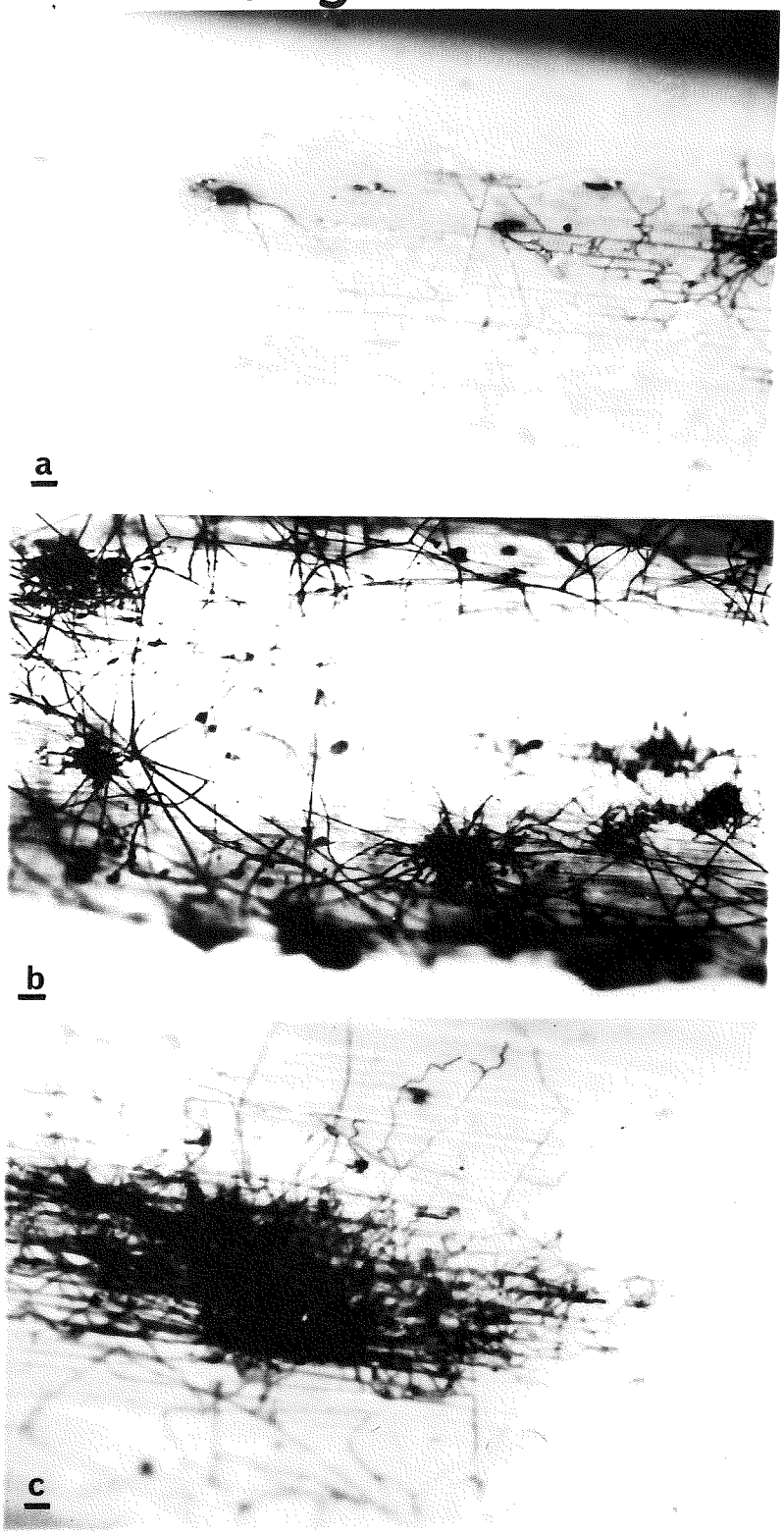
(i) Inoculated with isolate 48. Six hours after inoculation hyphae were seen to grow out of the agar inoculum and a number of hyphae had reached the host surface. 12 hours later, a thin mat of hyphae were seen on the side of the hypocotyl nearest the inoculum. A number of hyphae had grown over the hypocotyl. Some hyphae grew loosely over the host surface and others were closely appressed to the cuticle. Loose hyphae

Fig. 9: (a) Surface view of hyphae of isolate 68 on wheat coleoptile. Note the penetration of stomata by the hyphae. (x150.)

(b) Surface view of the distribution of infection cushions of isolate 69 on wheat coleoptile. Stomata are beneath the cushions. (x150.)

(c) Surface view of an infection cushion of isolate 68 on wheat coleoptile. Stomata is beneath the cushion. (x300.)

Fig. 9



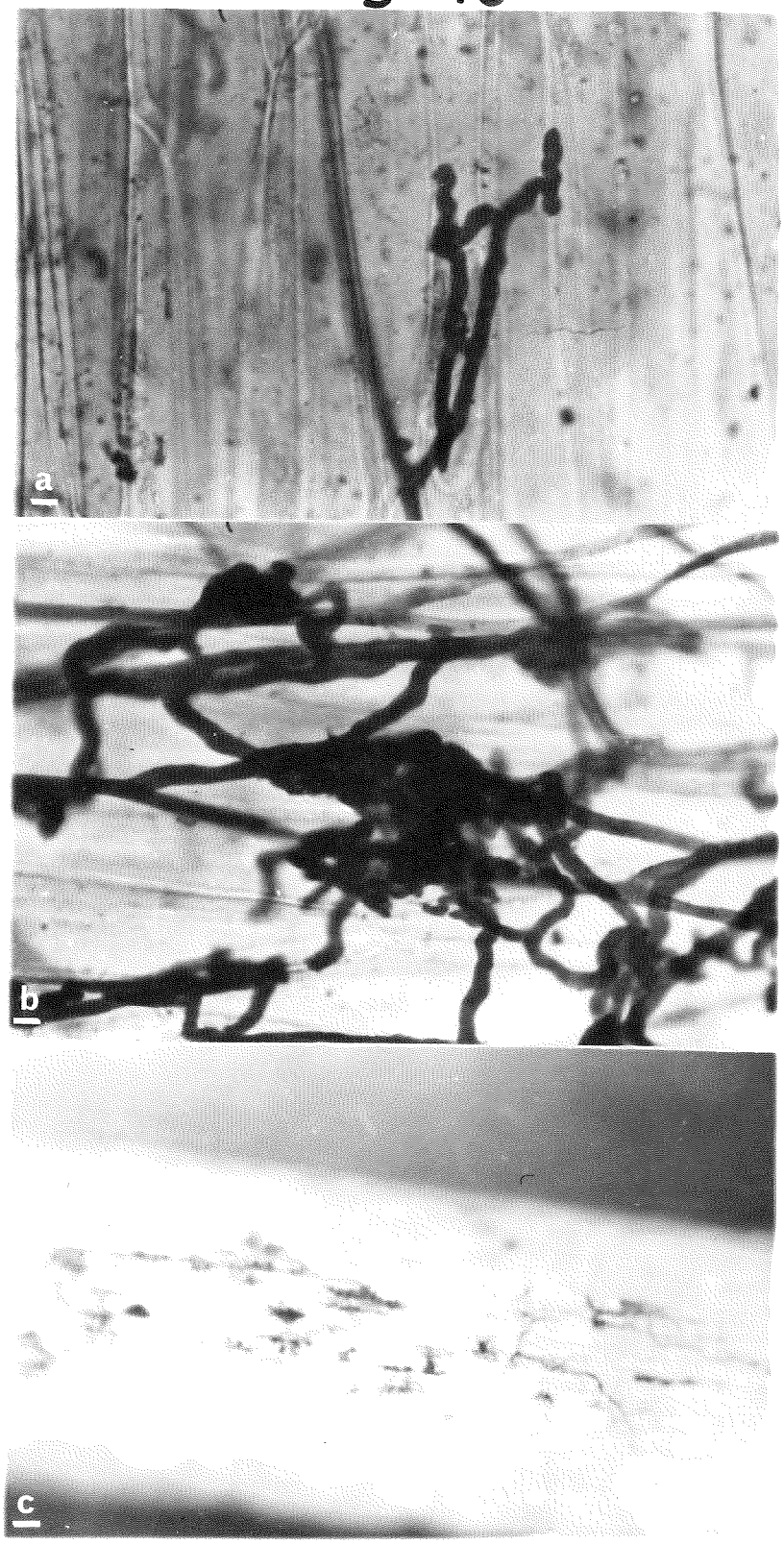
grew randomly and in some cases gave rise to hyphae that became appressed to the cuticle. Hyphae that were closely appressed to the host surface usually grew along the longitudinal grooves of the cuticle immediately above the junctions of the underlying epidermal cells. These hyphae were mostly fast growing and were called "runner" hyphae which gave rise to primary branches 0.4-1.6 mm behind the fungal tips and approximately at right angles to the main hyphae. These primary branches were either limited in growth similar to isolate 68 (Fig. 10a) or became "runner" hyphae. The primary branches usually grew over the sides of the underlying epidermal cells. Primary branches may or may not give rise to further branches. Inter-twining of repeatedly branched hyphae of limited growth resulted in fungal clumps termed infection cushions similar to isolate 68 (Fig. 10b). Side branches of one or more runner hyphae may give rise to infection cushions. Infection cushions of various sizes, similar to isolate 69 (Fig. 10c), were present. Infection took place beneath some of the cushions. There was no apparent discoloration or death of the host cells beneath the infection cushions before or immediately after penetration. Infection was not correlated with the sizes of the cushions. Infection took place beneath some small cushions and not underneath some big cushions. Injury to the epidermal hairs in

Fig. 10: (a) Surface view of isolate 68 on radish hypocotyl showing the initials of an infection cushion. (x560.)

(b) Surface view of a young infection cushion of isolate 68 on radish hypocotyl surface. (x560.)

(c) Surface view of the distribution of young infection cushions of isolate 69 on radish hypocotyl. (x150.)

Fig. 10



some cases induced the formation of infection cushions over the site of injury with the subsequent penetration of the host. Penetration into the host via stomata by single hyphae was rarely seen. The superficial parts of single hyphae which entered the host branched on the host surface and formed structures similar to infection cushions. A day after inoculation, a thick mat of hyphae was seen over the hypocotyl. There was an increase in the number of cushions and some of the cushions had coalesced to form chains. Water-soaked areas on the hypocotyls, due to gross injury to the host by the pathogen, were seen. Two days after inoculation, the hypocotyls collapsed.

(ii) Inoculated with isolate 68. Growth was similar to that of isolate 48 with a few exceptions. There were fewer "runner" hyphae and, therefore, a thinner mycelial mat on the host surface. There were also fewer infection cushions. Infection of the host was less severe than with isolate 48. Single hyphae were seen to enter stomata causing browning reactions in the surrounding tissue. However, entry via stomata was uncommon. Very frequently, hyphae were seen to grow over stomata without any effect (Fig. 11a). Two days after inoculation only parts of the hypocotyl collapsed.

(iii) Inoculated with isolate 69. Growth was similar to that of isolate 48 with a few exceptions. The infection cushions were formed of tighter fungal knots than in the case of isolate 48. Penetration of the host was seen beneath most of the cushions. Entry into the host via stomata by single hyphae (Fig. 11b) was rare. Hyphae were seen to grow over and near stomata without apparent effect on the hyphae. Stomata had been penetrated under a few cushions. Damage to the host was extensive.

Two days after inoculation with sclerotia, hyphae were seen to grow from the sclerotia onto the radish surface. Once on the surface of the hypocotyl, the hyphae grew in a characteristic manner and formed infection cushions (Fig. 11c). Host penetration was seen beneath these cushions. Only a small percentage of the sclerotial cells produced hyphae, those in contact with the hypocotyl surface and those on the surface of the sclerotia. The hyphae produced were mainly "runner" hyphae.

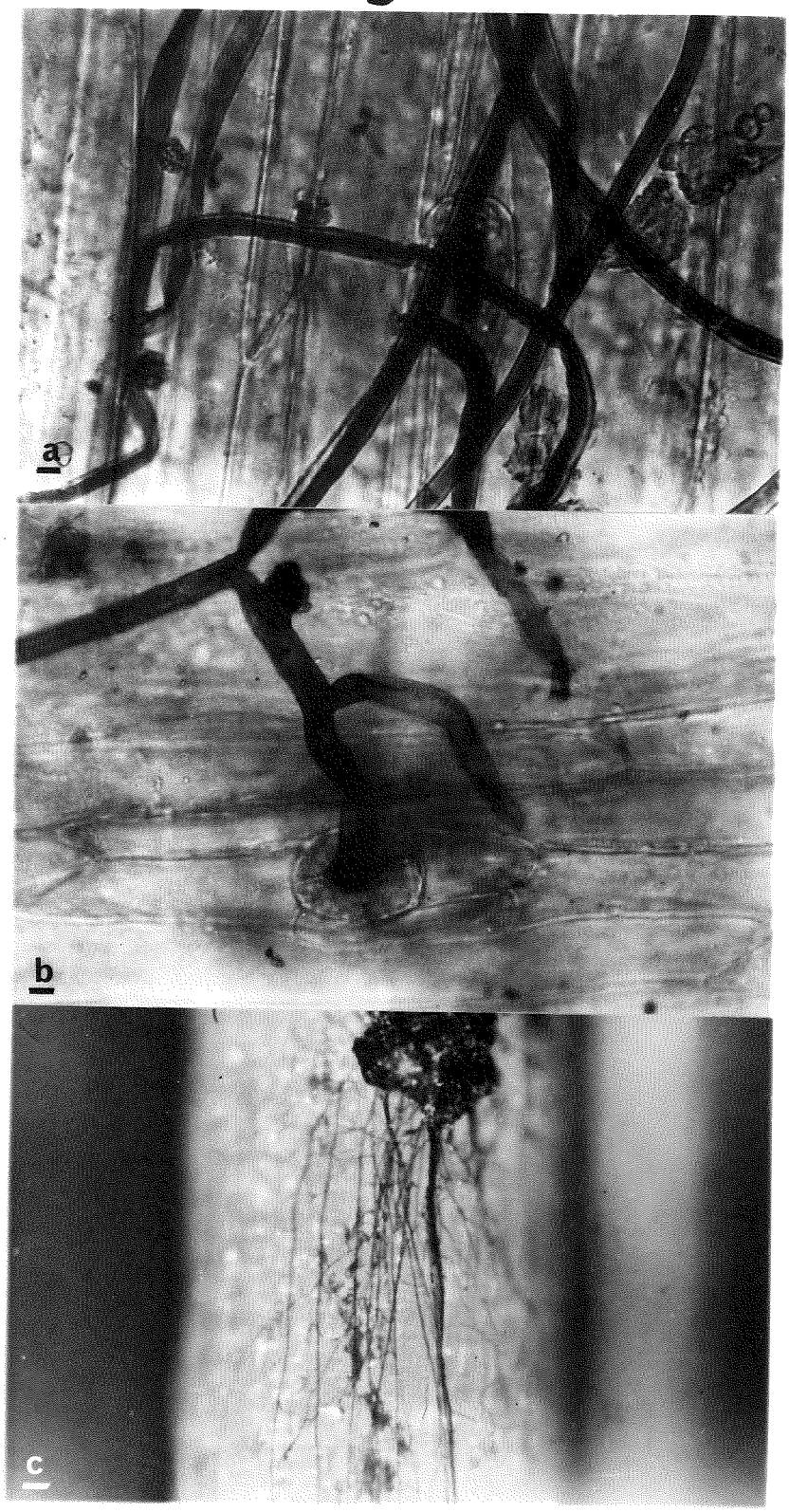
(iv) Inoculated with isolate R10. Twelve hours after inoculation, fungal hyphae were seen to grow out of the inoculum. A day after inoculation, hyphae were seen to be in contact with the host surface. A few of the

Fig. 11: (a) Surface view of a hyphae of isolate 68 lying on top of a stoma of radish hypocotyl. No apparent effect on the hyphae can be seen. (x560.)

(b) Surface view of a single hyphae of isolate 69 entering a stoma of radish hypocotyl. (x560.)

(c) Sclerotia of T. cucumeris, isolate 69, in contact with radish hypocotyl surface for two days. (x120, surface view.)

Fig. 11



hyphae followed junctions of the epidermal cells and behaved like isolate 48. Two days after inoculation, more hyphae were in contact with the host. A number of infection cushions were formed (Fig. 12a,b) and no penetration was seen beneath most of these cushions. When infection occurred, hypersensitive reactions were seen (Fig. 12c).

B. The fine structure of T. cucumeris on the surface of radish hypocotyl.

Platinum-carbon replicas of T. cucumeris hyphae on the surface of radish hypocotyls showed that the hyphal tip is rounded (Fig. 13) and the fungal surface is smooth in texture. At the site where the hyphal tip comes in contact with the host surface are many particles (probably mineral in origin). These particles may be held in position by a secretion from the hypha. The same secretion may account for hyphal attachment to the host surface.

Ultra-thin sections through hyphae of T. cucumeris growing on the surface of radish hypocotyls showed an outer amorphous coating (Fig. 14a, 15, 16a). This coating was either very thin or non-existent towards the apex (Fig. 14b) and foreign particles were embedded on its surface (Fig. 16a). The cell wall was thinnest

Fig. 12: (a) Surface view of the initials of an infection cushion of isolate R10 on radish hypocotyl surface. (x300.)

(b) Surface view of an infection cushion of isolate R10 on radish hypocotyl surface. (x150.)

(c) Surface view of an infection cushion of isolate R10 on radish hypocotyl surface causing hypersensitive reactions in the host. (x150.)

Fig. 12

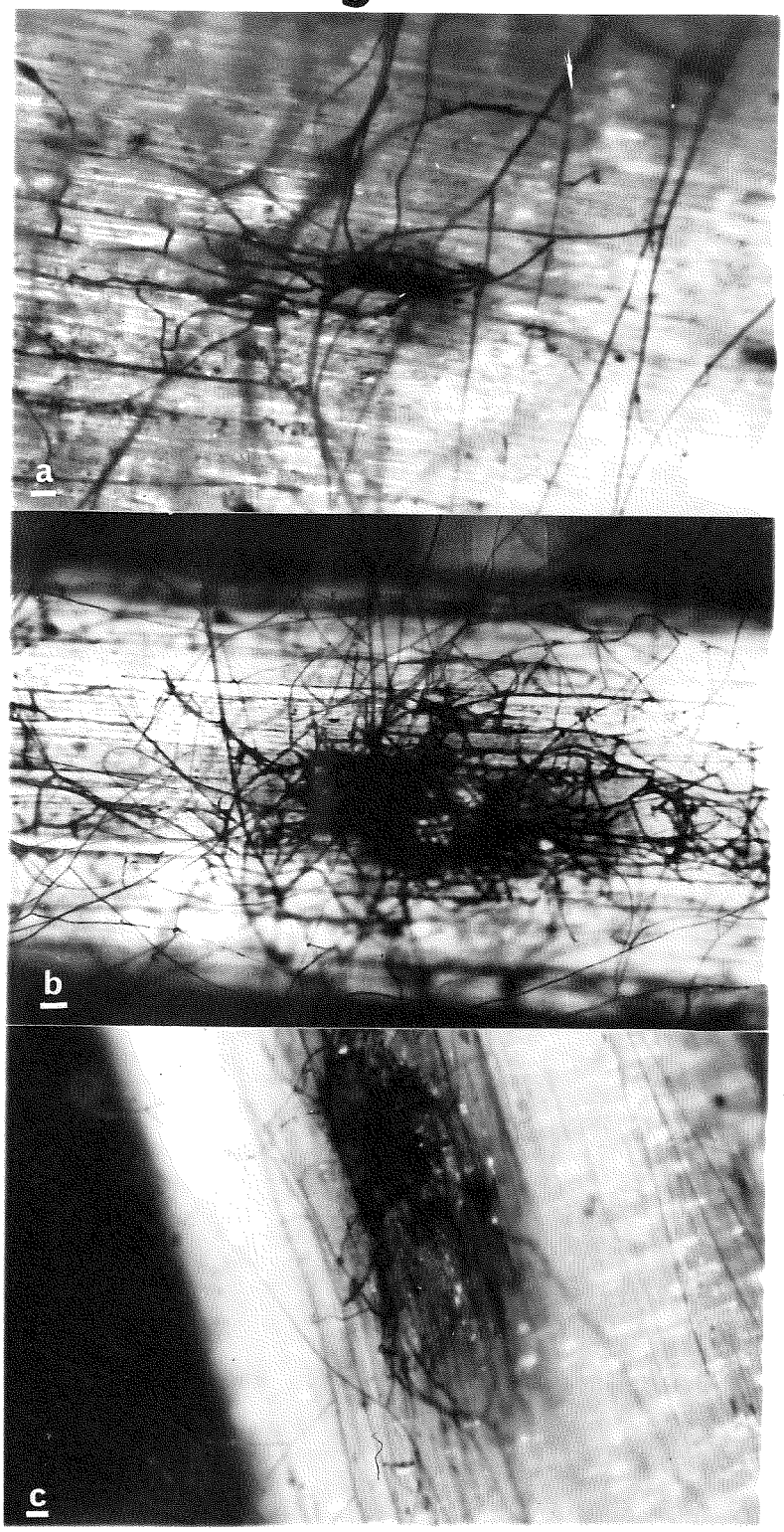


Fig. 13: Platinum-carbon replica of hyphal tip of T. cucumeris, isolate 69, on radish hypocotyl surface. Thick wrinkles on the hypha are probably due to drying during preparation of the replica. (x20,000.)

Fig. 13

Fig. 14: Ultra-thin sections through T. cucumeris, isolate 69, growing on radish surface.

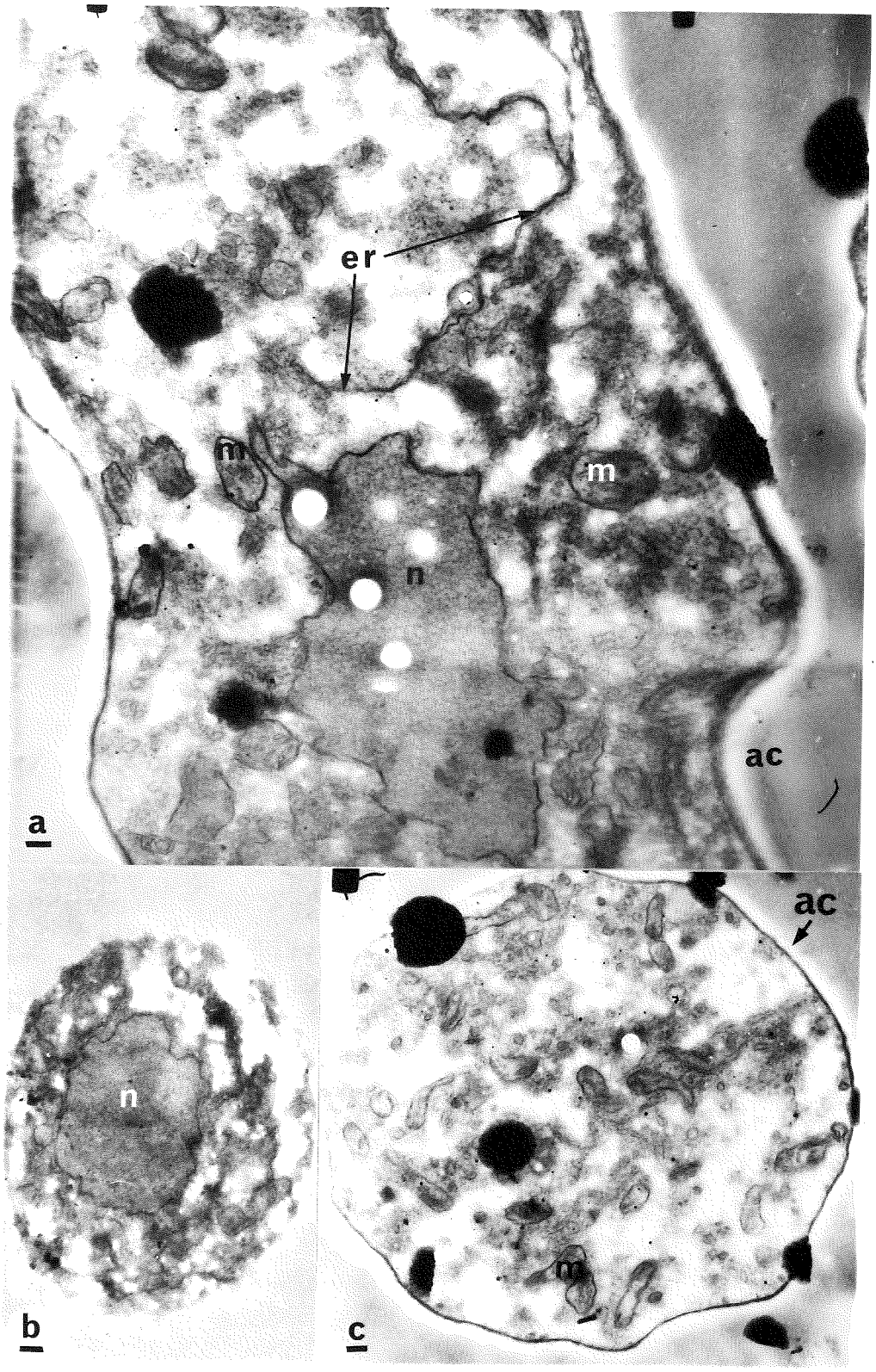
(a) Material fixed in OsO_4, stained with lead. (x28,000. L.S.)

(b) Material fixed in $KMnO_4$, unstained. (x10,000. T.S.)

(c) Material fixed in OsO_4, stained with lead. (x10,000. T.S.)

ac = amorphous coating
er = endoplasmic reticulum
m = mitochondrion
n = nucleus

Fig. 14



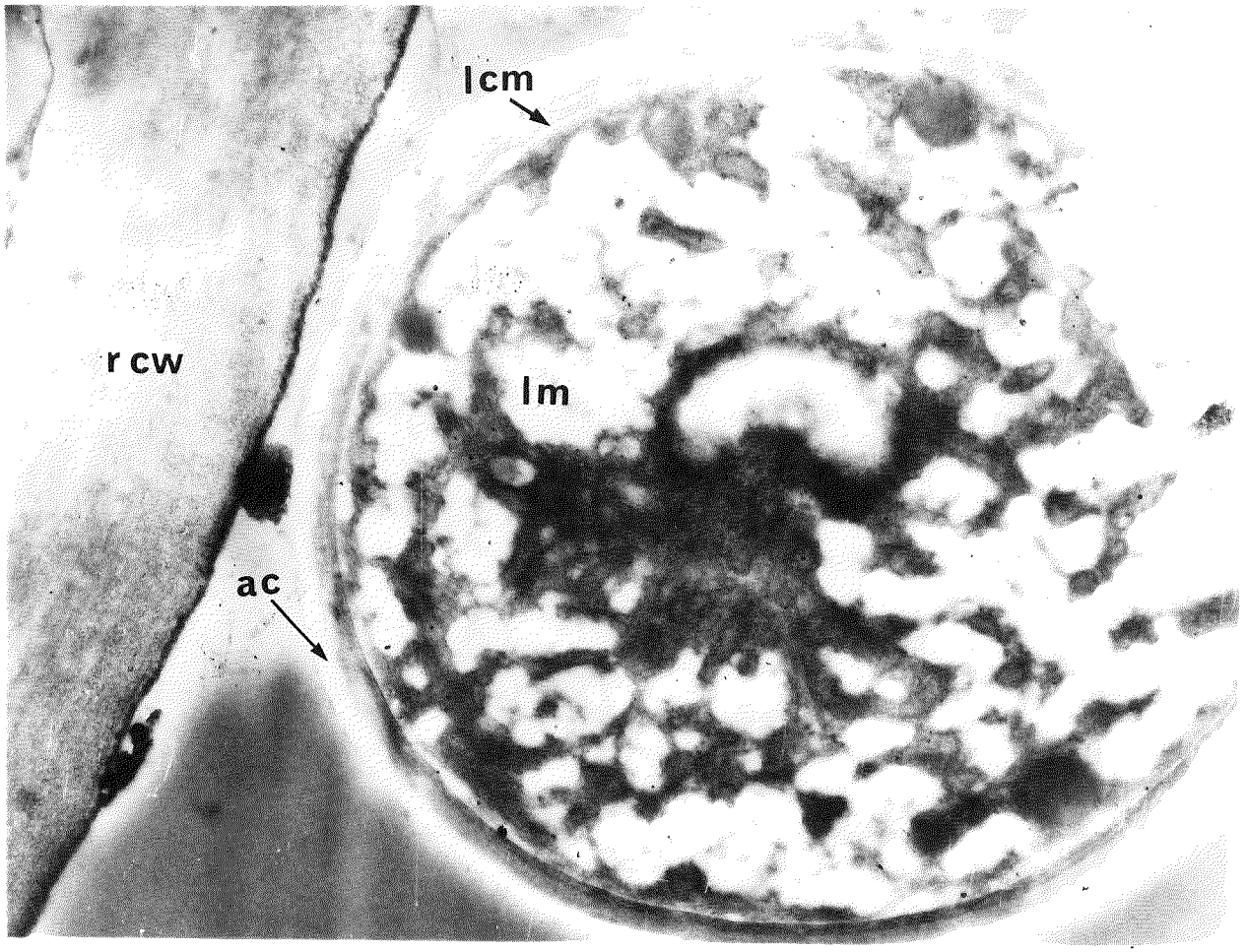
towards the hyphal tip (Fig. 14b). Its thickness increases away from the apex (Fig. 14a,c) or as hypha matures (Fig. 15, 17). The lateral cell wall consisted of electron dense lamellae. At intervals along the hyphae were septa. Each septum was highly complex and consisted of a cross wall and a central structure termed a dolipore (Moore & McAlear 1962) or septal apparatus (Bracker & Butler 1963; 1964). The cross wall was composed of two electron dense lamellated layers separated by an electron transparent central lamella (Fig. 16a). The electron dense layer is thinnest towards the centre and its thickness increases to a triangular thickening on joining the lateral wall (Fig. 16a,b). The dolipore consists of a dough-nut swelling near the centre of the septum surrounding the septal pore (Fig. 16a,c). On each side of the septal pore, there is an electron dense discontinuous membranous cap, termed the parenthesome (Moore & McAlear 1962). Cytoplasmic connections between adjacent cells are through the septal pore. No plasmodesmata were seen in the cross walls.

On both sides of the septum and internal to the lateral wall is the thin electron dense plasma membrane or the ectoplast membrane (Fig. 15, 16). In some areas between the ectoplast and the lateral wall or cross wall

Fig. 15: Ultra-thin transverse section through the hypha of isolate 48 growing on radish hypocotyl surface. Sections were fixed in OsO_4 and stained with Reynold's lead citrate. (x22,500.)

ac = amorphous coating
lcm = lateral cell wall
rcw = radish cell wall
lm = lipid material

Fig. 15



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are bodies of electron-transparent material (Fig. 16b) with or without vesicles or tubules of electron dense material. These electron-transparent bodies have been termed lomasomes (Moore & McAlear 1961).

The appearance of the cytoplasm near the hyphal tip (Fig. 14a) differed from older mature hyphae (Fig. 15). Near the hyphal tip, there was an abundance of mitochondria. These mitochondria were oval shaped, enclosed by double-membranes and had plate-like cristae. In mature fungal cells (Fig. 15) there was an abundance of lipid materials. However, there were many structures in common between the hyphal tip and the mature fungal cell. The nucleus (Fig. 14a) had a double membrane with pores and the nucleoplasm was granular. Besides mitochondria, lipid materials and the nucleus, the cytoplasm had endoplasmic reticulum (Fig. 14a), numerous vesicles, ribosomes and glycogen in alpha particles. The subcap matrix (Fig. 16a,c) within the bounds of the parenthosome was less electron dense than the ground cytoplasm of the rest of the cell even though it was continuous with it. This may be due to the low amount of ribosome and alpha particles of glycogen within the subcap matrix. Sections of fungal cells within the cushion show dense cytoplasm with a lot of alpha particles of glycogen (Fig. 17).

Fig. 16: Ultra-thin sections through the septa of *T. cucumeris* hyphae, isolate 48, on the surface of radish hypocotyl. Sections fixed in OsO_4 and stained with Reynold's lead citrate.

(a) x12,000, (b) x53,000, (c) x14,000.

ac = amorphous coating
cw = cross wall
d = dolipore
lo = lomasome
lcw = lateral cell wall
p = parenthesome
sm = subcap matrix
tt = triangular thickening

Fig. 16

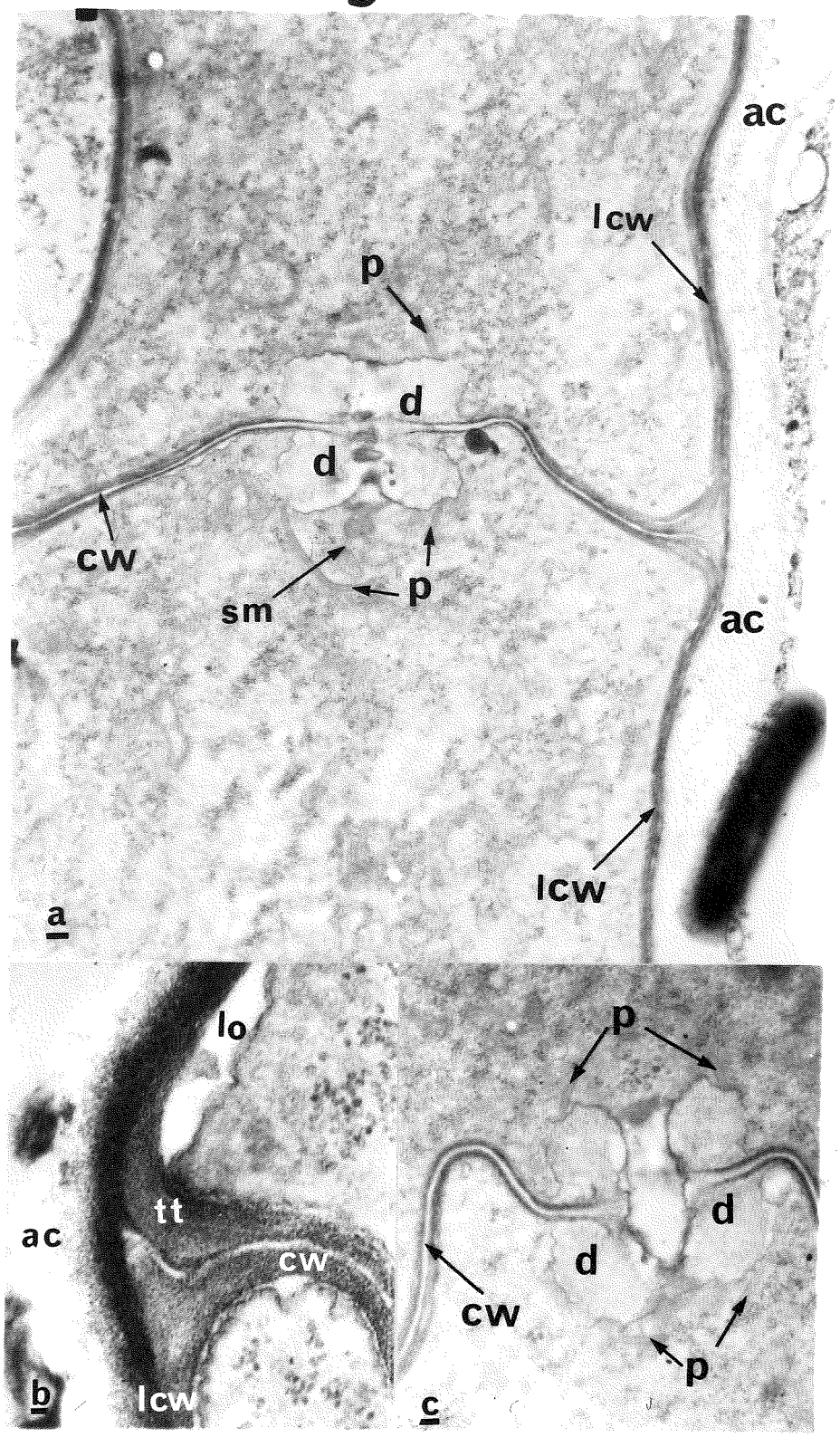
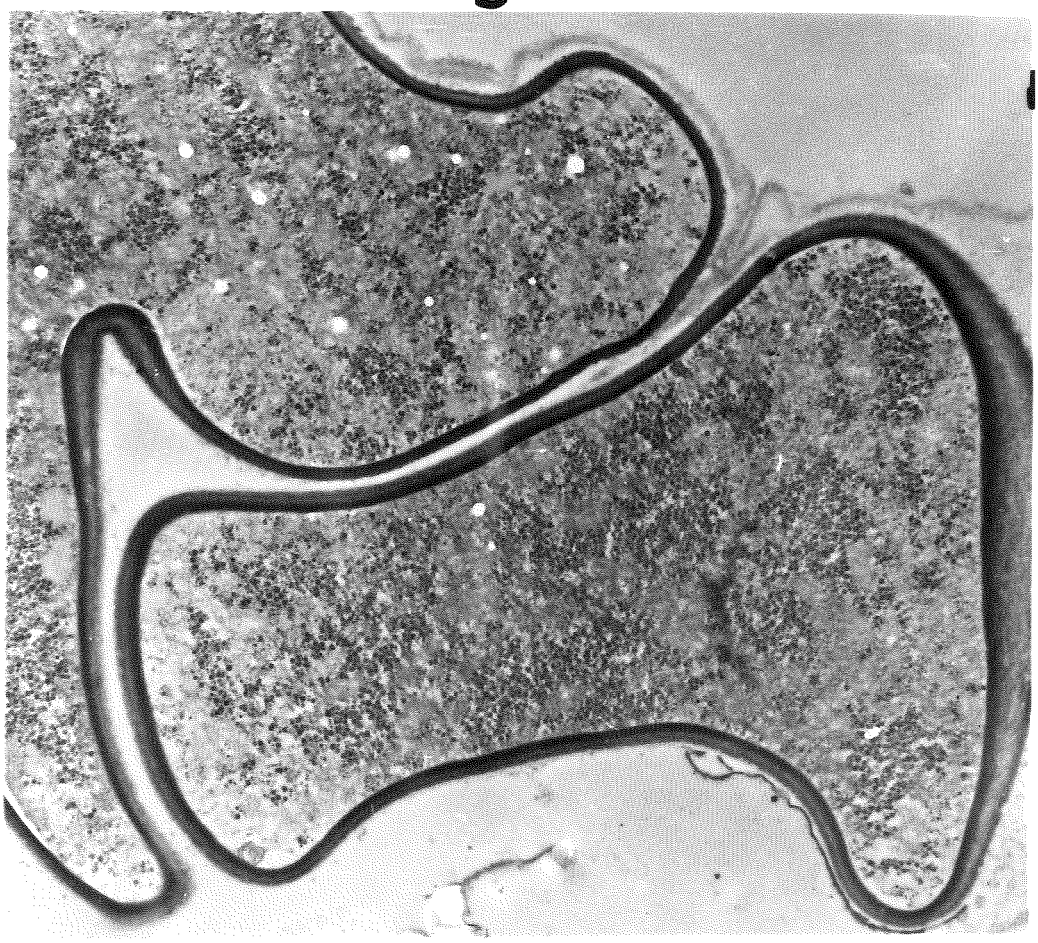


Fig. 17: Ultra-thin sections through infection cushion cells of isolate 48. Material fixed in OsO_4 and stained with Reynold's lead citrate. (x12,000.)

Fig. 17



C. Penetration into radish hypocotyls.

(i) The fungal cushion. Fungal cushions were examined in situ and after removal from the host surface. The parts of cushions in contact with the host surface consisted of compacted cells. The contour of surfaces of cushions in direct contact with the host follows closely the topography of the host surface (Fig. 18a).

Although sclerotia and fungal cushions are very similar in morphology wax-embedded sections, taken through areas of the radish hypocotyl in contact with the sclerotia, showed no penetration beneath the sclerotia (Fig. 18b, 19a).

(ii) The host surface. Pieces of radish epidermis stained with saturated alcoholic Sudan IV showed that the epidermal cells were long and tapering at both ends. The epidermal cells were covered with a thin cuticle which was stained pink. Occasional breaks in the cuticle were due to the presence of stomata. Small patches of orange to slightly reddish coloured material, probably waxes, were seen on top of the cuticle. The tapering ends of epidermal cells lie between the sides of two adjacent epidermal cells. Transverse sections through the ends of these epidermal cells show a small cell with two bigger cells on each side (Fig. 19b).

Fig. 18: (a) Side view of a detached infection cushion of isolate 68 formed on radish hypocotyl. (x600.)

s = surface that had been in contact with the host,

t = top of the cushion.

(b) Sclerotia of isolate 69 in contact with radish hypocotyl surface for two days. (x440, T.S.)

Fig. 18

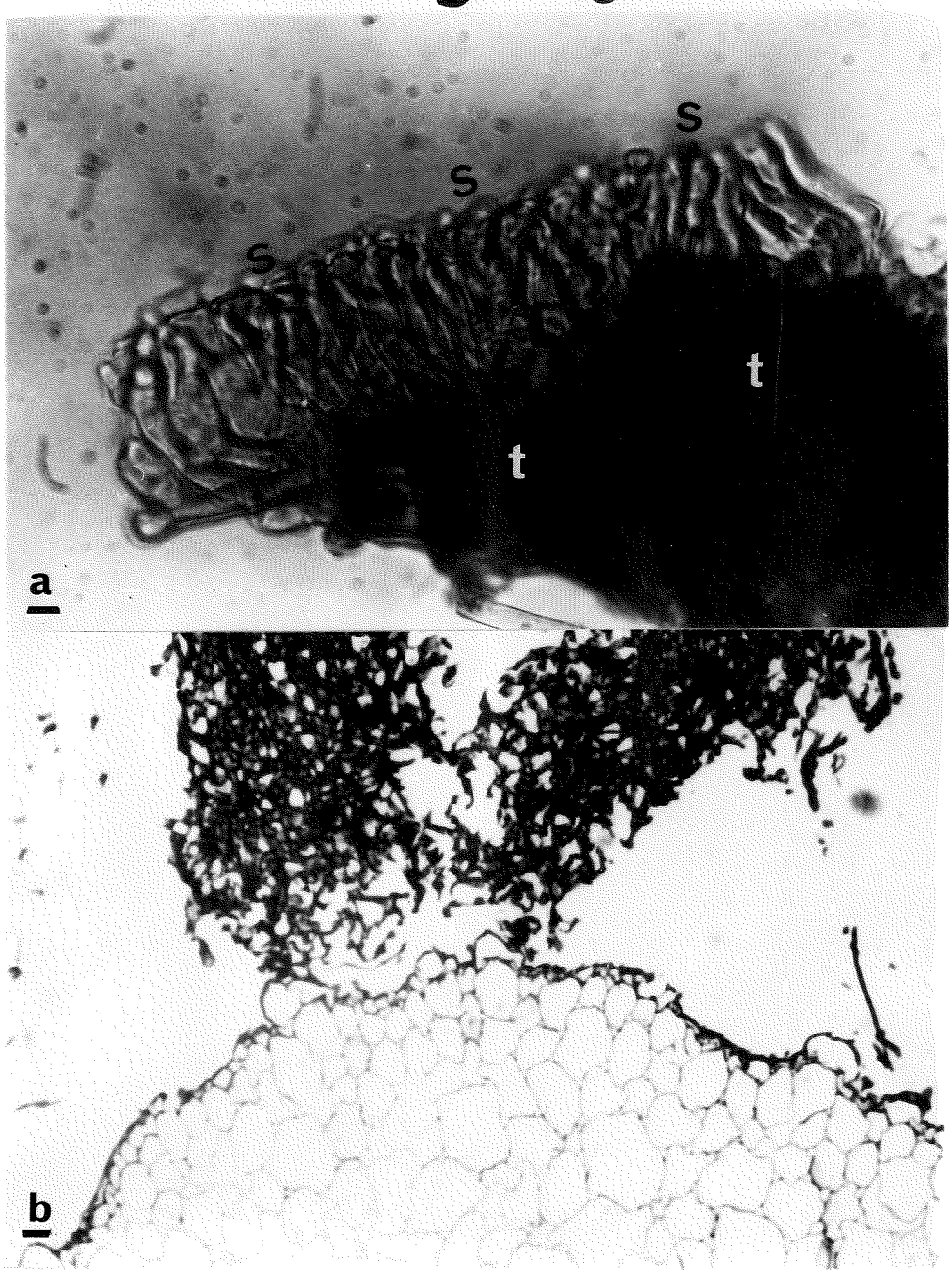
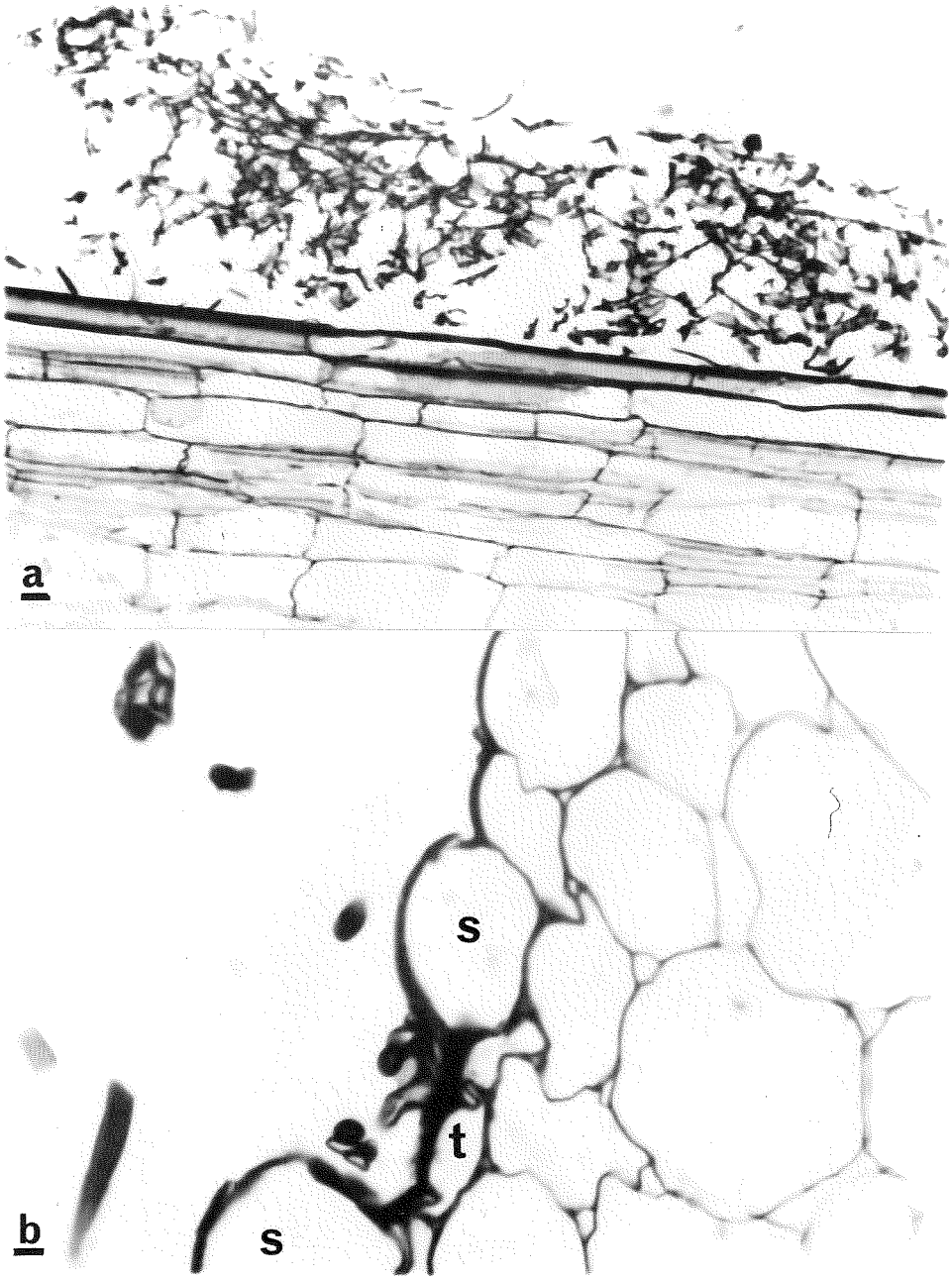


Fig. 19: (a) Sclerotia of isolate 69 in contact with radish hypocotyl surface for two days. (x440, L.S.)

(b) One of the many sites, on radish hypocotyls, which if occupied by an actively growing infection cushion would result in host penetration. (x600.) s, epidermal cells bordering the tapered end, t, of another epidermal cell.

Fig. 19



The importance of the positions of the ends of the epidermal cells in relation to the sides of the adjacent epidermal cells in the mechanics of host penetration by fungal cushions will be discussed later.

Platinum-carbon replicas of radish hypocotyl surfaces of one, two, four and six week old seedlings showed the presence of small irregular rossette-like wax platelets (Fig. 20). Wax platelets were sparsely distributed on one-week old radish hypocotyl surfaces. The density of these wax platelets increased as the seedling aged. The platelets almost covered the entire surface when seedlings were six weeks old. Whether or not a thin layer of wax covers the general surface is unknown. Treatment with xylene removed the rosette-like plates and the surface of the cuticle was smooth in some areas and granular in others.

Surface replicas of the sites of fungal cushion formation on the host surfaces were examined. These sites showed disorganisation of wax platelets, presence of bacteria and debris of mineral and organic origin (Fig. 21). After treatment with xylene, pits or pore-like structures (200-250 Å in diameter) in the host cuticle were seen at these sites of fungal cushion formation. Aggregates of pits were seen (Fig. 22) and these were either near (Fig. 22a) or away from (Fig. 22b)

Fig. 20: Carbon platinum replica of radish hypocotyl surfaces. (x35,000.)

(a) 1 week old seedling

(b) 2 week old seedling

(c) 4 week old seedling

(d) 6 week old seedling

ls = latex spheres

w = wax platelets

Fig. 20

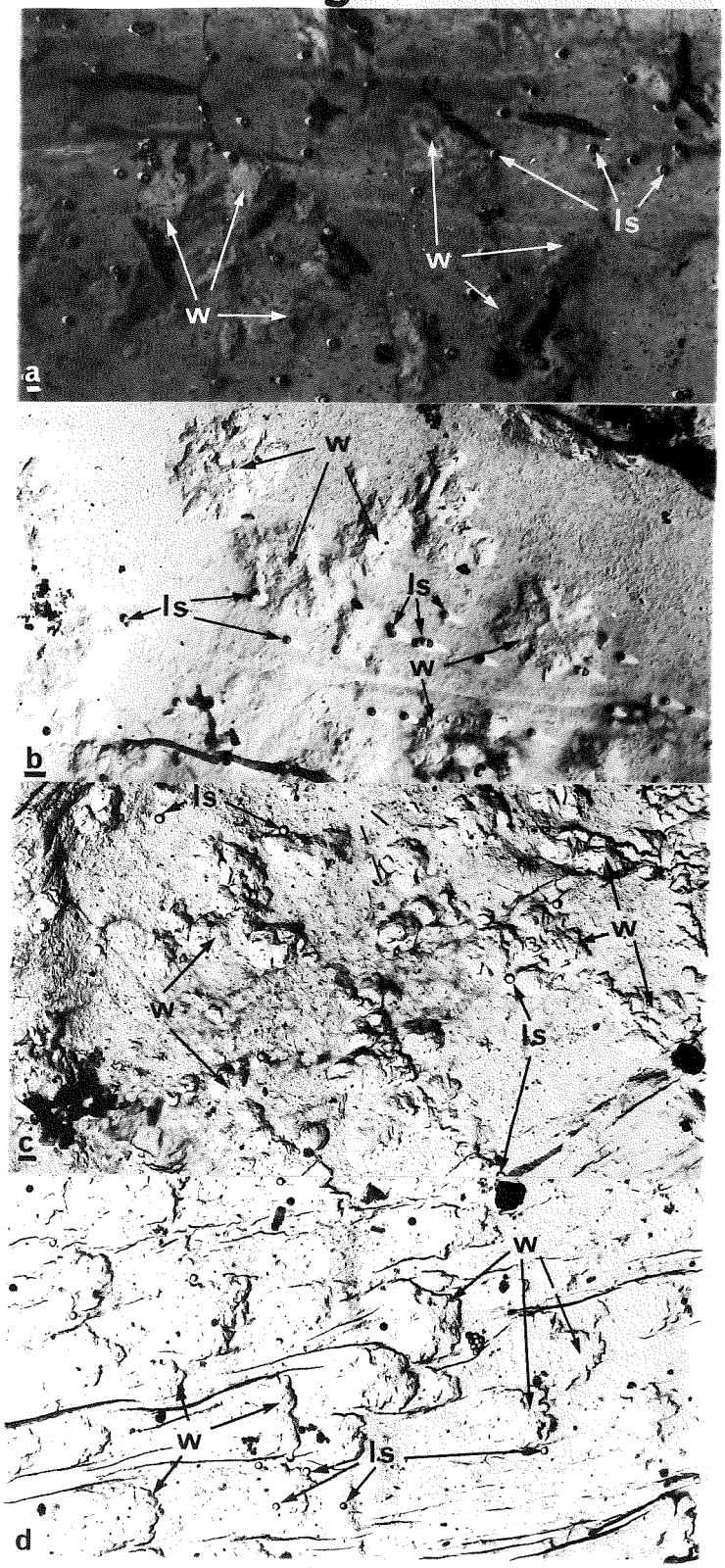


Fig. 21: Platinum-carbon replica of site of fungal cushion formation on radish hypocotyl surface. Note the disorganization of wax platelets, the presence of bacteria and debris of organic and mineral origin. (x8,000.)

b = bacteria
ls = latex spheres
w = wax platelets
o = organic debris

Fig. 21

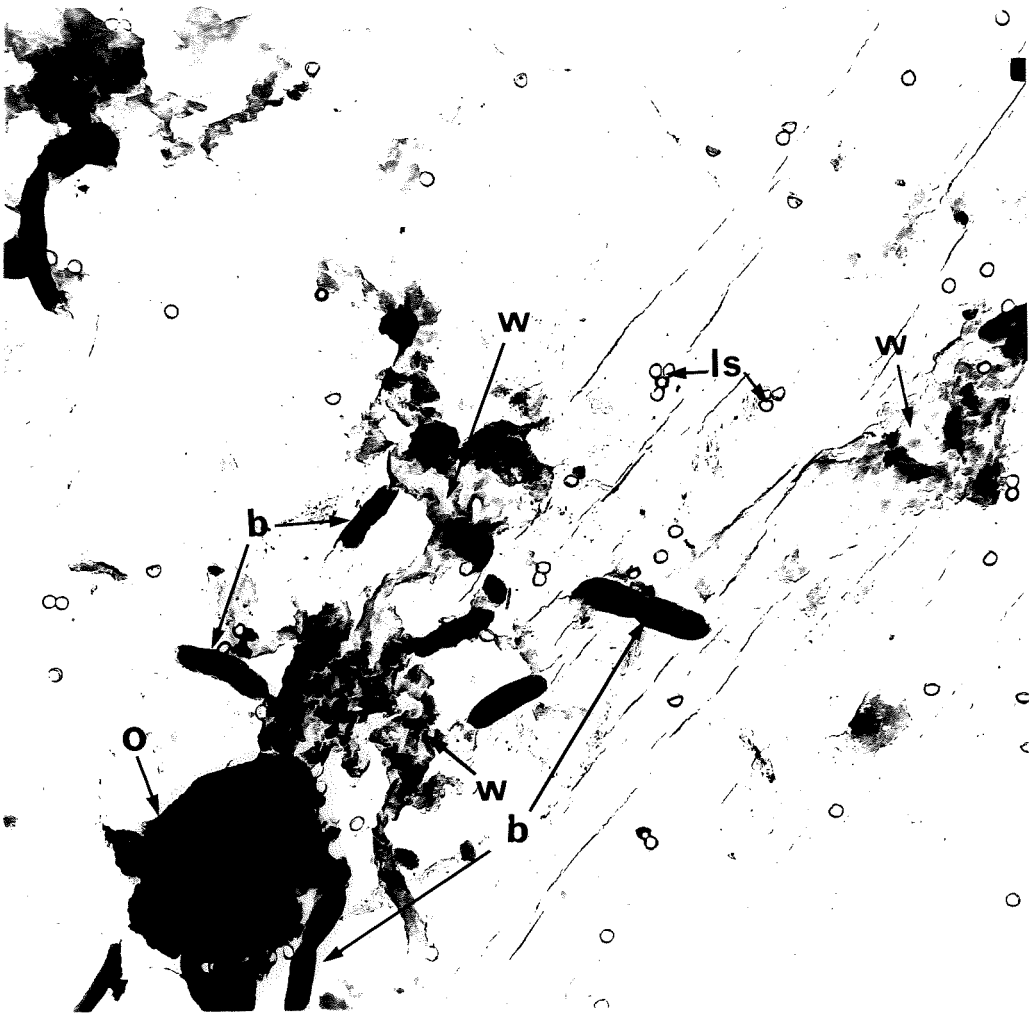


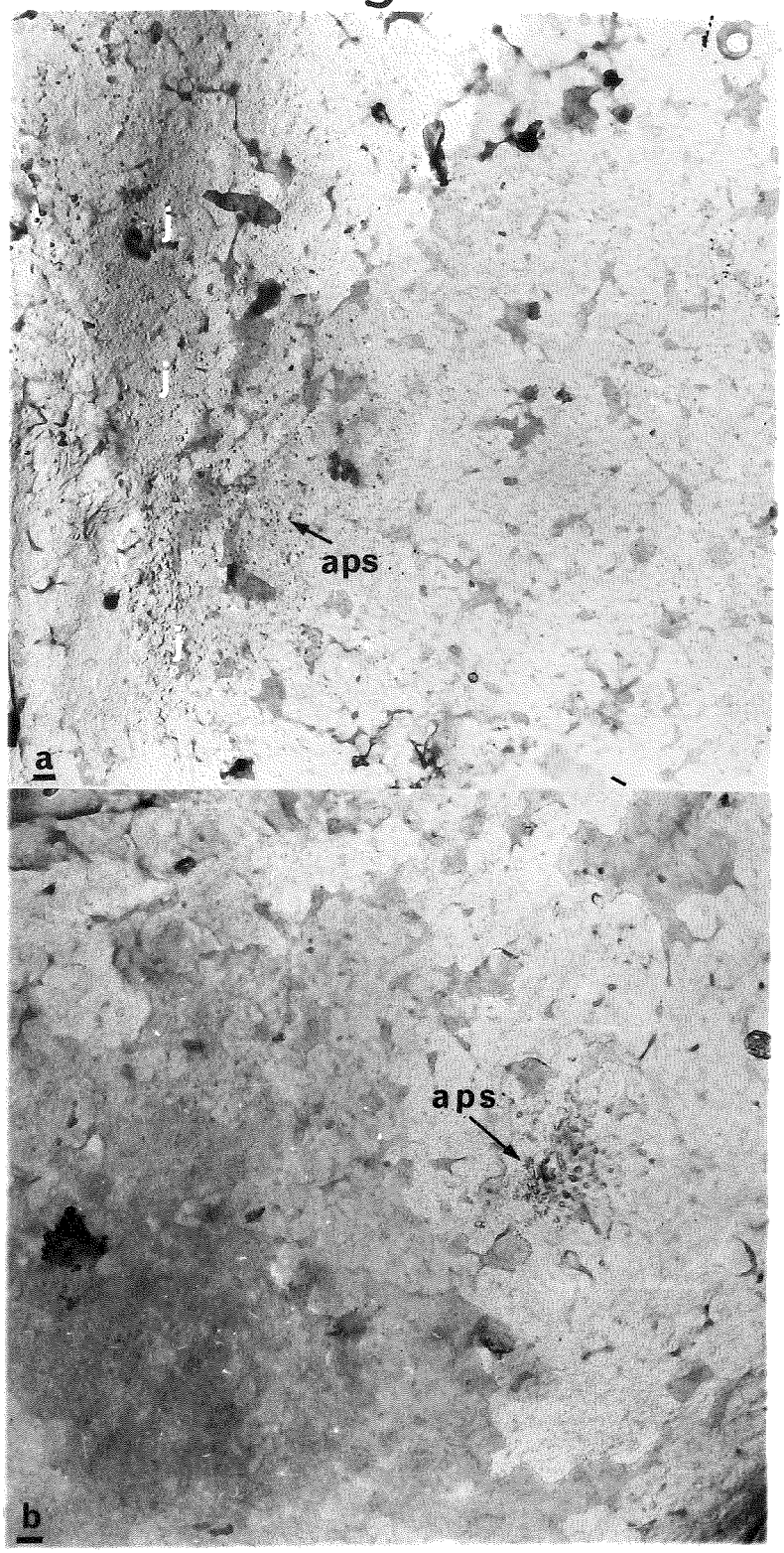
Fig. 22: Platinum-carbon replica of sites of fungal cushion formation on radish hypocotyl surface after the removal of wax by xylene to show the distribution of pits or pore-like structures.

(a) x12,000

(b) x20,000

j = cell junction
aps = aggregate of pore-like structures

Fig. 22



the junctions of epidermal cells. The pits were either associated with rough (Fig. 23a) or granular (Fig. 23b) surfaces. Aggregates of pits occupy an area measuring 0.5 to 1 micron in diameter. Ultrathin sections through the sites of fungal cushion formation showed that the cuticle was of uniform thickness and no obvious breaks in the cuticle to suggest pores were detected. This may be due to the absence of cuticular pores or if they were present, their diameters were smaller than the thickness of the sections or the sections missed the pores altogether.

Ultrathin sections were taken through the epidermal cells at the sites of fungal cushion formation after lead infiltration. These sections showed that parts of the outer cell walls contained more lead particles than other parts of the same cell wall (Fig. 24, 25). This indicates that parts of the cuticle covering the outer cell walls were more permeable to lead citrate solution than other parts of the cuticle. Cell junctions of epidermal cells contained more lead particles than other parts of the cell wall (Fig. 26). This suggests that the permeability of the cuticle to diffusing substances was greatest at cell junctions. Some of the sections were shadowed with carbon in an attempt to detect breaks in the cuticle. No such breaks were

Fig. 23: Enlargement of Fig. 22 to show the parts of the radish surface hypocotyl bearing pits or pore-like structures.

(a) x27,000.

(b) x40,000.

ps = pore-like structures

Fig. 23

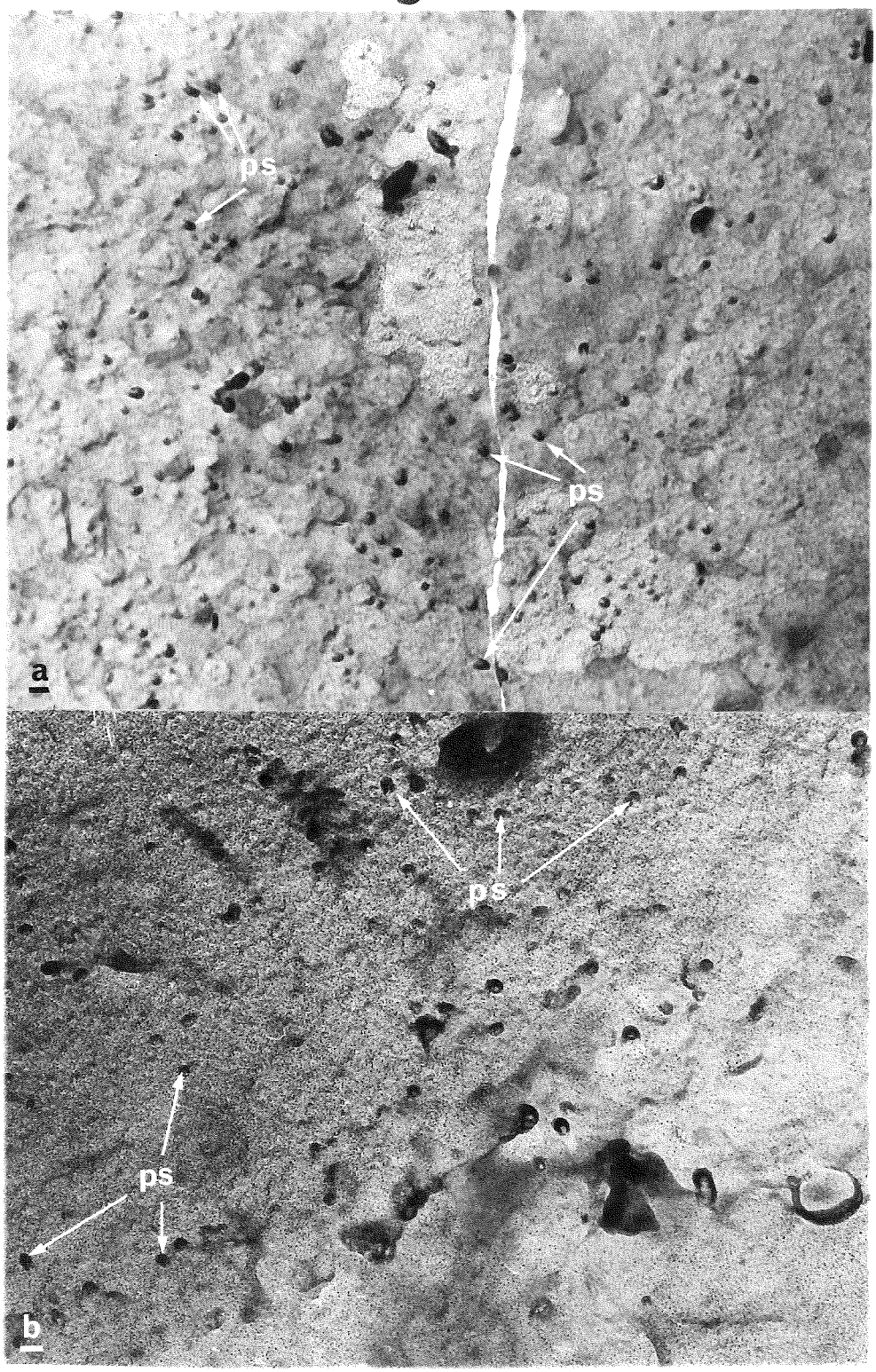


Fig. 24: Ultra-thin transverse sections of the outer wall of an epidermal cell of radish, infiltrated with lead, at the site of infection cushion formation. Note a greater amount of lead particles in the cell wall towards the top of the photograph. (x8,000.)

c = cuticle
p = part of cell wall with lead particles

Fig. 24

Fig. 25: Ultra-thin section of epidermal cell wall of radish infiltrated with lead. (x40,000.)

lp = lead particles

Fig. 25

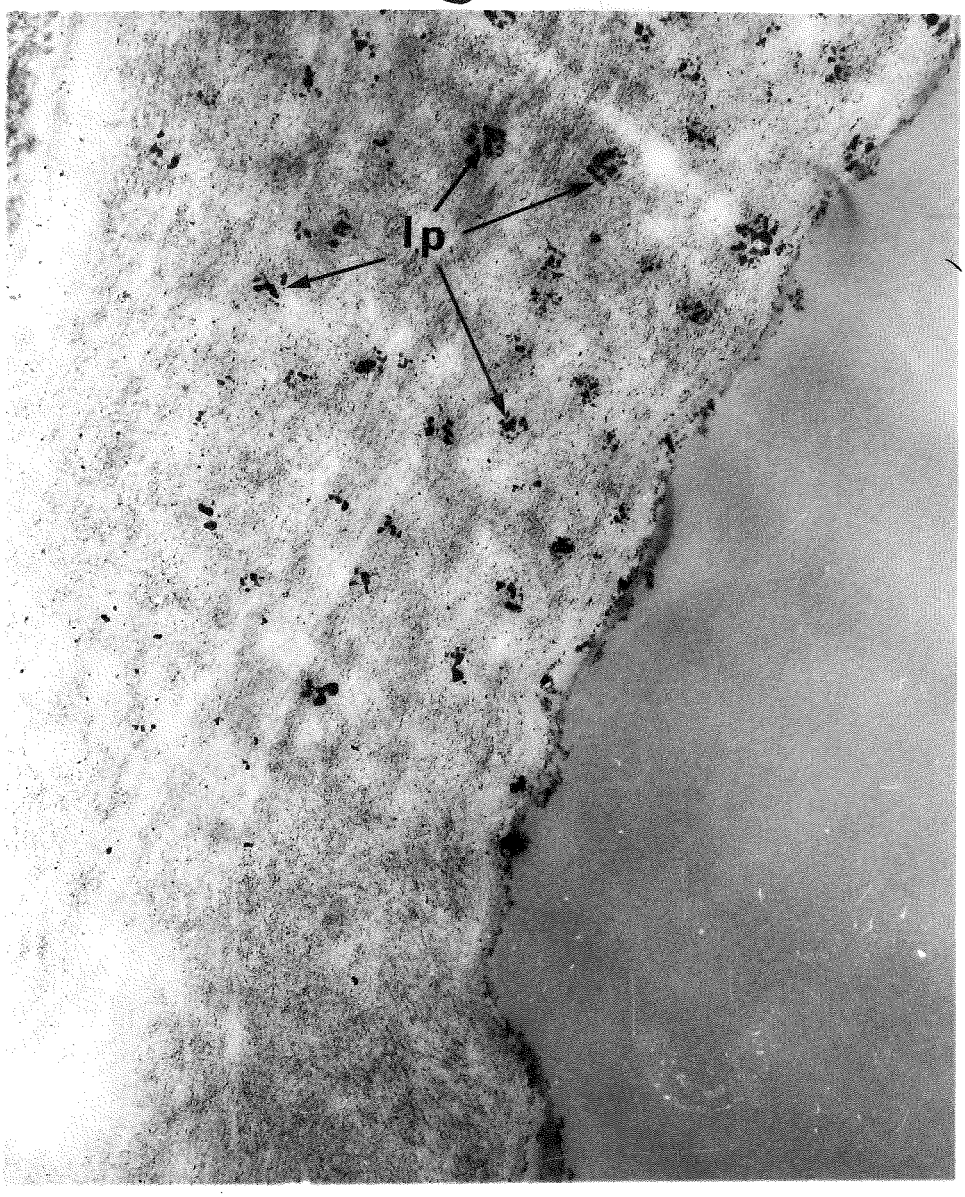
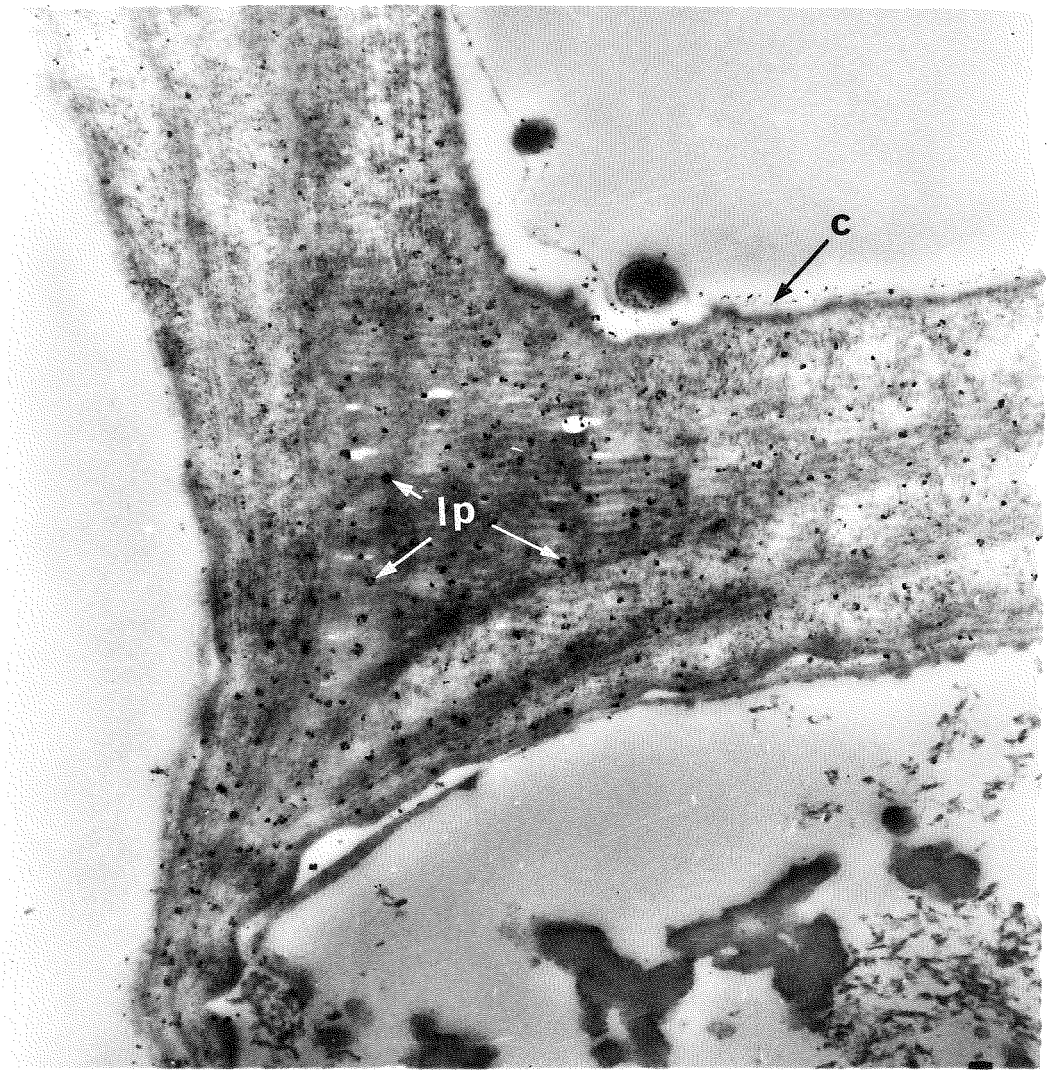


Fig. 26: Ultra-thin section of epidermal cell of radish hypocotyl infiltrated with lead. (x22,500.)

c = cuticle
lp = lead particles

Fig. 26



detected (Fig. 27). The particles of lead were found to lie in the spaces between the micells of the cellulose cell wall (Fig. 28).

(iii) The effect of host injury on penetration by the pathogen. Radish hypocotyls were punctured with a sterile needle and inoculated with 2 mm agar cubes bearing hyphae of a stem strain of T. cucumeris. After incubation for about 12 hours a higher number of infection cushions were formed at the edge of the punctures than at other areas. This may be due to the presence of an increased amount of plant sap as a result of injury. These cushions were removed before penetration from them into the host could be effected. Fungal hyphae grew over the edges of the punctures and entered the injured tissue (Fig. 29). Sections taken through the injured areas showed that the fungal pathogen could colonize tissue without the necessity of forming infection cushions (Fig. 30). The hyphae that entered the punctures in the radish hypocotyls were unconstricted. At a later stage, when more hyphae entered the punctures, sections through the punctures contained penetrating hyphae very similar to that developed from infection cushions (Fig. 30c).

Fig. 27: Ultra-thin section of radish hypocotyl cell infiltrated with lead and shadowed with platinum-carbon. (x42,000.)

c = cuticle
lp = lead particles in radish cell wall
ls = Latex sphere

Fig. 27

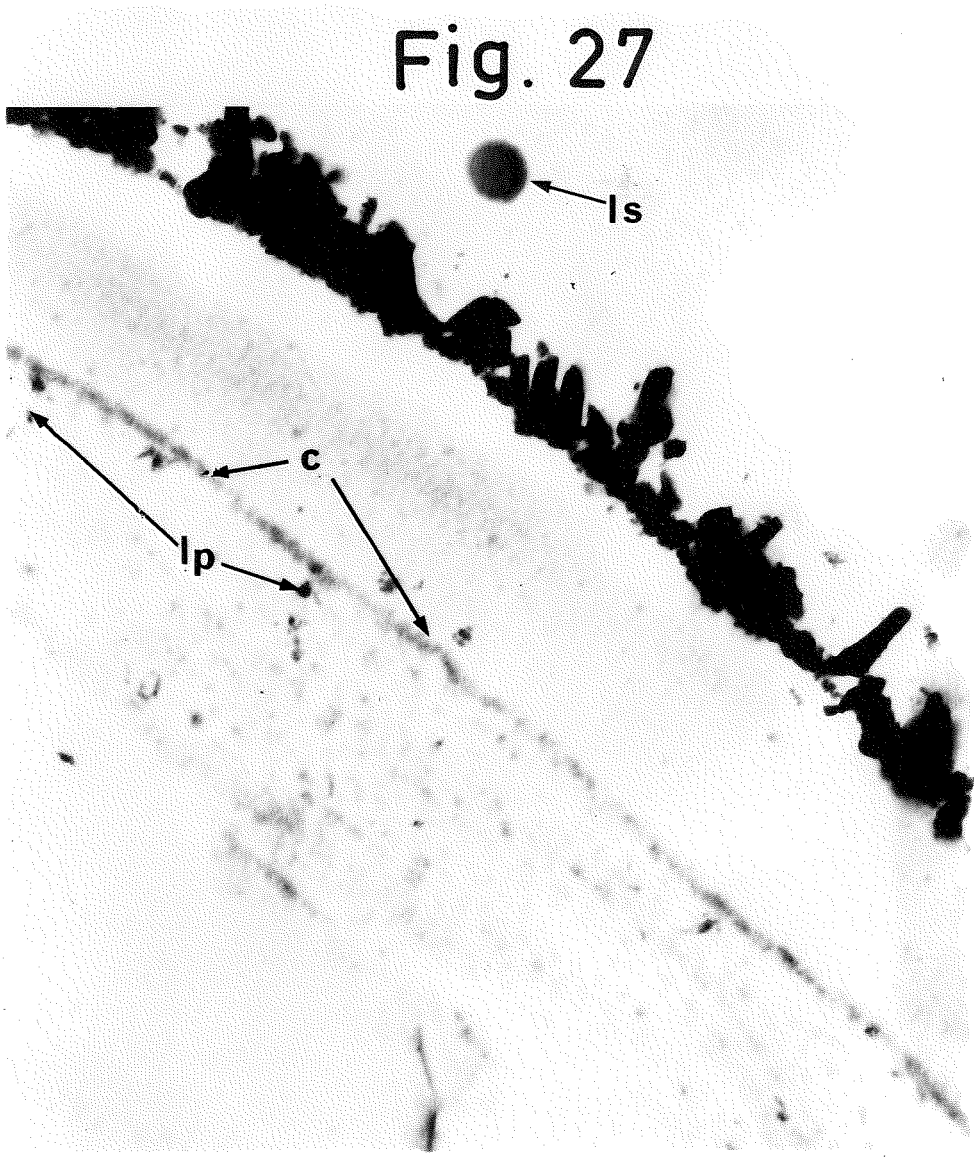


Fig. 28: Ultra-thin section of lead infiltrated epidermal cells of radish hypocotyl at the site of fungal infection cushion formation. Sections were shadowed with platinum carbon. Note the positions of the lead particles in relation with the longitudinal cellulose fibres. (x27,000.)

cl = cell lumen
lp = lead particles in cell wall

Fig. 28

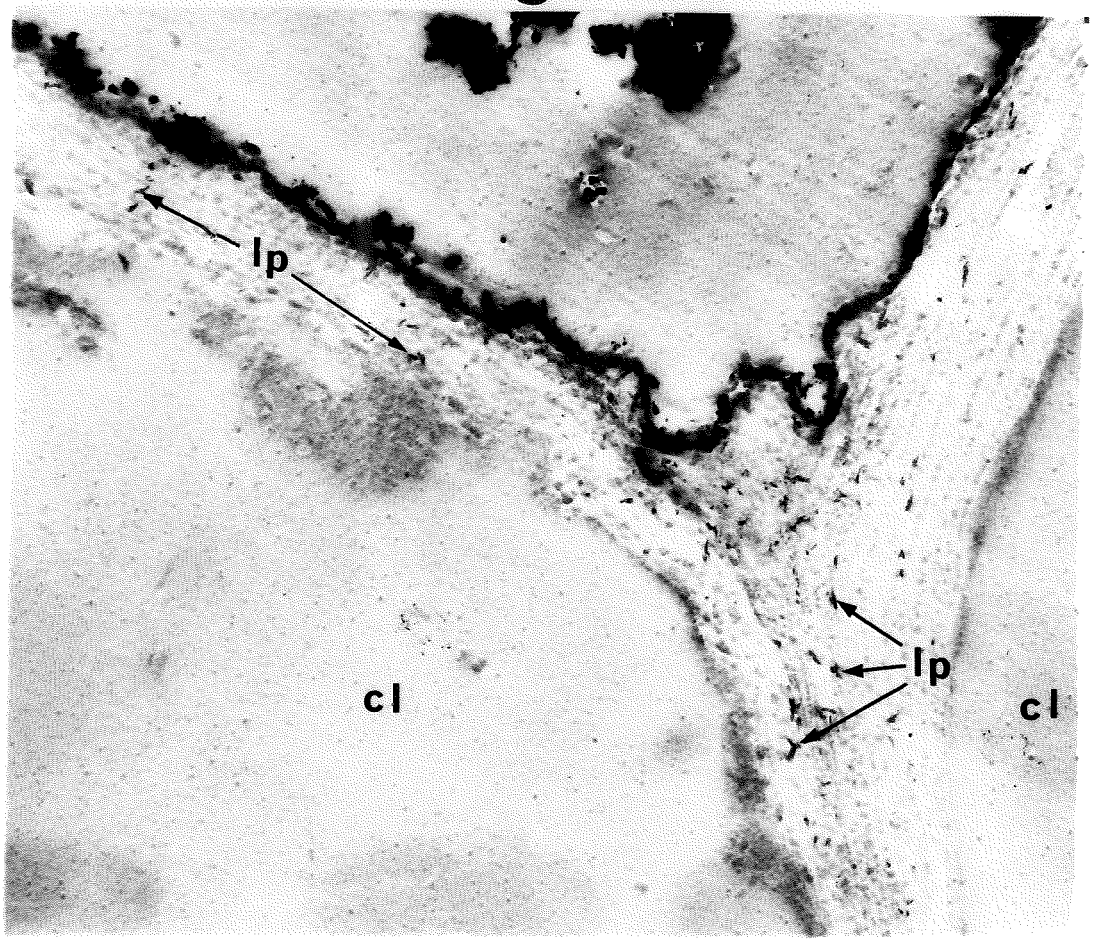


Fig. 29: Surface view of hyphae of *T. cucumeris* entering injured areas on radish hypocotyl surface. (x270.)

(a) Early stage, inoculated with isolate 69.

(b) Later stage, inoculated with isolate 48.

Fig. 29

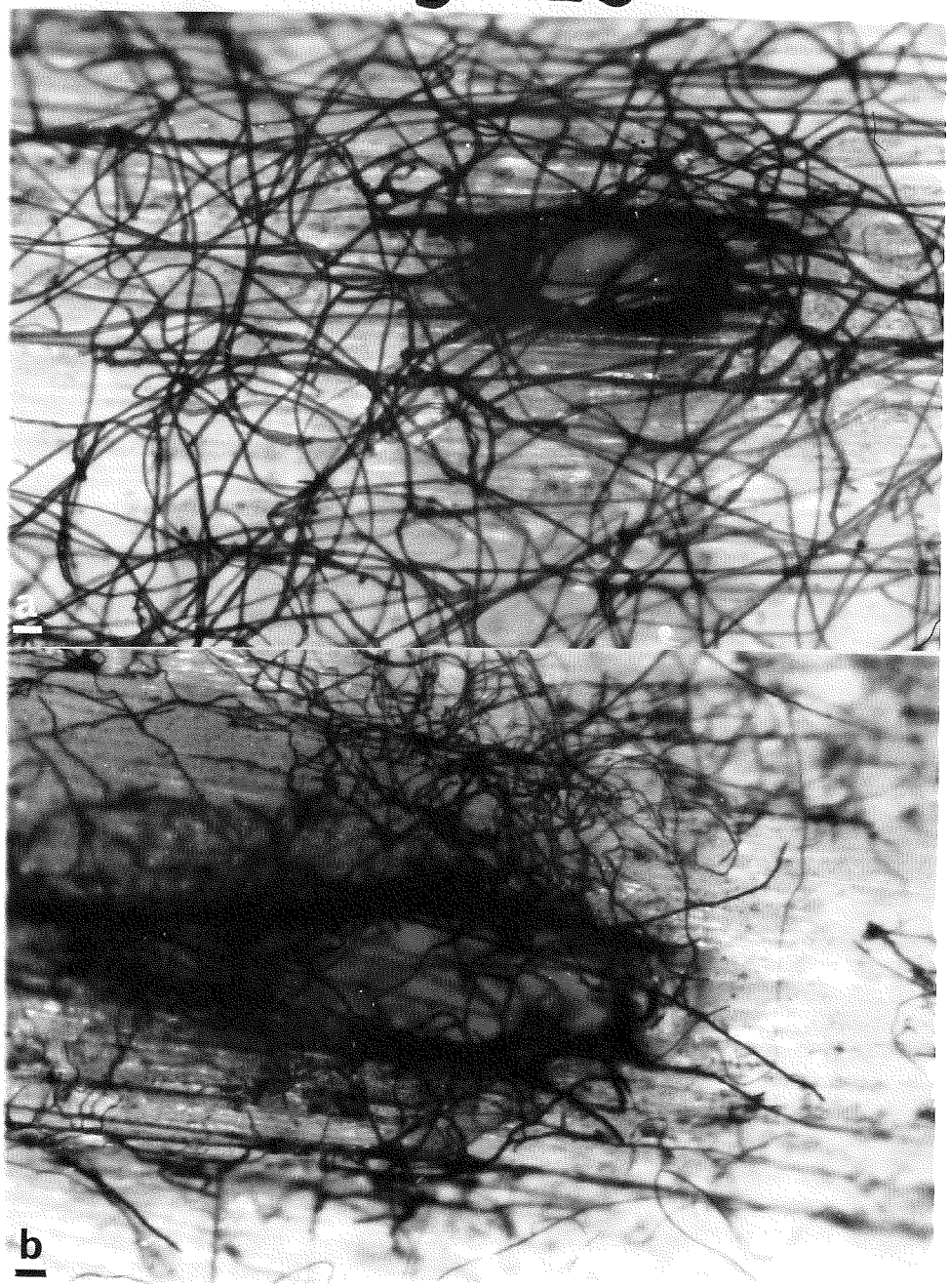


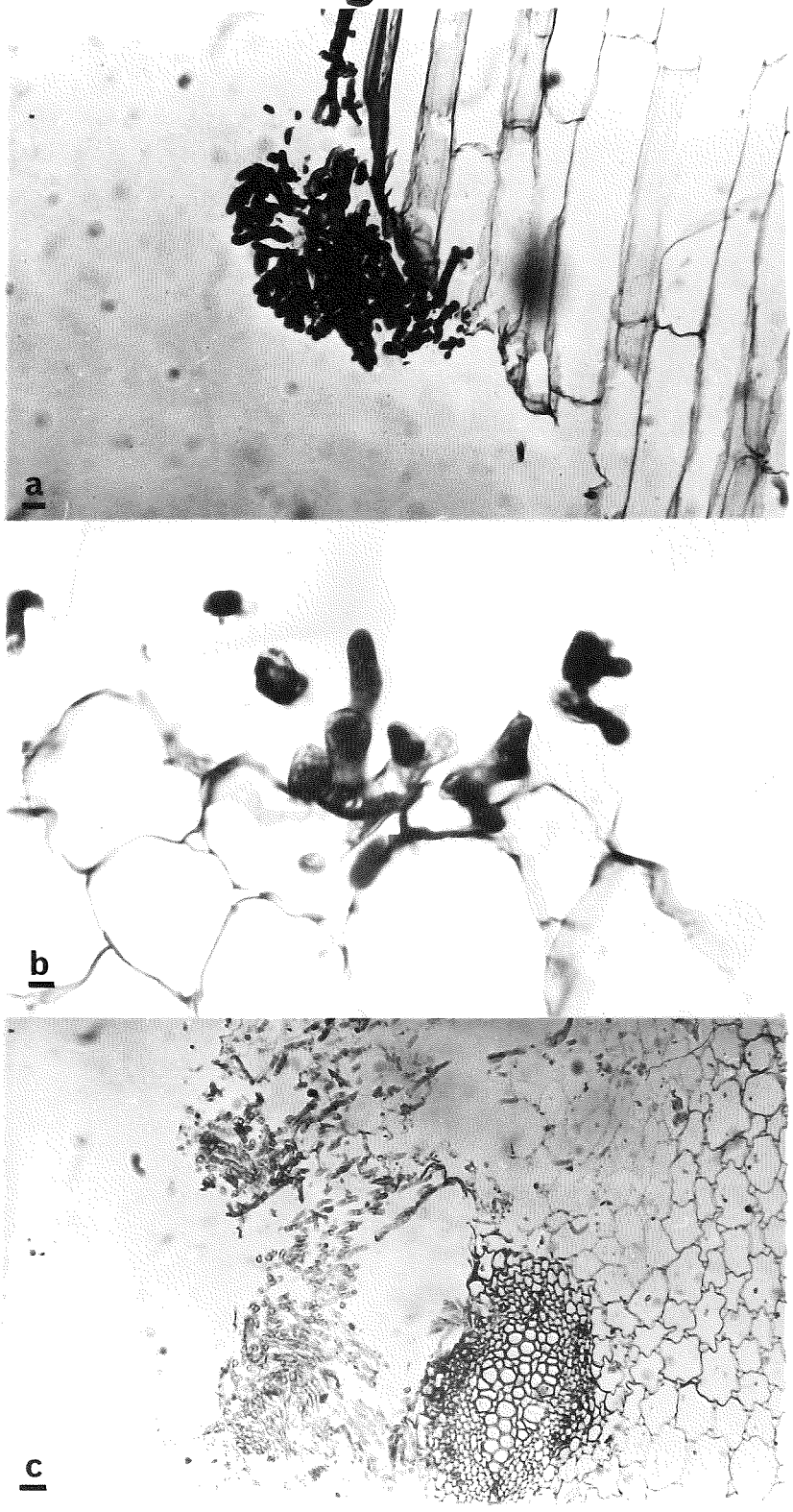
Fig. 30: Sections through injured areas on radish hypocotyl, showing that hyphae of T. cucumeris, isolate 69, are capable of parasitizing the cortical cells directly when the cuticle is damaged.

(a) Early stage. (x530. L.S.)

(b) Early stage. (x560. T.S.)

(c) Late stage. (x400. T.S.)

Fig. 30



(iv) Relationship between the location of fungal cushions and areas of infection.

Infection cushions on radish hypocotyls were displaced with a dissecting needle and the damage to the host surface in relation to the size and site of the cushion noted. No definite correlation between the size of the infection cushions and the amount of penetration beneath these cushions was found. Some areas beneath very small cushions were penetrated while areas beneath some fairly large cushions were not penetrated. Almost all the large cushions occupied sites that were penetrated. Small infection cushions beneath which infection had occurred were usually found in narrow spaces in between two epidermal cells. These narrow spaces had been shown to be the tapering ends of epidermal cells in between the sides of adjacent epidermal cells (see Section C, ii). In a few cases, infection cushions were found over penetrated stomata. The infected areas beneath most small cushions occupied a position which coincided with the centres of the cushions. This was not true in the case of large infection cushions which had initial penetration beneath them. Penetrated areas, after removal of the fungal cushions, were usually found to be elongated in shape (Fig. 31) and longitudinal splits were seen. These

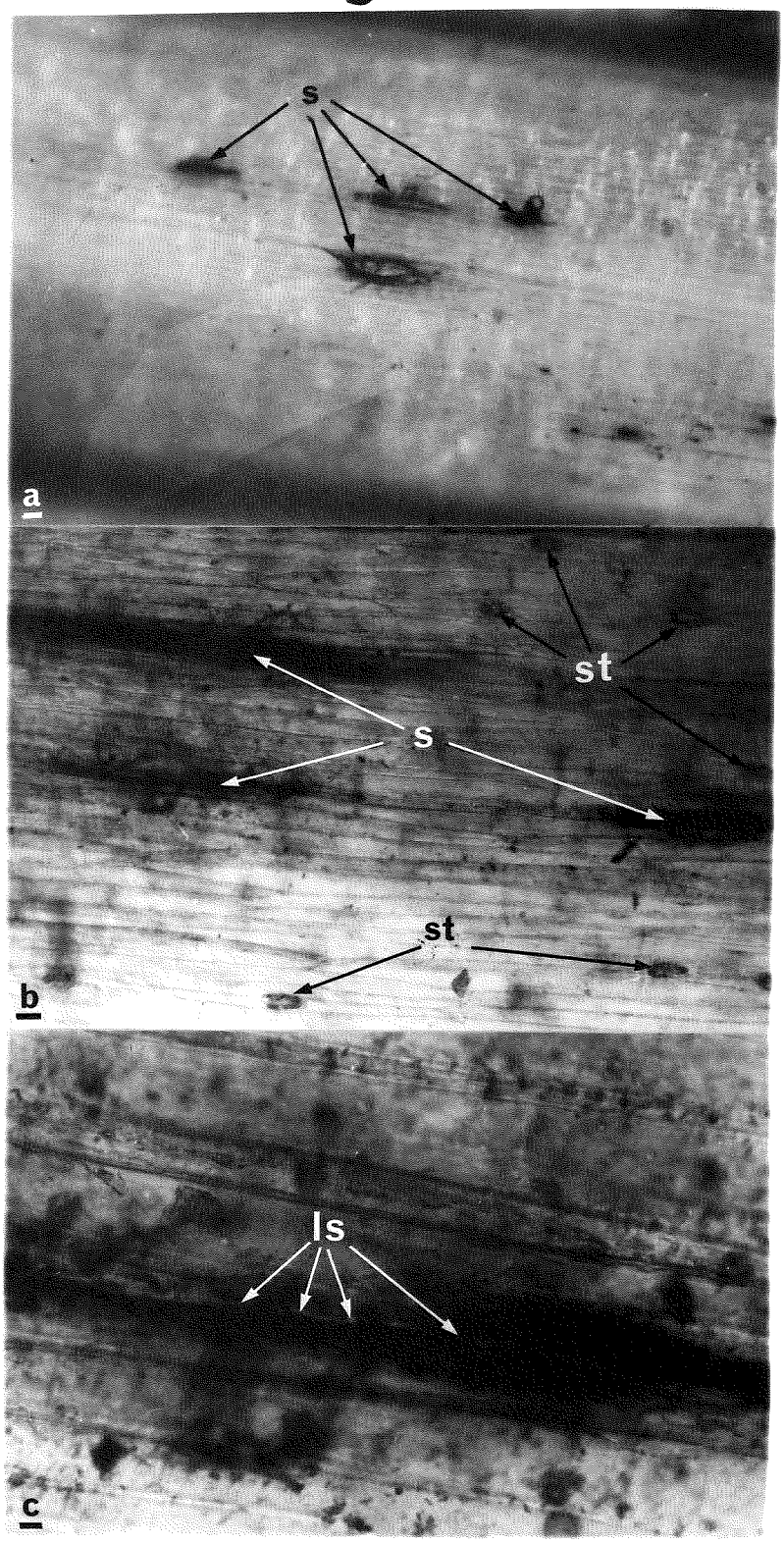
Fig. 31: Surfaces of radish hypocotyls inoculated with isolate 69 with infection cushions removed.

(a) Reflected light photograph of part of a whole mount stained with trypan blue. Stomata are not clear. (x100.)

(b) & (c) Transmitted light photographs of dissected epidermis stained with trypan blue and mounted in aqueous IKI. Note the frequent occurence of stomata in (b). (c) is an enlargement of (b) to show the longitudinal splits caused by penetrating hyphae beneath a small cushion. (b) x400. (c) x560.

ls = longitudinal split
s = site of infection
st = stomata

Fig. 31



longitudinal splits were usually associated with young vigorously growing infection cushions suggesting that penetrating hyphae acted as a wedge.

D. The spread of a stem strain, isolate 69, of the pathogen within radish hypocotyls.

Dark coloured lesions were formed in the host tissue 12-24 hours after penetration by T. cucumeris, isolate 69. Transverse sections showed that two or three layers of cells immediately below the site of penetration had been completely filled with fungal hyphae. Penetrating hyphae entered the host beneath infection cushions in thick strands in between the epidermal cells (Fig. 32). Epidermal cells in between wedges of hyphae were either grossly distorted or crushed. Crushed epidermal cells were invaded by hyphae. No constriction of the penetrating hyphae and no production of slender penetration pegs were seen beneath the cushions (Fig. 33a). Penetration into the host was effected from single infection cushions or from cushions on different parts of the hypocotyl or from cushions on adjacent parts of hypocotyls (Fig. 32a). There was an enormous proliferation of fungal tissue at the site of penetration and three or four cortical cell layers immediately below. This region was mainly colonized intracellularly.

Fig. 32: Transverse sections of radish hypocotyl through the sites of infection by T. cucumeris, isolate 69.

(a) Host penetration beneath two infection cushions. (x130.)

(b), (c), (d) Note that host penetration is by thick strands of fungal hyphae. Some host cells were destroyed and others grossly distorted. (x400.)

Fig. 32

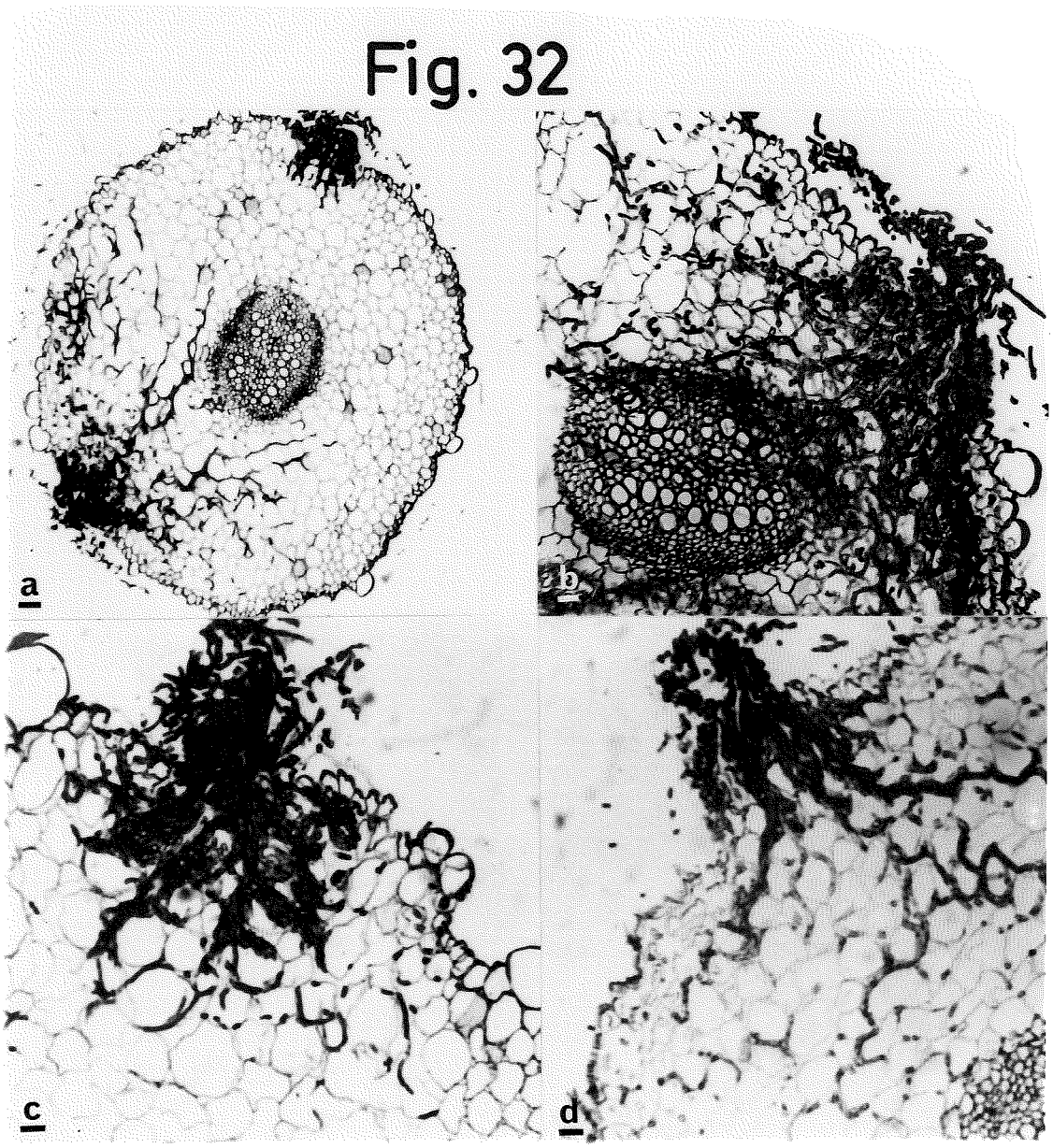
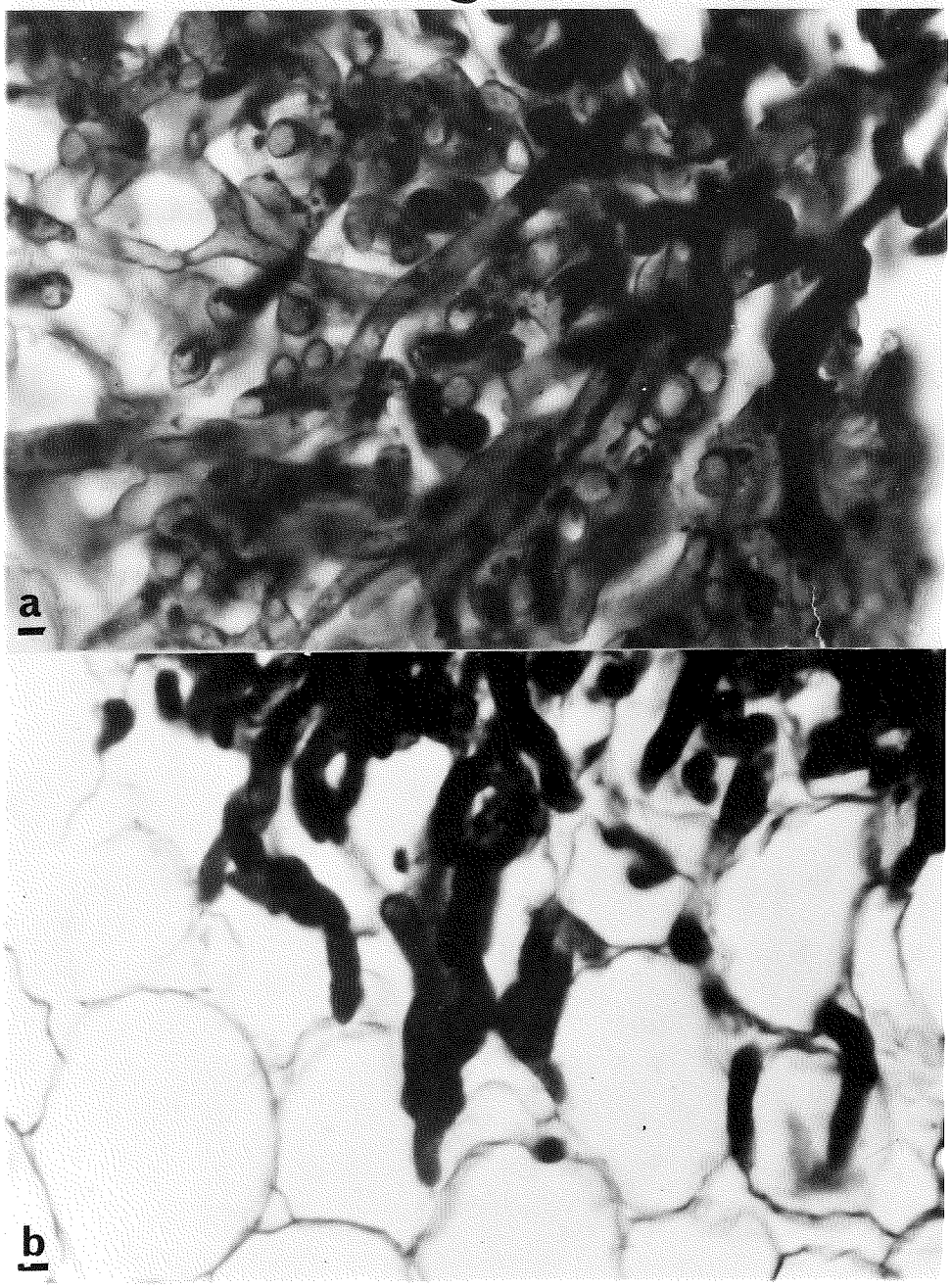


Fig. 33: Intra-cellular hyphae of T. cucumeris, isolate 69, at the site of entry into radish hypocotyl.

(a) Enlargement of Fig. 32b. Note that there is no change in diameter of penetrating hyphae. (x600.)

(b) Enlargement of Fig. 32c. Advancing intra-cellular hyphae form a wedge in colonizing cortical cells. (x600.)

Fig. 33



Intracellular colonization was by means of hyphae coming together in the form of a wedge (Fig. 33b). The area occupied by intracellular hyphae is referred to as the lesion.

Longitudinal sections showed that internal hyphae spread faster towards the centre than longitudinally within hypocotyls (Fig. 34). Transverse sections through the edges of the lesions showed that the fungal cells were tightly packed within the lumen of each infected host cell and that they grew mainly along the long axis of infected cells (Fig. 35a). Strands of hyphae spread along the hypocotyl first intracellularly from above and below the lesion (Fig. 34a) and then fungal growth was mainly intercellular (Fig. 35b, 37a). Longitudinal spread of the pathogen in between epidermal cells and the cuticle caused these layers to separate (Fig. 35c, 36a, 37a). A large amount of hyphae caused the cuticle to rupture, especially immediately next to sites of penetration, allowing more hyphae to enter the host with corresponding increases in the sizes of cushions and lesions.

Ultrathin sections through hyphae of isolate 69 within radish cell walls showed areas of cell walls, next to the hyphae, which were more electron transparent than

Fig. 34: Longitudinal sections of radish hypocotyls through the sites of infection by T. cucumeris, isolate 69. (x440)

(a) Strands of hyphae spread along the hypocotyl intracellularly from the point of infection.

(b) Hyphae spread mainly intercellularly towards the centre of the hypocotyl.

Note that the spread of the pathogen is faster towards the centre than along the hypocotyl.

l = lesion

Fig. 34

Fig. 35: Transverse sections through radish hypocotyl tissue infected by T. cucumeris, isolate 69.

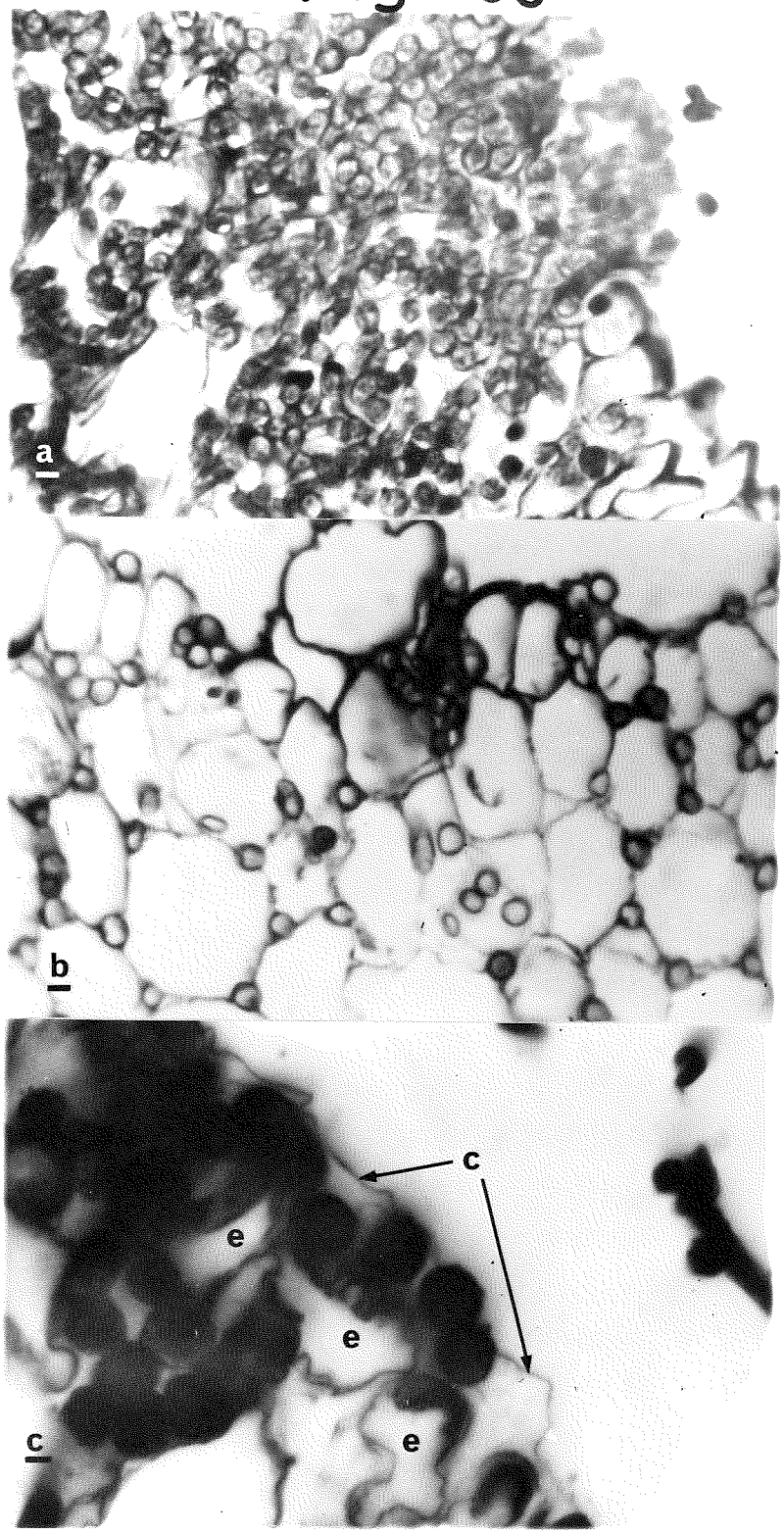
(a) Intracellular hyphae at the edge of the lesion. (x560.)

(b) Outside the lesion, hyphae are mainly intercellular. (x560.)

(c) Hyphae growing in between the epidermal cell and the cuticle. The epidermal cells are distorted and the cuticle lifted away from the epidermal cells. (x1250.)

c = cuticle
e = epidermal cell

Fig. 35



the other parts of the walls (Fig. 36b). This suggests that cell wall material was degraded by enzymes from the hyphae. Hyphal cell walls were thin and highly electron opaque (Fig. 36c). A number of small areas on the cell walls were electron transparent. It is not known whether or not these areas were artifacts. Covering the fungal cell wall was a thin amorphous fibrillar, almost electron transparent, layer. The significance of this layer is unknown and will be discussed later.

From lesions spread of the pathogen towards the centres of hypocotyls was mainly intercellular. Some hyphae grew around the cortical cells (Fig. 37b). Hyphae may grow between five or six cells in the plane of transverse sections (Fig. 37c). These hyphae branched in a characteristic Rhizoctonia manner and they possessed dolipore septa (Fig. 37b). There were no obvious structural changes in the tissue in advance of the hyphae or within tissue colonized intercellularly. No resistant structures were developed by the host to contain the pathogen. When cortical cells were penetrated intracellularly by hyphae there was no constriction of hyphal diameter (Fig. 37b,c). When cortical cells were penetrated no lignitubers, callosities or calluses formed. With the exception of sites of lesion formation, where the host cells were

Fig 36: Ultra-thin transverse sections of hyphae of isolate 69 within the radish hypocotyl cell wall.

(a) Hyphae growing in between the cellulose wall of the epidermis and the cuticle. Fixed with glutaraldehyde and osmium tetroxide and stained with lead citrate. (x6,000.)

(b) Inter-cellular hypha. Note degradation of host cell wall. Fixed with potassium permanganate, stained with uranyl acetate and lead citrate. (x4,000.)

(c) Enlargement of (b). Arrows show electron transparent areas. (x12,000.)

c = cuticle

Fig. 36

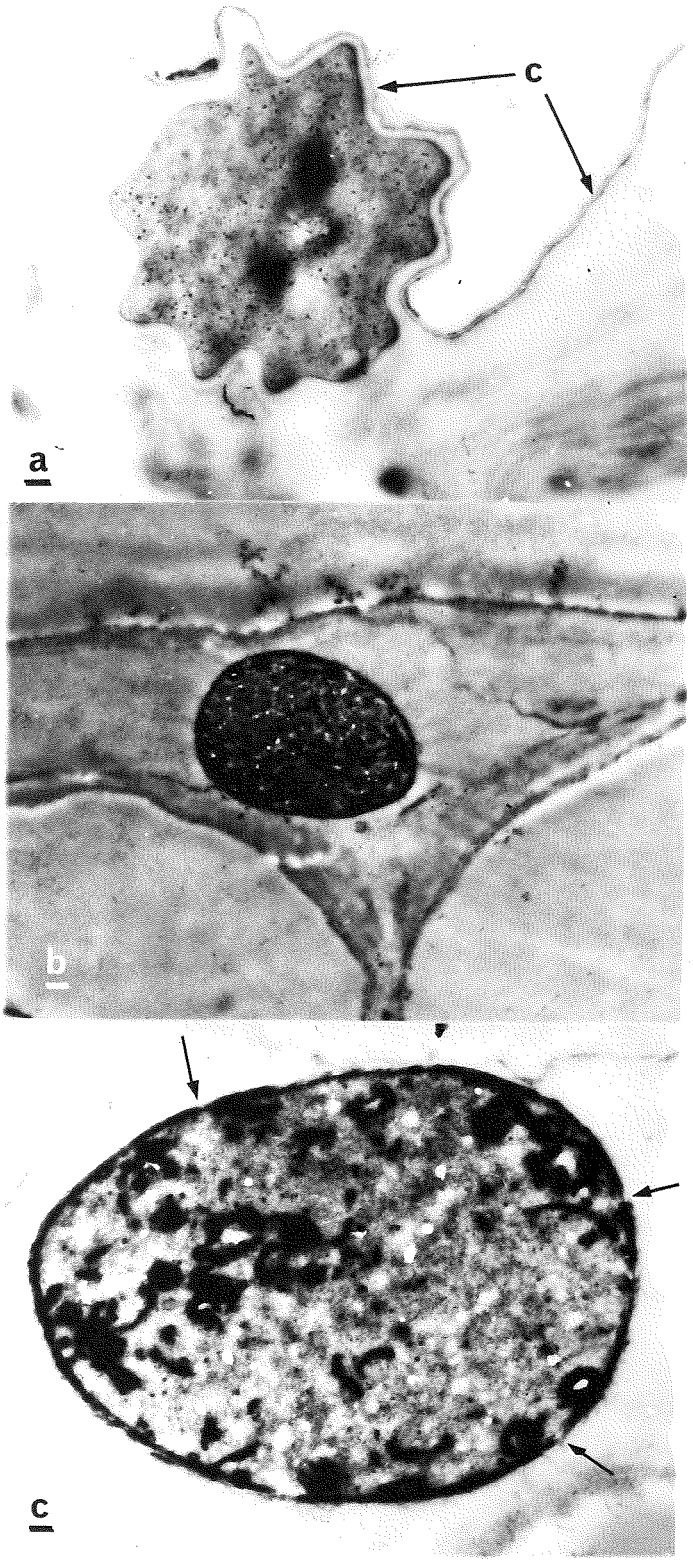


Fig. 37: Transverse sections showing rapid intercellular colonization of radish cortical tissue by hyphae of T. cucumeris, isolate 69, outside the lesion. (x560.)

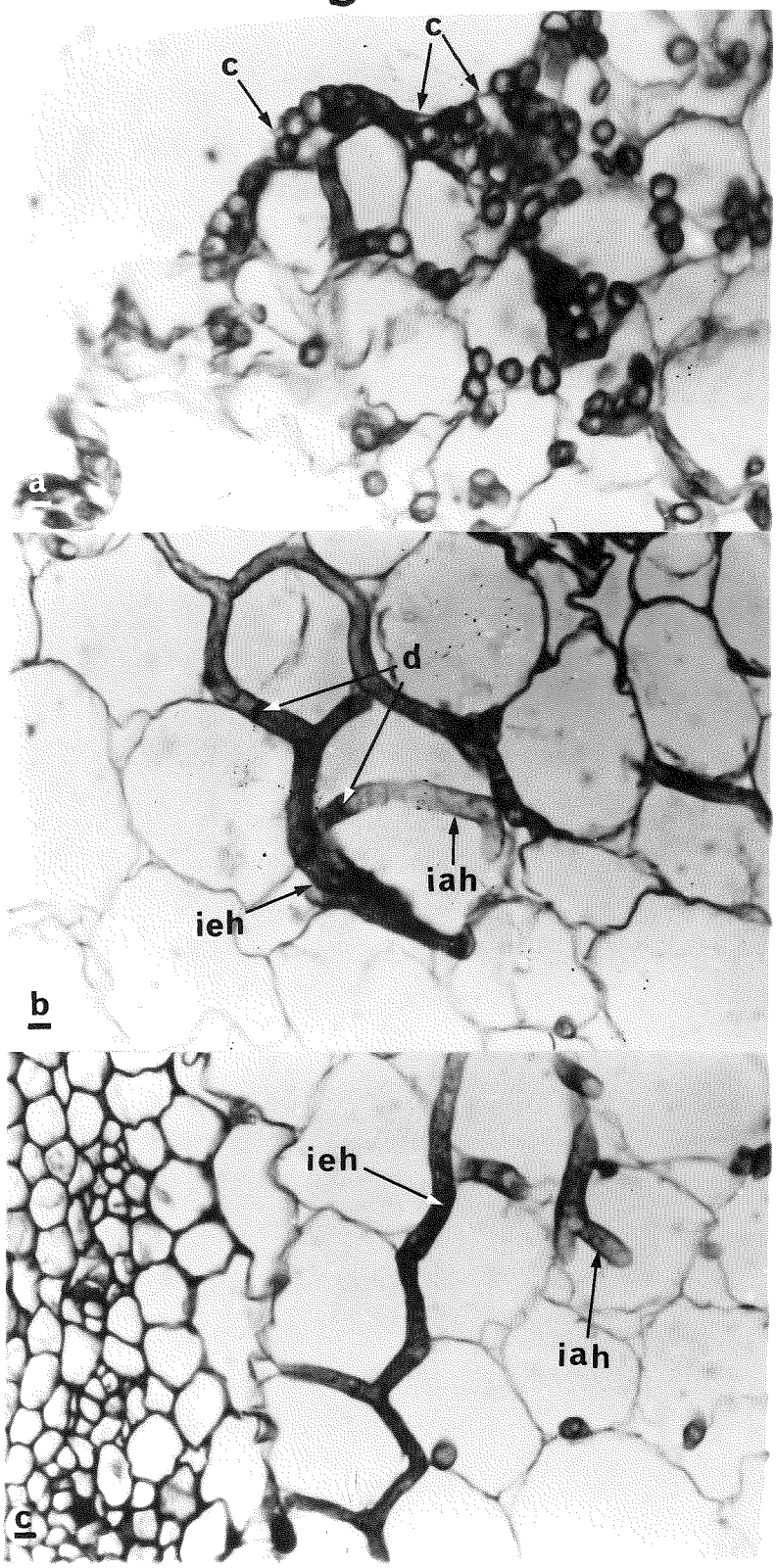
(a) Longitudinal spread of hyphae above the lesion.

(b) Intercellular hyphae encircling host cells. Note the presence of dolipores in the fungal septa.

(c) Intercellular hyphae spread towards the stele. Note intracellular penetration without constriction of the fungal hyphae.

c = cuticle
d = dolipores of fungal septa
e = epidermal cell
iah = intracellular hypha
ieh = Intercellular hypha

Fig. 37



destroyed, sections of colonized cortical tissue were very similar to sections of some ectotrophic mycorrhizal tissues.

Forty-eight hours after inoculation there was an increase in lesion size. A water-soaked halo was seen around the infection cushion. Transverse sections through sites of infection revealed that the whole of the cortex had been colonized, mainly intercellularly (Fig. 38). Most of the intercellular hyphae grew along the axis of the seedling (Fig. 38b). At this stage, hyphae began penetrating cortical cells and the cytoplasm of penetrated host cells appeared granular (Fig. 39a). Coils of hyphae were found around (Fig. 40a) and inside (Fig. 40b,c) cortical cells. Some of the host cells were completely filled with intercellular hyphae (Fig. 39b). The endodermis was penetrated both inter- and intra-cellularly (Fig. 40c, 41a,b). Steles were colonized before hypocotyls collapsed. Intracellular hyphae were present in the phloem but not in the xylem vessels (Fig. 41b). The cytoplasm of the phloem cells were granular and withdrawn from the cell wall (Fig. 41c). No other symptoms were detected in the phloem cells. Tyloses and gums were not observed in xylem vessels.

About two and a half days after inoculation, the

Fig. 38: Transverse sections through radish hypocotyls extensively colonized by hyphae of T. cucumeris, isolate 69.

(a) x440.

(b) Enlargement of (a). (x600.)

Fig. 38

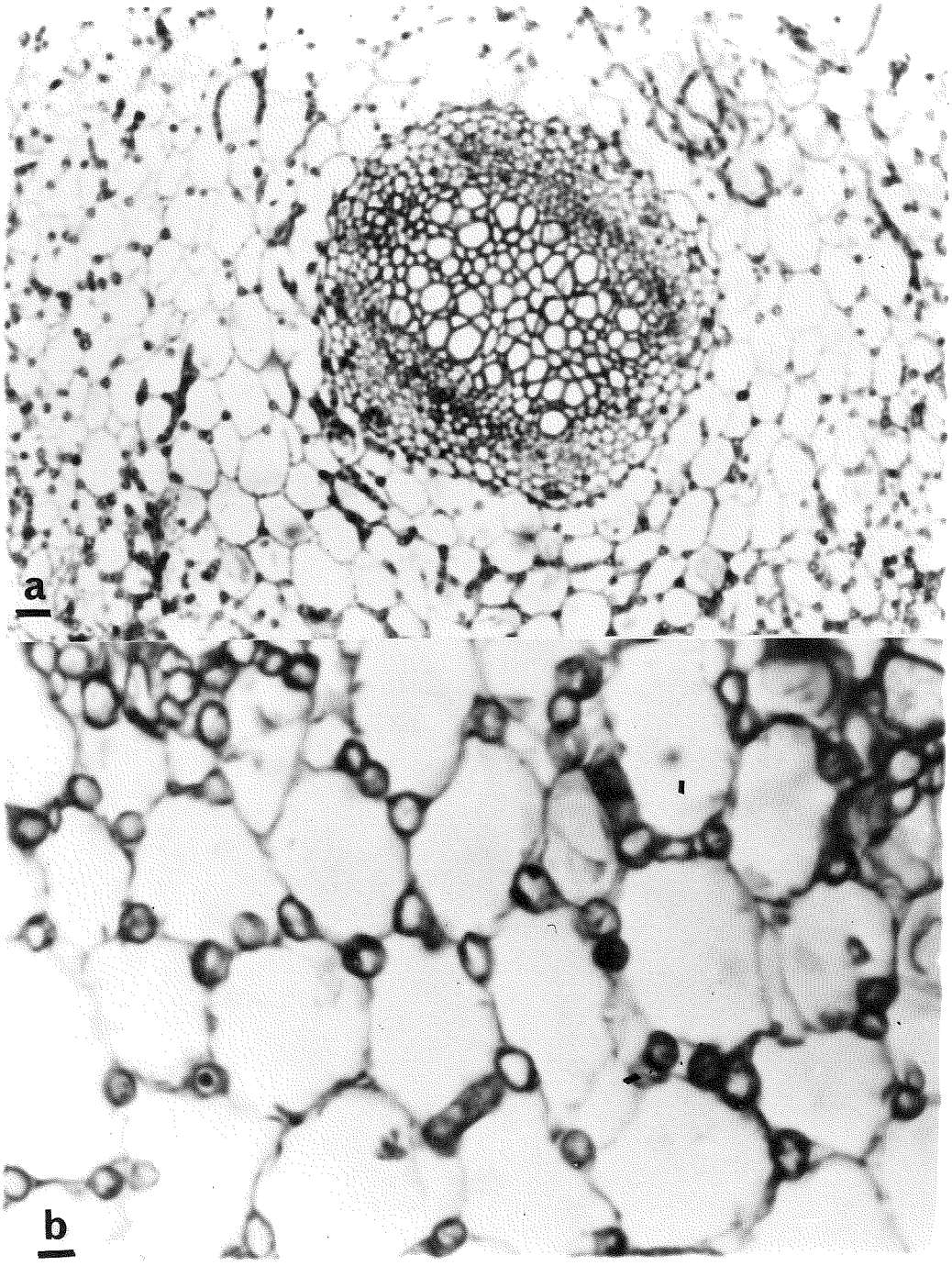


Fig. 39: Transverse sections of radish hypocotyls colonized by isolate 69. (x600.)

(a) Intracellular hyphae induce granular appearance of host cytoplasm.

(b) Coils of hyphae within host cells.

Fig. 39

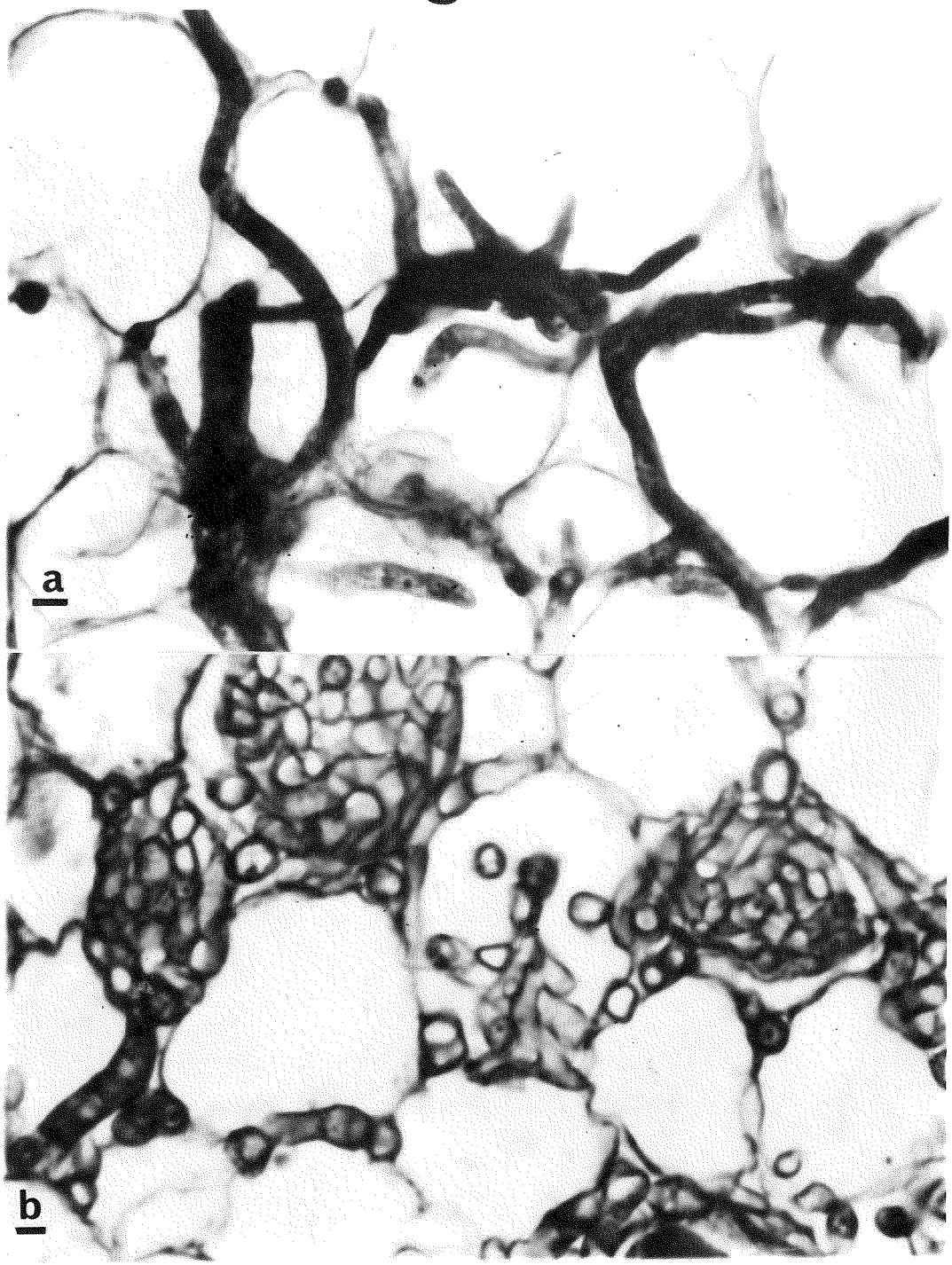


Fig. 40: Longitudinal sections through radish hypocotyls infected with isolate 69. (x560.)

(a) Hyphae coiled around host cells.

(b) Hyphae coils within host cells.

(c) Penetration into the stele.

Fig. 40

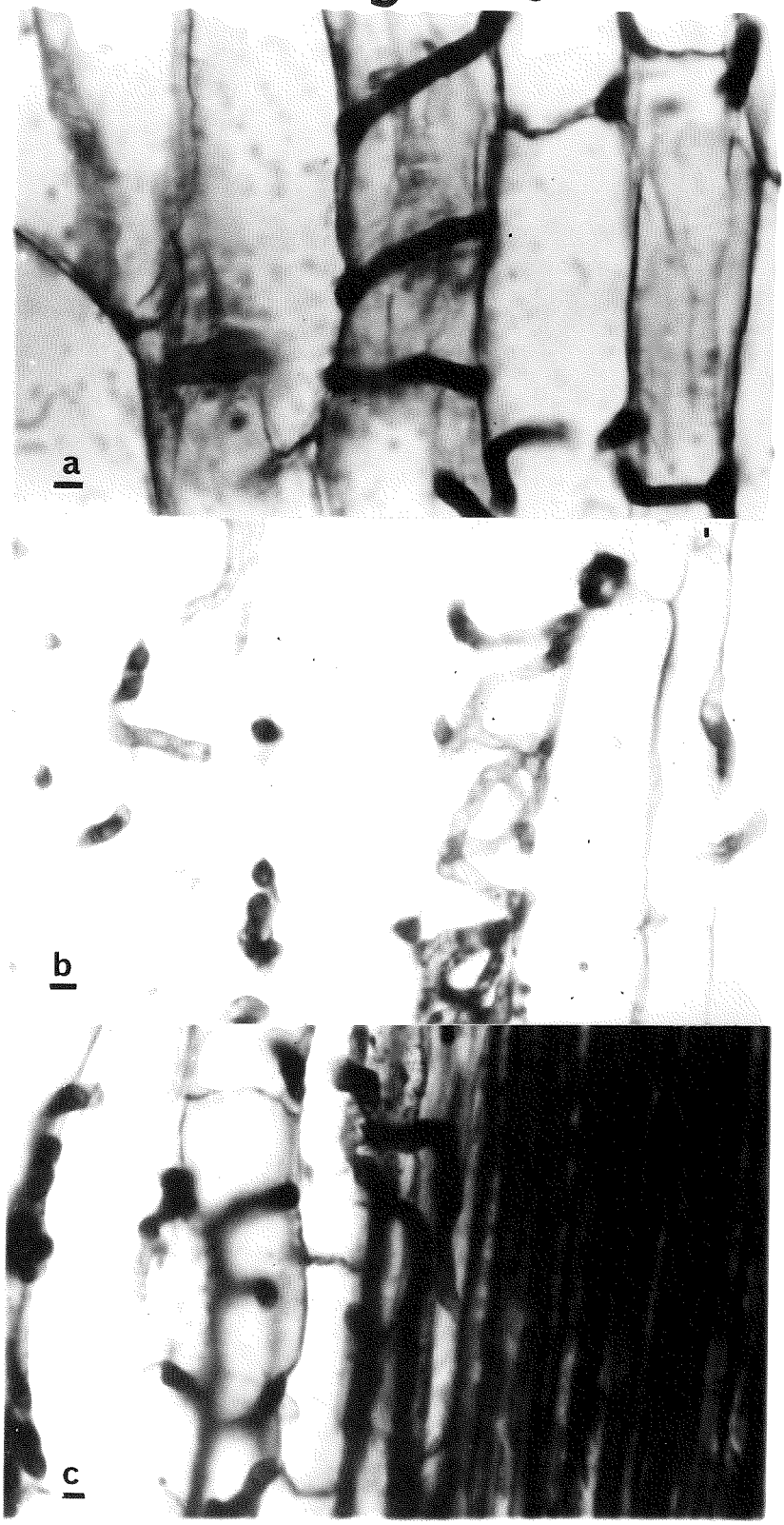


Fig. 41: Transverse sections of radish hypocotyls infected with isolate 69.

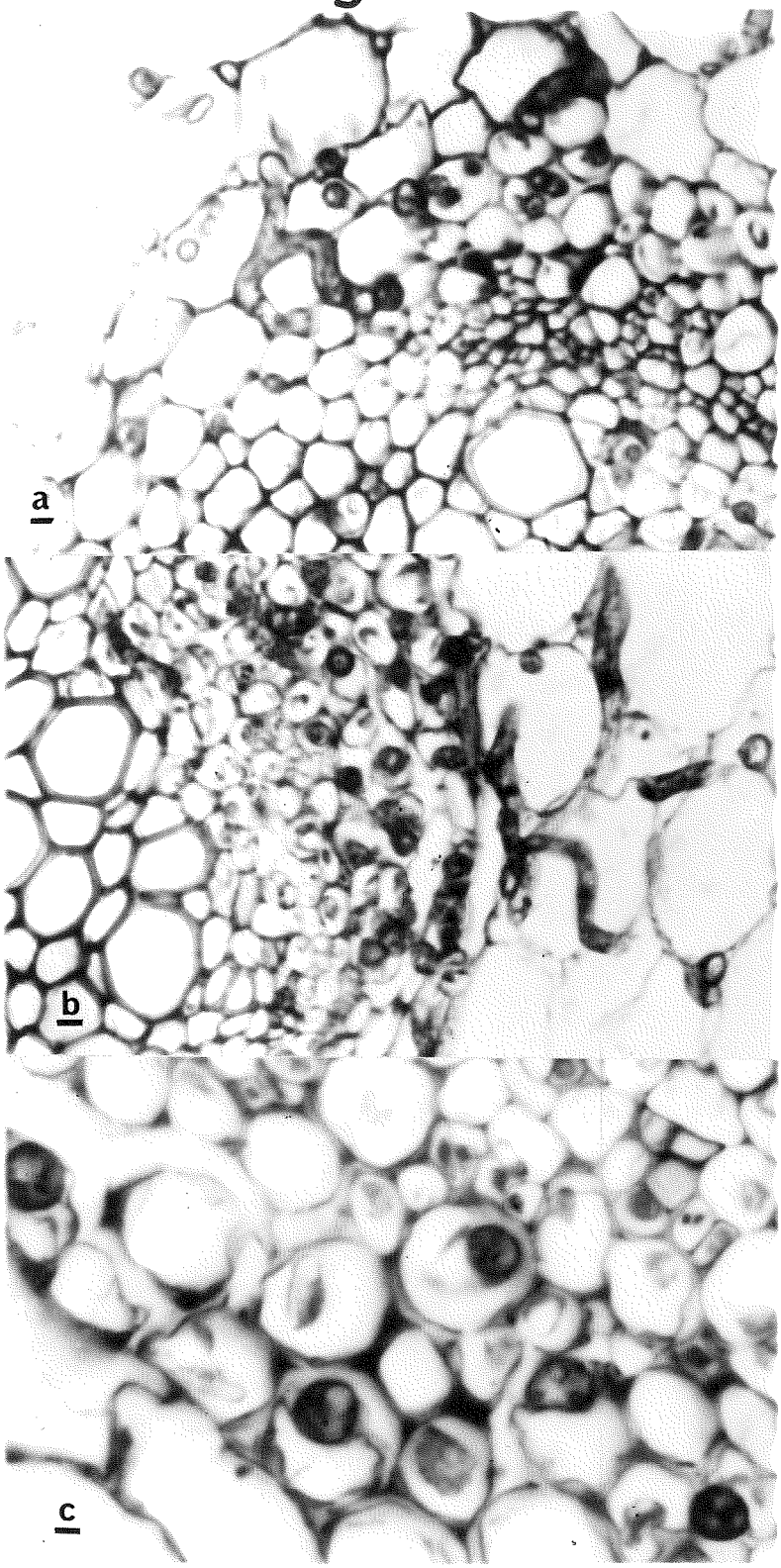
(a) Intercellular penetration of the endodermis into the stele. (x560.)

(b) Intracellular penetration of the endodermis. (x560.)

(c) Intracellular hyphae in the phloem cells. The cytoplasm is granular and shrunken away from the cell wall. (x1250.)

Note that no hyphae enter the xylem vessels.

Fig. 41



hypocotyl collapsed although the root and foliage remained healthy.

E. Spread of pathogen within radish and wheat roots.

(i) Radish root infected by a root strain of T. cucumeris. Hyphae grew on the surface of roots and on root hairs (Fig. 42a). Fungal hyphae induced an intense host reaction at the site of contact. Intracellular penetration into the root hairs by slender penetration pegs were seen (Fig. 42b). The root tip and the piliferous layer were penetrated either by hyphae from the lumen of root hairs (Fig. 42c) or by direct penetration by hyphae growing on the surface via slender penetration pegs. Spread of the pathogen through the cortical cells was mainly intracellular with the production of slender penetration pegs (Fig. 43). The endodermis and the phloem were colonized intracellularly (Fig. 44). Dark host reaction, granular appearance of the host cytoplasm and retraction of the cytoplasm from the cell wall were seen. Xylem vessels were rarely invaded. Gummy substances were produced in a few xylem vessels.

(ii) Radish root infected by a stem strain of T. cucumeris.

Hyphae were found to attack the root tip region and

Fig. 42: Penetration of root hairs of radish by a root strain, R10, of T. cucumeris. (x560.)

(a) Hyphae growing on the surface of a root hair.

(b) Intracellular penetration of root hair by a slender infection peg.

(c) Penetration of the piliferous layer by hyphae growing through the lumen of a root hair.

c = constriction of fungal hypha
h = hypha
p = piliferous cell
rh = root hair

Fig. 42

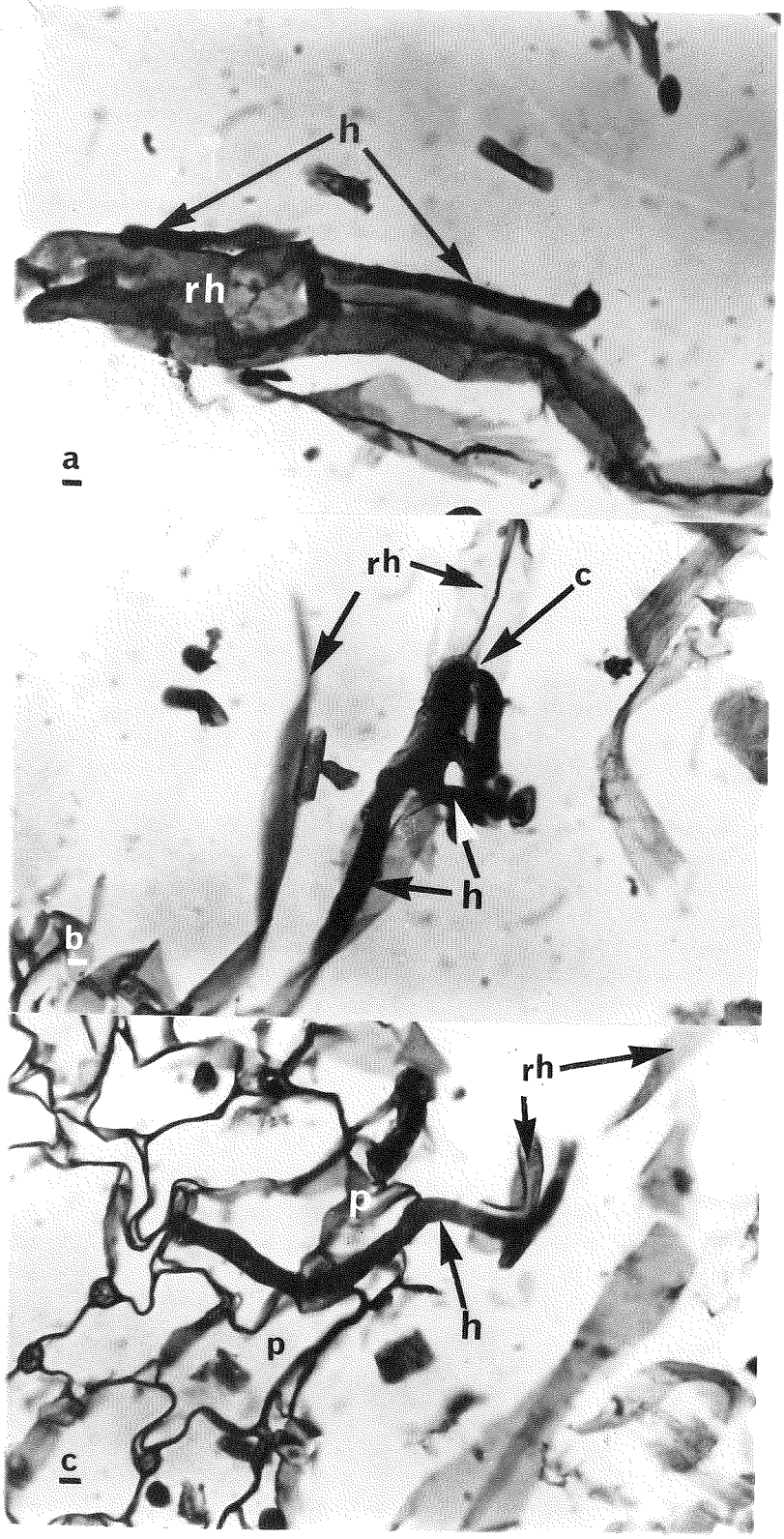


Fig. 43: Transverse sections of radish roots infected with a root strain, R10, of T. cucumeris.

(a) Inter- and intra-cellular hyphae. (x560.)

(b) Penetration through cortical cells by slender infection pegs. (x1250.)

(c) Intracellular penetration. (x1250.)

c = constrictions of hyphae
iah = intracellular hyphae
ieh = intercellular hyphae

Fig. 43

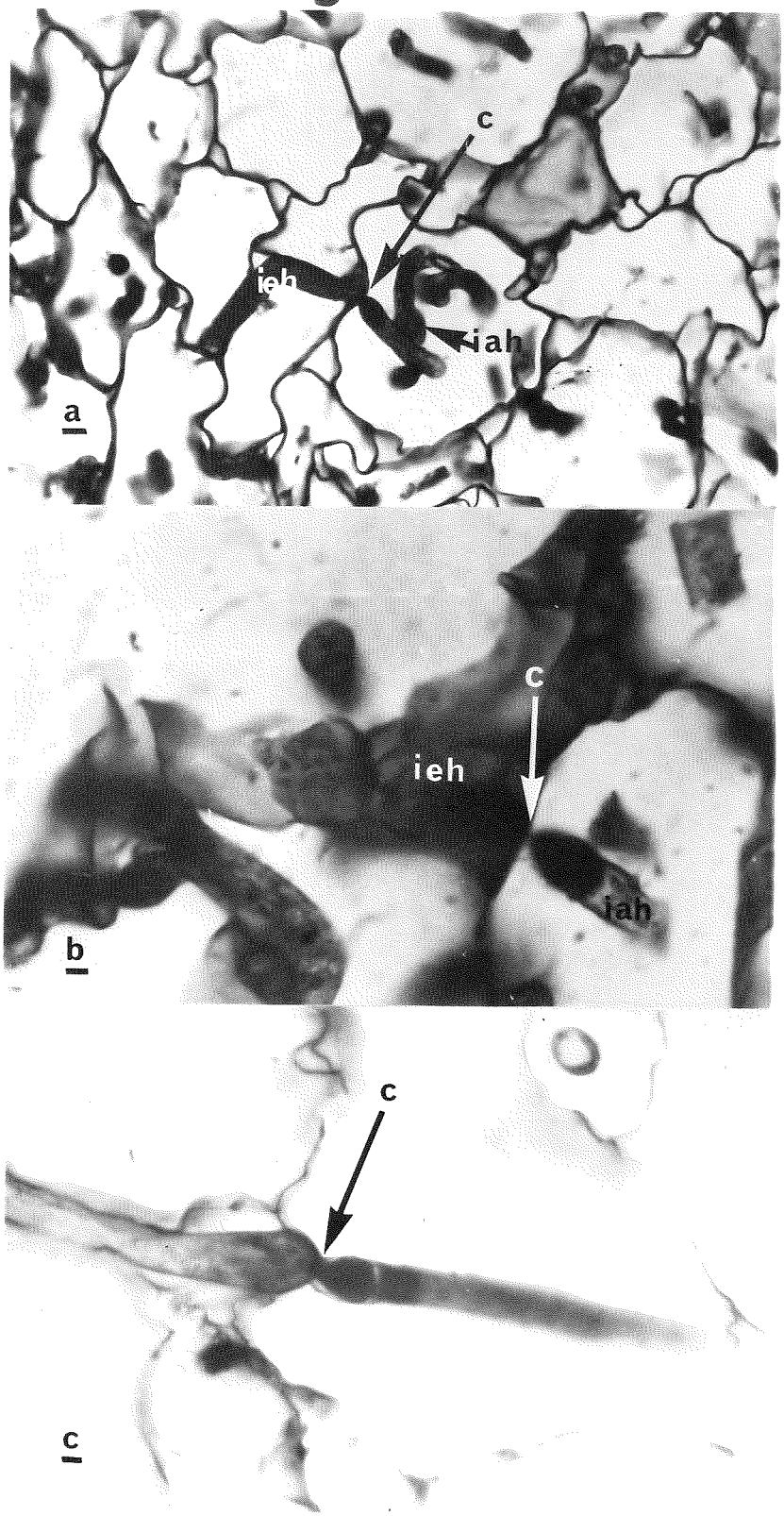
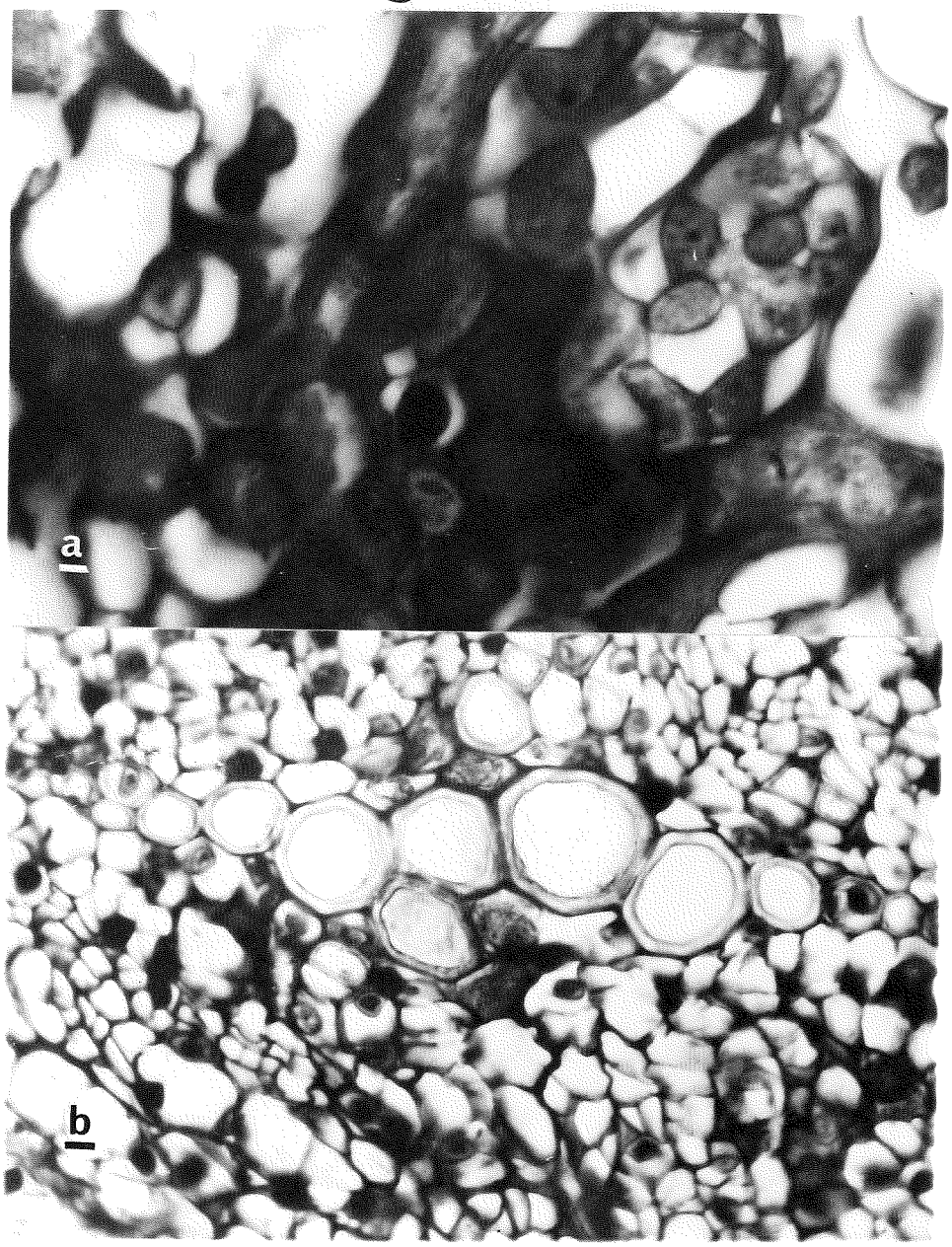


Fig. 44: Transverse sections of radish roots infected by a root strain, R10, of T. cucumeris. (x600.)

(a) Intracellular penetration of the endodermis.

(b) Intracellular penetration of the phloem.

Fig. 44



the root hairs intracellularly. Entry into the older parts of the root was mainly via wounds caused by emerging side roots. Tips of emerging side roots were rapidly colonized intracellularly. Very often infected side roots led to the colonization of the stele of the main root (Fig. 45a,b). Phloem cells were extensively colonized and hyphae penetrated a few vessels (Fig. 45b,c).

(iii) Wheat root infected by a root strain of T. cucumeris.

The roots of wheat seedlings infected with a root strain, R10, of T. cucumeris were usually covered with a web of external hyphae. Transverse sections through the root hair region showed that the root hairs were encircled by hyphae and intracellular hyphae were found inside some of them (Fig. 46a,b). Hyphae in direct contact with the piliferous layer induced dark host reactions at the site of contact (Fig. 46c). Entry into the root tip and the piliferous layers was accomplished intracellularly by single hyphae which formed slender penetration pegs during passage through the cell walls. Cortical cells were also colonized intracellularly by hyphae forming slender penetration pegs (Fig. 47c, 48a). Upon entry into the cell lumen, penetrating hyphae assumed their normal diameters. Uncolonized cells in the infected zones had granular cytoplasm and the

Fig. 45: Sections through the roots of radish seedlings infected by T. cucumeris, isolate 69.

(a) Extensive colonization of the phloem. (x400. T.S.)

(b) Enlargement of (a). (x560.)

(c) Colonization of the phloem and xylem vessels. (x560. L.S.)

Fig. 45

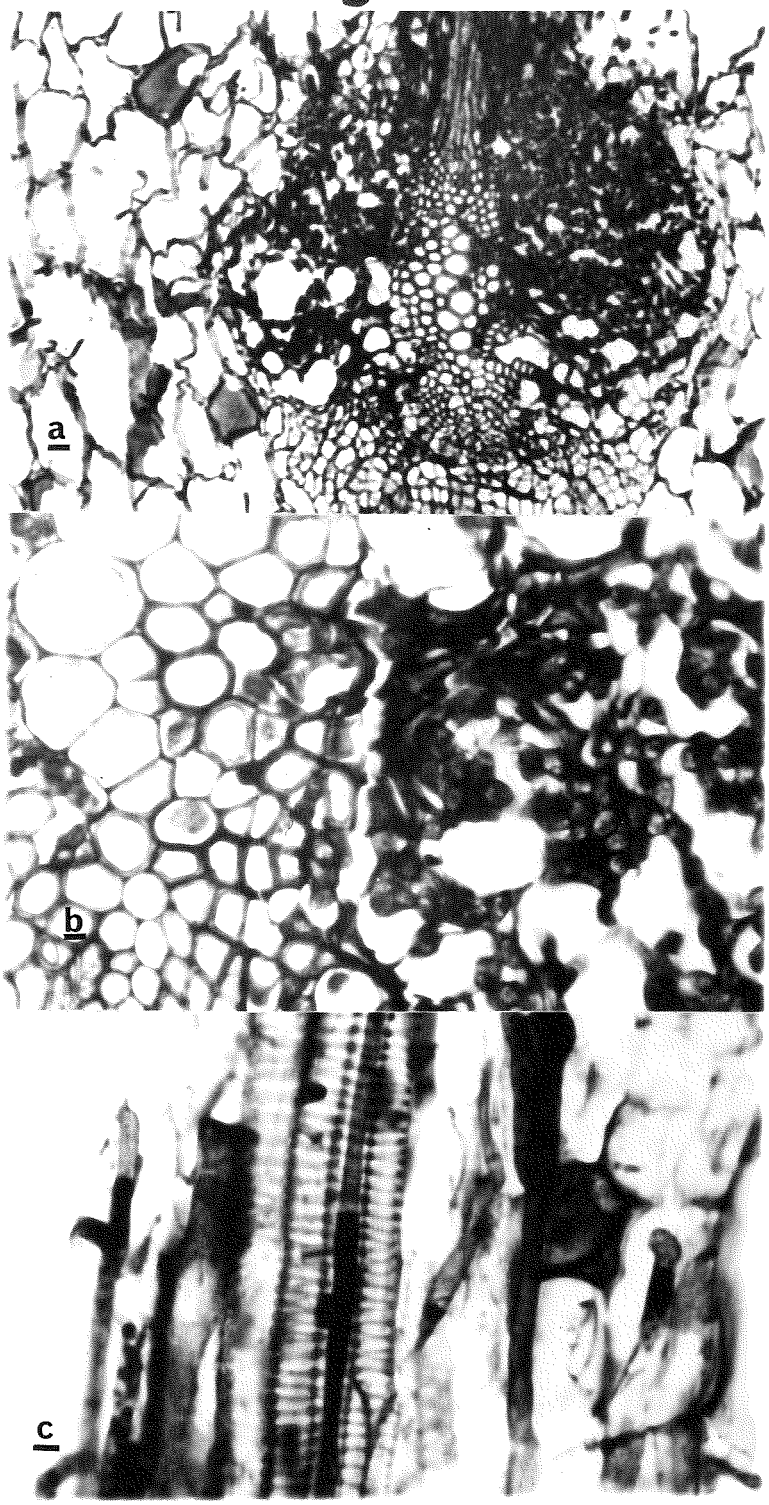


Fig. 46: Wheat roots infected with a root strain, R10, of T. cucumeris. (x560.)

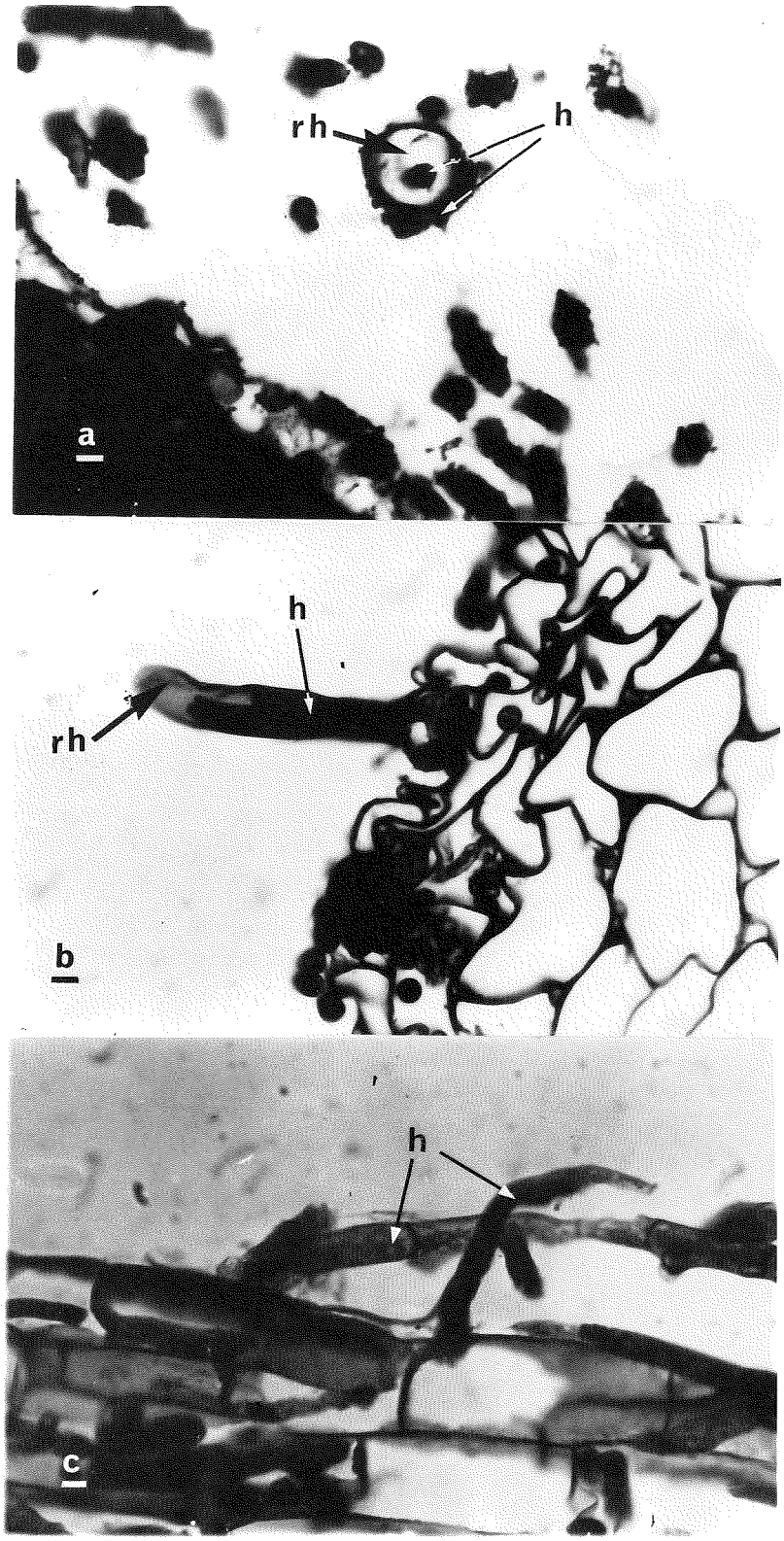
(a) Transverse section through a root hair showing hyphae on the surface and within.

(b) Transverse section of infected root showing two hyphae within the lumen of a root hair.

(c) Longitudinal section showing hyphae in contact with the piliferous layer inducing dark host reactions.

h = hyphae
rh = root hair

Fig. 46



cytoplasm was retracted from the cell walls (Fig. 47b). Most of the hyphae spread towards the centre of the root. At the regions of the root where the endodermis was absent or not lignified, the hyphae were able to enter the stele via the endodermis (Fig. 47a, 48a). Entry into the endodermis was by inter- and intra-cellular hyphae. Colonization of the young stelar tissue was mainly intracellular (Fig. 48a). Upward spread of the pathogen into the stele after full development of the endodermis was from colonized root tips (Fig. 48b). If the point of infection of the root was at or above the region where the endodermis was fully matured, the pathogen was confined to the cortex (Fig. 49) and hyphae were unable to penetrate fully matured endodermis. Although no hyphae entered the stele, gummy substances were formed in adjacent xylem vessels (Fig. 49c).

(iv) Wheat root infected by a stem strain of T. cucumeris.

The pathogen was capable of colonizing the whole of the root tip (Fig. 50a). Some of the root hairs and the cells of the root tip and the piliferous regions were colonized intracellularly by means of infection pegs. Piliferous cells that were colonized were filled with fungal hyphae (Fig. 50b, 51a). Subsequent spread of the pathogen through the cortex was mainly intracellular

Fig. 47: Sections through wheat roots infected with T. cucumeris, isolate R10, at the region before the endodermis was lignified.

(a) T.S. x400.

(b) T.S. Infected piliferous layer and cortex. (x560.)

(c) L.S. Constriction of hyphal diameter during passage through cortical cells. (x1250.)

c = constrictions of hypha
p = piliferous cells

Fig. 47

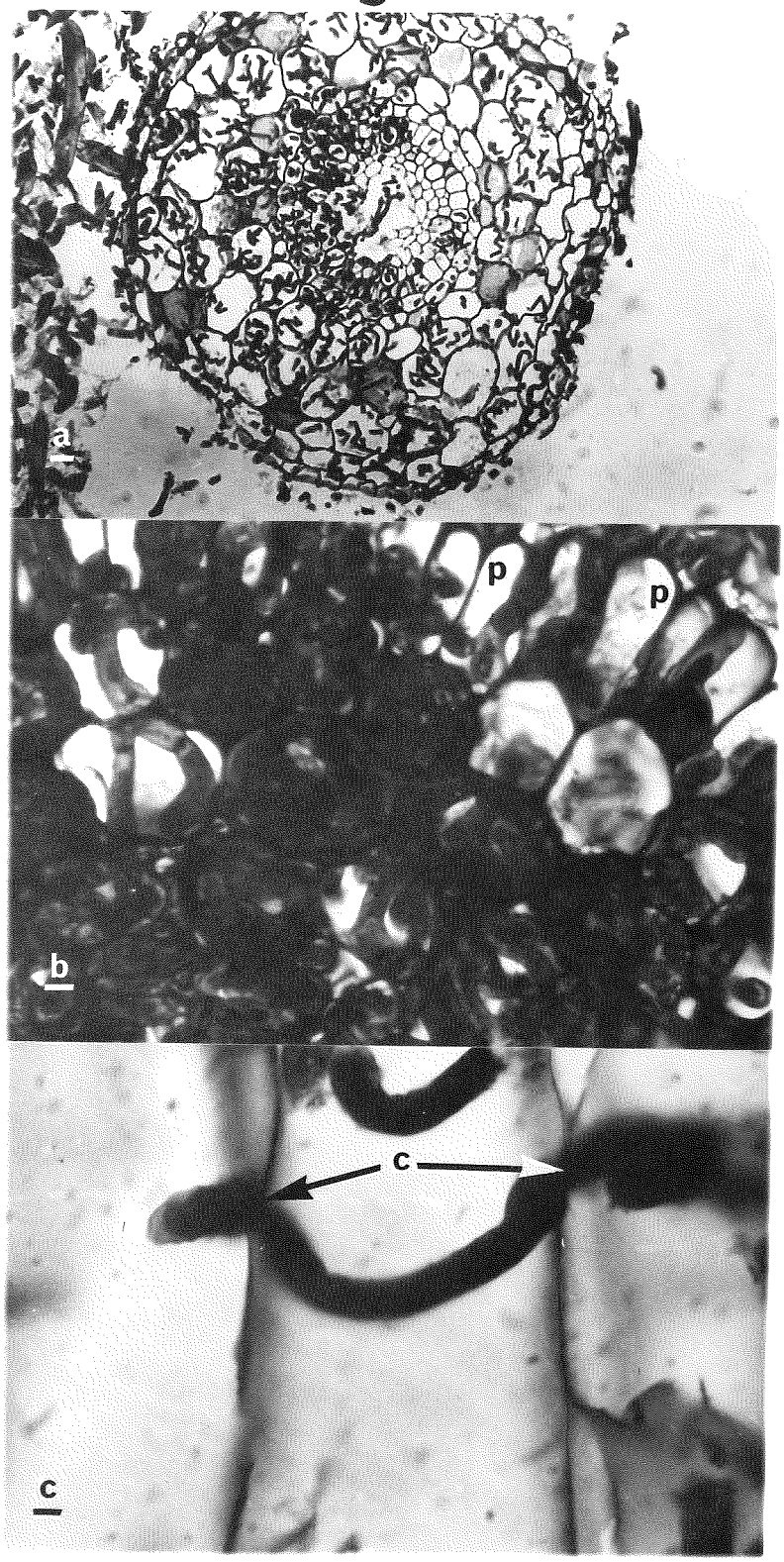


Fig. 48: Wheat roots infected with T. cucumeris, isolate R10. (x600. T.S.)

(a) Entry into stele via the endodermis before its lignification.

(b) Entry into stele via infected root tip after lignification of the endodermis.

e = endodermal cell

Fig. 48

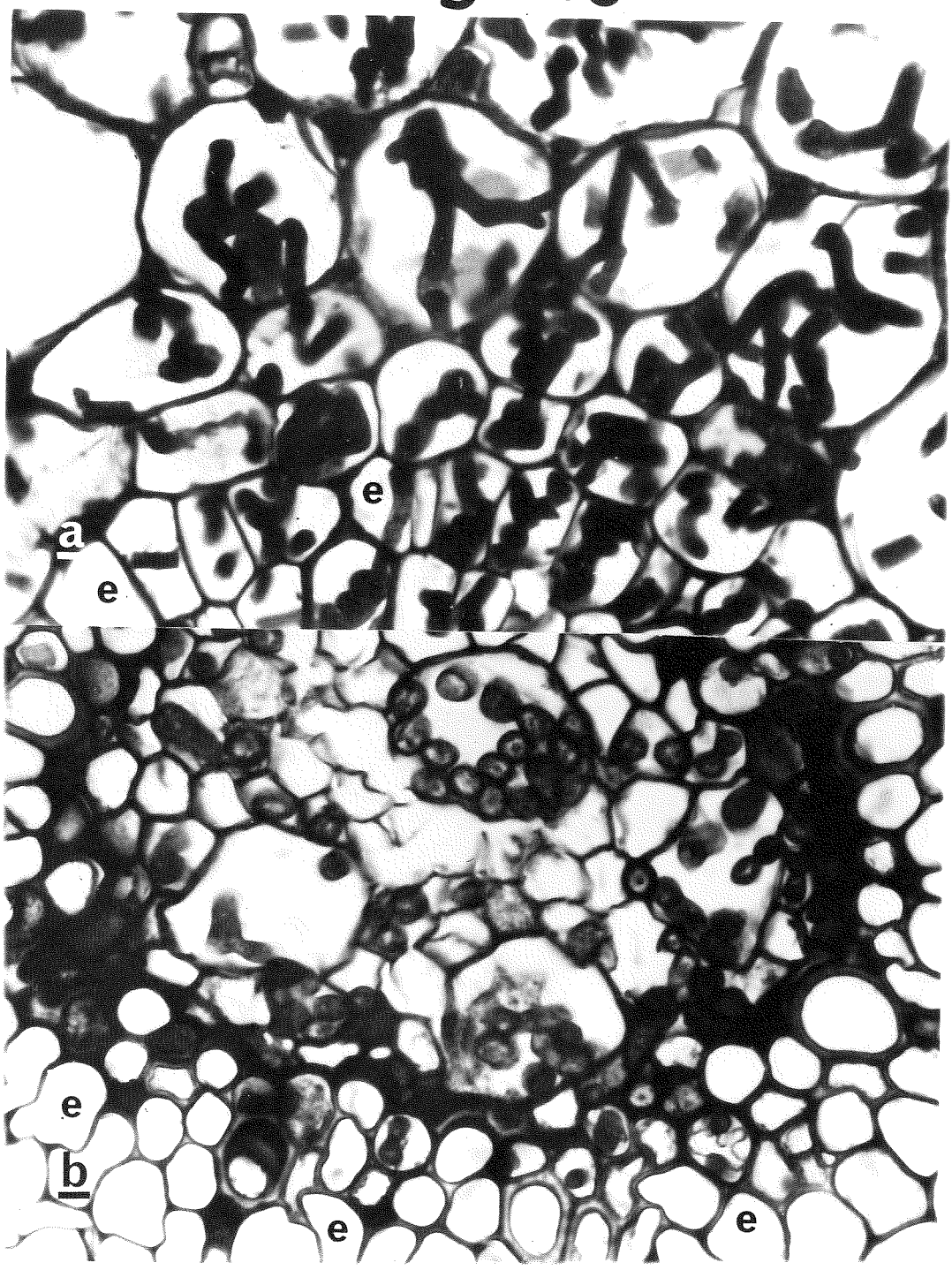


Fig. 49: Sections through wheat roots infected with a root strain, isolate R10, of T. cucumeris.

(a) x400. L.S.

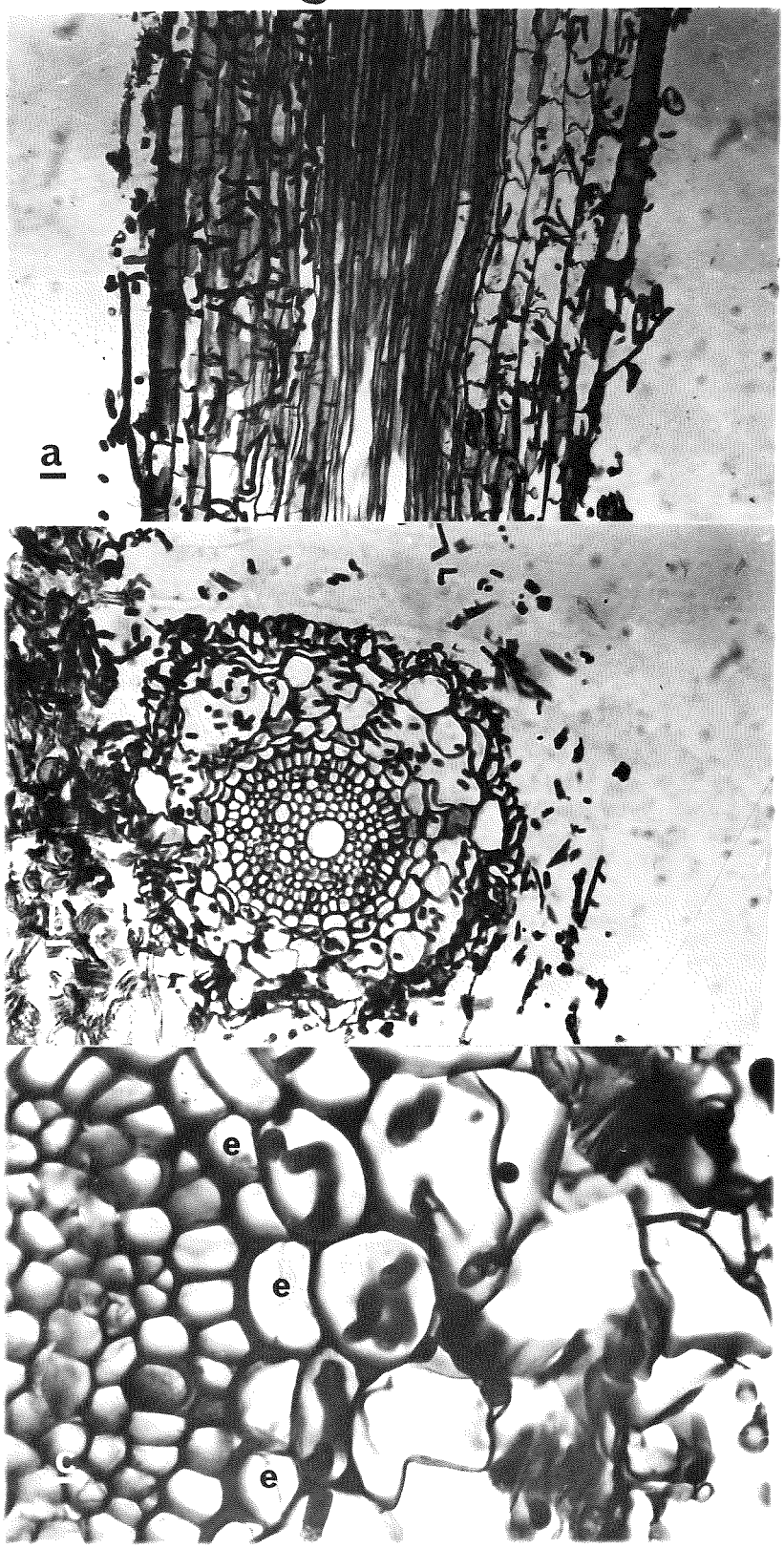
(b) x400. T.S.

(c) x560. T.S.

Note that the pathogen is confined to the cortex by the lignified endodermis.

e = endodermal cell.

Fig. 49



(Fig. 51b). Some of the cortical cells of the outer two layers and the two layers next to the endodermis had their lumens packed with fungal cells (Fig. 50b, 51b). Few of the other cortical cells were infected. Unlignified endodermis was penetrated intracellularly. The phloem was extensively colonized and hyphae penetrated xylem vessels (Fig. 51c).

Fig. 50: Sections of wheat roots infected with *T. cucumeris*, isolate 48. (x400.)

(a) L.S.

(b) T.S.

Fig. 50

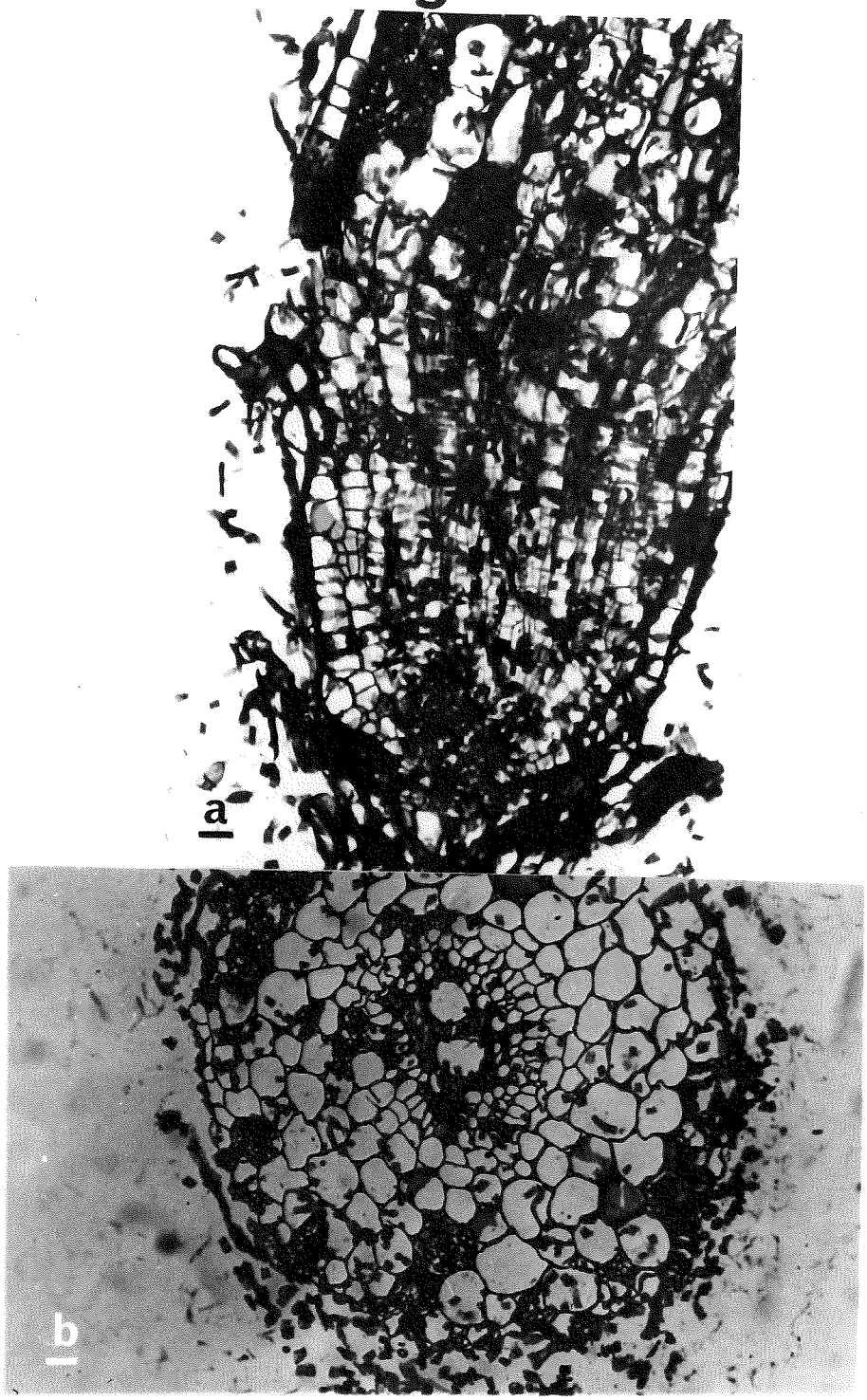


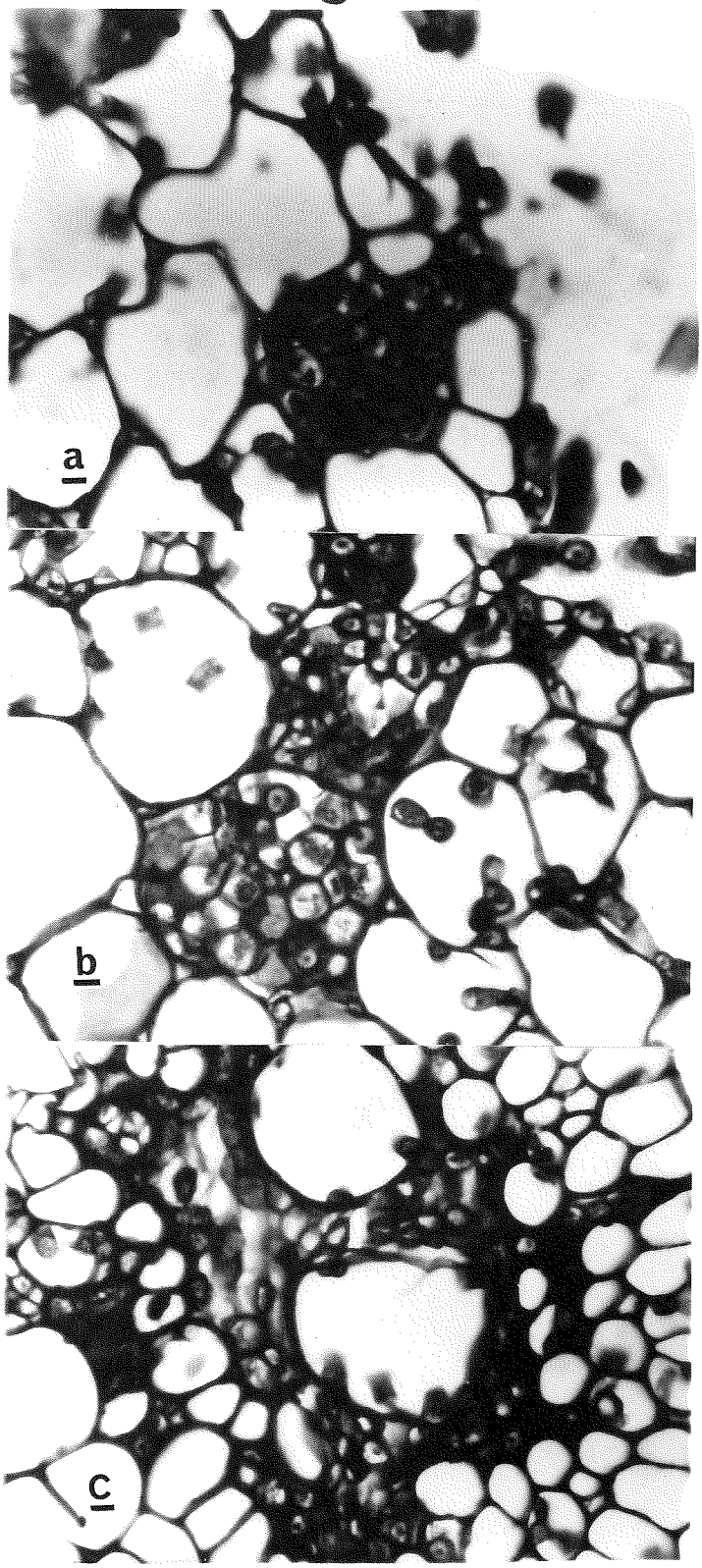
Fig. 51: Transverse sections of wheat roots infected with a stem strain, isolate 48, of T. cucumeris. (x560.)

(a) Intracellular penetration into the piliferous layer.

(b) Masses of intracellular sclerotial-like fungal cells in the cortex.

(c) Invasion of the stele.

Fig. 51



Discussion.

In part I of this dissertation, the present author discussed the variability of T. cucumeris and it's capability of causing a large variety of plant diseases. A survey of the literature shows that detailed information about the histology of host-pathogen relations of plant diseases caused by T. cucumeris is limited. It is clear that conclusions based on the examination of the interactions between a fungal strain and its host are not necessarily the same as that between other fungal strains and their host plants, let alone for different types of plant diseases caused by the same pathogen. However, there seem to be some disease processes that are common to plant diseases caused by T. cucumeris.

Differential susceptibility of closely related plants to fungal diseases constitutes one of the most interesting problems in plant pathology. One variety may be heavily infected, but another, equally exposed to different races of the same pathogen may behave differently. Many hypothesis have been put forward to explain the phenomenon. Perhaps, the greatest influence of the host on the pathogen during the pre-penetration stage of pathogenesis is when the pathogen comes in contact with the host. As such, the resistance or susceptibility of the host to a pathogen may be determined at this stage.

In the majority of the studies on the host pathogen relations, little or no consideration was given to the nature of the host surface. In fact, the host surface, had until recently, been widely accepted as an inert layer to be penetrated mechanically by the pathogen before pathogenesis can take place.

The present author has examined the behaviour of three stem strains and a root strain of T. cucumeris on the surfaces of seven test plants. All the three stem strains are crucifer-attacking strains. The hyphal growth on the surface of the two crucifer hosts and the mode of formation of infection cushions were essentially similar to that described by other authors (Abdel-Salem 1933, Flentje 1957, Christou 1962, Gonzalez & Owen 1963, Khadga et al. 1963, Dodman 1965). The behaviour of the three stem strains on the surface of five non-crucifer test plants varied considerably from hyphae which were closely appressed to plant surfaces and following the junctions of epidermal cells to loose fungal mats with or without the formation of fungal aggregates. Similar results were obtained with the root strain except that growth was very much slower. This is contradictory to the results obtained by Flentje (1957) who found that the root strain did not react to stems of cabbage, tomato and lettuce.

The ultra-structure of T. cucumeris on the host surface was studied by the present author. Using platinum-carbon replicas, the fungal tips were found to be covered by a secretion, probably mucilaginous. This covering is amorphous in nature when chemically fixed and viewed under the electron microscope. This amorphous layer is present on the outside of hyphae growing on the host as shown in the present investigations. Wood (1967) in reviewing the penetration of plants by fungi, noted the presence of mucilaginous sheaths in Botrytis cineara for attachment before penetration, around appressoria of Sclerotinia libertiana and for attachment just behind the penetrating tips of the rhizomorphs of Armillaria mellea. Mucilaginous sheaths have also been reported to surround Pellicularia filamentosa (T. cucumeris) hyphae before the formation of appressoria (Flentje 1957) although Nakayama (1960) found no such sheaths. Mucilaginous sheaths surrounding hyphae are reported for some non-pathogenic fungi, e.g. the "jelly" fungi (Moore 1965). Such sheaths are also found on fungi in artificial culture (Bracker & Butler 1963, Tanaka & Yanagita 1963). There are a number of reports of good adhesion of hyphal tips to the host surface without mucilage or a sticky substance. It is not clear how this happens. However, Dickinson (1960) suggested that such adhesion may be due to intermolecular forces between two

surfaces in close contact. Extra-cellular coatings are also found on fungal haustoria within hosts (Bracker 1967), parasitic hyphae (Boyer & Issac 1964, Schmid 1966) and mycorrihizal hyphae (Foster & Marks 1966). The importance of extra-cellular sheaths has been stressed by Bracker (1967) since enzymes and toxic substances have to pass through them before reaching the host. The nature of sheaths is unknown but they are probably secreted by the hyphae. The present author has examined the hyphae of T. cucumeris within the host cell walls and found that certain areas on the hyphal cell wall are more electron transparent than others indicating differences in permeability. Similar electron transparent areas in hyphal cell walls of wood-inhabiting and wood-destroying fungi were reported by Schmid (1966).

The fine structure of T. cucumeris on the host surface have been studied by the present author and are similar to those reported for R. solani grown on artificial media (Bracker & Butler 1963, 1964).

Almost all the ultra-structural reports of fungus-host interactions concern diseases caused by aerial plant pathogens and these are mainly confined to studies of haustoria. Ultra-structural information on the relations on soil-borne fungal parasites to host cells is limited.

The present author has examined the hyphae of T. cucumeris within host cell walls and found that the cell walls of the fungi, inside the host, are different from that growing on the outside. The cell walls of the fungi within the cell walls of the host are thin and possess electron transparent areas which may be the sites through which large amounts of enzymes are secreted. Production of enzymes is inferred because of the clearing of that part of the host cell walls next to hyphae.

Orientation of the hyphae on plant surfaces is due to the chemical and physical nature of the surface. The characteristic growth of T. cucumeris on the host surface before the formation of infection cushion is seen on isolated radish cuticle without a supply of exudate (Kerr & Flentje 1957) but not on smooth artificial membranes supplied with the host exudate as illustrated by photographs published by Flentje et al. (1963a). Hence the importance of the physical nature of the plant surface. Maheshwari and Hildebrandt (1967) found that the germ tubes of urediospores of Puccinia antirrhini are orientated at right angles to the parallel cuticular ridges of the leaf surface of snapdragon plants. Such oriented growth could also be obtained on collodion and cellulose acetate replicas of the leaf surface and they contended that such growth directs germ tubes towards stomata.

The mode of penetration of T. cucumeris isolates on seven test plants was studied by the present author. Three types of penetration were noted. Penetration by single hyphae into stomata of pea and wheat, via natural wounds caused by adventitious roots in lettuce and beneath infection cushions on turnip and radish was commonly observed. All three types of penetration have been reported in the literature. The three crucifer stem strains rarely penetrate stomata of their crucifer hosts although stomata were quite numerous. Similar results were obtained by Flentje (1957) and Khadga et al. (1963).

The present author examined the areas of the host surface covered by the infection cushions. There was no apparent discoloration or death of the host cells beneath the cushions before penetration. Similar results were obtained by Flentje (1957), Christou (1962) and Khadga et al. (1963). Linskins and Haage (1963) reported induced cutinase production by R. solani. Wood (1967) has reviewed the literature on the probability of involvement of cutinase in the penetration of the cuticle by fungi. He pointed out that since cuticle does not accumulate to any degree in nature, there must be a number of micro-organisms that are able to degrade cutin. However, as far as parasitism is concerned, there is an obvious contrast between the slow though continuous

degradation of the cuticle in soil and the rapid dissolution of cuticle required when pathogenic fungi penetrate cuticles in a matter of hours.

The importance of the host surface of radish seedlings in inducing the production of infection cushions by T. cucumeris has been studied by Flentje et al. (1963a). They found that there are discrete areas on young radish seedlings that induced infection cushion formation. These areas become fewer as the seedling ages. However, by rubbing the hypocotyls with or without an organic solvent, they found that the number of discrete susceptible areas increases and returns an old resistant seedling to the susceptible conditions. This suggests that a greater amount of wax on the older seedlings cover up areas of localized source of exudate so that these areas are not available to stimulate cushion formation. The present author made a detailed study of the radish surface in relation to host penetration by T. cucumeris. Using platinum-carbon replication methods, the surfaces of one to six-week old radish seedlings were examined under the electron microscope. One-week old seedlings have sparse wax platelets while six-week old seedlings have their surfaces almost covered by wax platelets. This finding strengthens Flentje et al.'s (1963a) hypothesis that rubbing the hypocotyls of radish seedlings

increases the discrete susceptible areas and returns old resistant seedlings to susceptible conditions. Plant waxes are variable and consist mainly of mixtures of long chain paraffins, alcohols, ketones, esters and acids. It seems likely that their compositions are characteristics of the species (Purdy & Truter 1961, Eglinton et al. 1962a;b). Waxes have been reported to possess growth-regulators (Vlitos & Cutler 1960) and materials which inhibit spore germination (Martin et al. 1957, Heather 1967). The effects of the surface waxes of plants on the hyphae of T. cucumeris are unknown. However, its presence on the surface seemed to mask areas that induce the formation of infection cushion by T. cucumeris.

The present author found that the cuticle covering the epidermis of the hypocotyl of radish is thin and uniform in thickness. Goodman (1962) has studied the structure of the apple leaf cuticle. According to him, the cutin forms the framework of the cuticular membrane, with waxes embedded within it and extruded from its surface, pectin and cellulose dilute the cutin as the membrane merges into the outer walls of the epidermal cells. Cuticular pores and ectodesmata are subjects of controversy. Early studies of plant surfaces with the electron microscope failed to show cuticular pores through which wax might reach the surface (Schieferstein & Loomis

1959, Juniper 1960). However, later, evidence was obtained of pores in leaf cuticles associated with waxy extrusions on their surfaces (Hall & Donaldson 1962, Hall 1967a;b). Pores in the epidermal cell wall lined with cytoplasm and plugged by cutin covered with wax have been reported (Scott et al. 1958) and these plasmodesmata are thought to be anchored in the cuticle of the outer walls of all epidermal cells (Scott 1966). Franke (1967) reviewed the literature on plant ectodesmata. He reported that ectodesmata are submicroscopic structures which extend from the cuticle, which they do not perforate, through the wall to the lumens of the epidermal cells. These ectodesmata are non-protoplasmic and, therefore, not comparable with that of plasmodesmata. The ectodesmata of leaves were described by Franke as being filled with liquid excretion of the epidermal protoplasts, somewhat broadened intermicellar spaces and they can be demonstrated using a special chemical treatment. In leaves, they are predominantly found along anticlinal epidermal cell walls, in some hairs, in the basal cells of hairs, in epidermal cells surrounding hairs, above, beneath and on both sides of veins and in guard cells. Ectodesmata are thought to be sites of foliar absorption as well as foliar excretion and cuticular transpiration.

The discrete susceptible sites on young radish

seedlings were further studied by the present author using the initials of infection cushions on the host surface as indicators. These sites were studied using platinum-carbon replication methods after removal of the surface wax, ultra-thin sectioning and lead infiltration followed by ultra-thin sectioning. Most of the replicas showed single pit-like structures generally distributed over the surface. However, after scanning through many replicas, aggregates of pits covering an area of diameter 0.5 - 1μ were found. A small cushion covers an area of approximately 1 mm in diameter. Therefore, aggregates of pits cover an area of about one thousandth that covered by a small cushion. It is not known whether these pits are surface plasmodesmata, ectodesmata or pores through the cuticle. Since these pit-aggregates are found on the host surface beneath infection cushions, they could be associated with a greater diffusion of plant exudates than the surrounding areas. It is not known whether or not these aggregates of pits serve as passage for waxes. Ultrathin sections through the sites of cushion formation failed to reveal any pores. The reasons may be that the sections were thicker than the diameters of the pits and the sections could have missed the aggregates of pits altogether.

Sections of lead infiltrated cells at the sites of

cushion formation showed that certain cells contained more lead particles at one part of their outer cell wall than the rest of the cell wall. This suggests that one part of the outer epidermal cell wall is more permeable to solutions than are other parts. Some of the lead infiltrated sections were shadowed with platinum-carbon but no breaks in the cuticles were detected. Also, certain cells show more lead particles at their junctions than others. This is interesting because the hyphae of T. cucumeris on the surface of susceptible hosts tend to follow the junctions between the epidermal cells. Also, the sites of appressorium formation of aerial pathogenic fungi tend to be at the grooves between adjacent epidermal cells. Examples are Erysiphe polygoni on red clover leaves, Peronospora parasitica on cauliflower leaves (Preece et al. 1967) and Gloesporium musarum on banana fruits (Meredith 1964). Yeast and bacteria too tend to accumulate at junctions between the epidermal cells (Ruinen 1961). The present author's findings of pit-aggregates and differential areas of permeability at the sites of cushion formation lend support to the claims of Flentje et al. (1963a) that on the stems of young radish seedlings there are discrete areas of localized exudation which stimulate the production of infection cushions by T. cucumeris. The permeability of the cuticle may be affected by environmental factors such as

weathering and degradation from exposure to sunlight, rain, buffering by wind and the activities of insects. Cracks in the cuticle can result (van Overbeck 1956, Currier & Dybing 1959) and Boosalis (1950) has suggested that natural cracks are avenues of entrance of R. solani hyphae into the hosts. Since radish seedlings grow to a height of approximately six centimetres from soil level in about a week after sowing, it is not unreasonable to suppose that tiny cracks exist in the cuticle as a result of growth through soil and a rapid extension of the hypocotyl.

Kerr and Flentje (1957) inoculated radish seedlings, from which strips of epidermis and cuticle had been removed or which had been punctured in several places by fine glass needles, with the radish strain of P. filamentosa (T. cucumeris). They found that both treatments increased the number of infection cushions produced, these structures being formed not on the exposed cortical cells, but on the cuticle immediately alongside such cells and penetration was always through cuticle and epidermis never through the wounded tissues. The present author punctured the cuticle of radish seedlings and removed any residual cuticle over the punctures before inoculation to determine the relative importance of the cuticle in the colonization of the injured cortex by hyphae of T. cucumeris. The treatment

increased the number of infection cushions formed at the edges of the punctures. No cushions were formed on the top of the exposed tissue. This confirms the findings of Kerr and Flentje (1957). However, if the cushions were removed before penetration occurred beneath them, it was found that hyphae of T. cucumeris were able to enter the wounds and successfully colonize the injured cortical tissue. This is in variance with the conclusions of Kerr and Flentje (1957) that penetration was always through cuticle and epidermis never through the wounded tissue. The fact that hyphae of T. cucumeris are able to colonize tissue through wounds may be an important factor in their survival and wide distribution. Non-host plants do not stimulate T. cucumeris to form infection cushions. If such plants were wounded, naturally or otherwise, they become predisposed to attacks by T. cucumeris hyphae. Such may be the case of the entry into wheat roots by the root strain, R10, in a synergistic relationship with nematodes as discussed in part one of this dissertation. Entry into non-hosts was also noted by the present author as a result of wounds caused by emerging adventitious and side roots. Although only limited areas of the non-host plants were usually attacked, such attacks may supply sufficient nutrient for the pathogen to carry over to the next season when susceptible plants are available.

Since appressoria of aerial fungi and infection cushions of soil-borne fungi are associated with penetration into plants, these structures have been regarded to function solely as penetrating organs (Flentje 1959, Dickinson 1960, Wood 1960; 1967). It is apparent from the reviews by the authors cited above that both appressoria and infection cushions are structures induced in the case of appressoria of aerial fungi, by a contact stimulus while infection cushions of soil-borne fungi are induced by chemical stimulus. There is evidence that appressoria have other functions in addition to penetration of hosts. Young (1926) found that Alternaria appressoria often do not produce penetration hyphae which infect cells below them. He also found that a majority of the specialized appressoria of Colletotrichum nigrum seen on wheat were not associated with infection. Similarly, Meredith (1964) found that only a minor proportion of the total appressoria of Gloesporium musarum present on banana fruits gave rise to lesions. It is likely that the dormant appressoria are able to respond to favourable conditions at a later date in the event of penetration hyphae from other appressoria failed in parasitizing the host. If this is the case, then appressoria contribute to the survival of the pathogen. In the case of T. cucumeris, the surface morphology of

big infection cushions is almost similar to that of small sclerotia developed on the host surface. Whether or not infection cushions have any survival value is unknown.

The present author used sclerotia of T. cucumeris to inoculate radish seedlings to see if sclerotia could act as infection cushions. No penetration was seen beneath the sclerotia. This may be due to poor contact between the sclerotia and the host surface. Only a small percentage of the sclerotial cells germinated to produce hyphae which formed infection cushions. This is in agreement with the observation of Pitt (1964b). Pitt found that sclerotia of R. solani are able to regerminate at least ten times and he contended that such a property enhances the survival of the pathogen.

Kerr and Flentje (1957) found that no penetration of "cellophane" ever occurred in response to plant exudate by Pellicularia filamentosa (T. cucumeris) even though good infection cushions were formed. They suggested that the nature of the surface on which organization occurred might be important in bringing about penetration. The present author found that the stem strains of T. cucumeris penetrated the radish surface with no constriction of the penetration hyphae. This was also reported by Boosalis (1950), Dodman (1965) and Dodman et al. (1968b). Wood (1967) summarised the current view in regard to infection

cushion and penetration. He pointed out that in diseases of plants at or below soil level there is a marked tendency for hyphae to come together resulting in a large area of adhesion so that the hyphae are not pushed away when penetration begins. The extent and strength of adhesion depends on the hardness and toughness of the surface to be penetrated and on the diameter of the infection hyphae. In the case of T. cucumeris the present author found that infection cushions of the stem strains on the surface of radish seedlings prior to infection could easily be displaced using a needle. Therefore, the current view that infection cushions of T. cucumeris provide the means of exerting mechanical force on the cuticle prior to penetration is inadequate in explaining the entry of the stem strains into radish.

The present author has studied the morphology of the radish seedling hypocotyls in relation to penetration from infection cushions formed by the stem strains of T. cucumeris and as a result suggests the following hypothesis to explain the relations between the infection cushions and host penetration. Hyphae of the stem strain of T. cucumeris come in contact with a crucifer seedling and grow along the hypocotyl following the longitudinal junctions of the epidermal cells. Hyphae in the vicinity of areas of greater diffusion of plant exudate are stimu-

lated to form infection cushions. As such the morphogenic changes of the hyphae at the bottom of the infection cushion are greater than that on the top. A dislodged infection cushion shows that the parts nearest the surface are compact whilst that on the top the cells are not as compact. If the site of the development of the fungal cushion is in a confined space between projecting epidermal cells, a tremendous force could be exerted on the side walls of the area occupied by dividing and expanding fungal cells. A state will be reached when the projecting epidermal cells are forced apart laterally and the break in the host cuticle beneath the infection cushion will allow fungal hyphae to penetrate the host with little or no change in diameter and the minimum amount of preassure exerted directly onto the surface. This is in agreement with the observation that mycelium of T. cucumeris are able to penetrate into wounds on radish hypocotyls. The epidermal cells are long and tapering. Therefore, areas on the host surface, which could serve as leverage for causing a break in the host cuticle by expanding fungal cushions, are common. These consisted of depressions where the narrow tapering ends of epidermal cells lie between the protruding central parts of two adjoining epidermal cells. This hypothesis of host penetration incorporates the report of Flentje

et al. (1963a) that chemical stimuli control the development of infection cushions on the host surfaces by T. cucumeris. The hypothesis is supported by the observation of Kerr and Flentje (1957) that there were no penetration by well defined cushions on "cellophane" in response to host exudate. The present author has examined numerous infection cushions on radish seedlings and found that in all cases (except those above stomata) when small infection cushions had effected penetration, they were confined to narrow spaces between epidermal cells. However, sites of localized diffusion of exudate may not coincide with sites for effective penetration. In these cases, the infection cushions could reach a size when their edges come to occupy sites for effective penetration and penetration is then effected. If these cushions were examined, their centres do not coincide with sites of infection. It is important to examine only cushions with initial infection since at a later date, due to an increase in exudation at the site of penetration, the original centres of the young cushions are lost. Therefore, old cushions will have their centres over the sites of penetration. The present author also found that newly penetrated areas, after removal of the fungal cushions were elongated in shape. This suggests a wedge-like action by penetration hyphae at a single point after initial entry into the host. If penetration were to

take place simultaneously by the production of fine infection pegs beneath cushions, penetrated areas would take the approximate shape of the cushions, not the shape observed during this investigation.

It would appear that there are two mechanisms by which hyphae of T. cucumeris enter the host beneath infection cushions. One in which the cushion acts as a composite appressorium in which hyphae in contact with the host surface penetrates into the host simultaneously by means of fine infection hyphae. The other, hitherto unrecorded, in which expanding cushions apply lateral force to projecting epidermal cells thereby forcing them apart and causing breaks in the cuticle. This, then, allows hyphae from beneath the cushion to enter the host unconstricted.

The mechanism of entry by T. cucumeris into roots differed significantly from the mechanism of entry into stems. In the case of roots, infection cushions are either absent or poorly developed. The piliferous layer of the root consists of the epidermis and at the root-hair region, there are numerous root hairs. The root hairs extend out into the soil and very often they are the first to come into contact with the pathogen. The root hairs as possible sites of penetration into the host have been largely ignored by plant pathologists.

Flentje (1957) in studying the parasitism of Pellicularia filamentosa (T. cucumeris) on cabbage, tomato, lettuce and wheat roots noted that hyphae became mixed with the root hairs. Chi and Childers (1963) studied the infection process on the roots of alfalfa and red clover. They did not consider the root hairs. The present author studied the infection process of T. cucumeris of a stem and a root strain on the roots of wheat and radish. In all cases, the hyphae were found to grow on the surfaces of root hairs. An intense dark host reaction was induced before infection. Entry into root hairs was by fine infection pegs. On entry into the lumen, the hyphae resumed their normal diameter. Hyphae were seen to grow along the lumens of root hairs towards the bases. It is not known whether or not hyphae are capable of entering the lumens of broken and senescent root hairs. The fact that hyphae of T. cucumeris are capable of infecting root hairs and then multiply at the root hair bases point to the importance of root hairs as avenues of infection. The piliferous layers of wheat and radish were penetrated by single hyphae by means of fine penetration pegs when inoculated with a root or a stem strain of T. cucumeris. However, in the case of radish roots inoculated with a stem strain, entry was mainly via wounds caused by emerging side roots.

When hyphae enter radish hypocotyls a lesion is formed in the epidermis and outer cortex immediately below infection cushions. These lesions consist of host cells filled with intra-cellular hyphae. Such a lesion is apparently absent when cotton hypocotyls were infected with T. cucumeris (Khadga et al. 1963) and the photographs published of sections through infected tissue did not show such a lesion. Lesions formed on bean hypocotyls were different from those formed on radish hypocotyls. The lesions on beans were limited (Christou, 1962) i.e. both the intra- and inter-cellular hyphae were usually contained within the first few layers of the cortical cells although no morphological barriers were seen to develop in the host tissue. In the case of the cotton hypocotyls (Khadga et al. 1963), the epidermis was penetrated intracellularly and the whole of the cortex was invaded intercellularly. Hyphae were seen to grow from the edge of the lesion in radish in between the cuticle and the epidermis thus dislodging the cuticle from the epidermis. Similar results were obtained by Christou (1962) and Khadga et al. (1963). From the lesion, hyphae were seen to spread rapidly in the cortex of radish intercellularly. At a later stage, some of the cortical cells were penetrated intracellularly without a change in hyphal diameter. The endodermis was penetrated both inter- and intra-cellularly. The phloem was

penetrated intracellularly while the xylem was not colonized. Similar results were obtained by Khadga et al. (1963) with cotton hypocotyls. Christou (1962), however, found that infection of bean hypocotyls was confined to the cortex. At the completion of infection, the present author found no sclerotia formed in the cortex. Similar results were obtained by Khadga et al. (1963) with cotton hypocotyls. However, sclerotia was found in bean hypocotyls (Christou 1962). Flentje (1957) studied the invasion of the crucifer strain and the composite strain of T. cucumeris on their respective hosts and found that entry and subsequent spread of the pathogen in the host were intracellular by means of fine infection pegs.

The present author found that in the case of the wheat and radish roots inoculated with a root or stem strain of T. cucumeris penetration into the root was either through the piliferous layer or from the base of root hairs by hyphae producing fine infection pegs. The lesions as seen in the radish hypocotyls were absent. Subsequent spread of the pathogen into the cortex, endodermis and phloem but rarely into the xylem vessels was mainly intracellular by means of infection pegs. However, in the case of wheat roots, depending on the site of entry, the pathogen could be confined to the

cortex. When wheat root is inoculated with a stem strain, sclerotial fungal cells filled some of the cortical cells. Flentje (1957) studied the modes of penetration of the root strain of Pellicularia filamentosa (T. cucumeris) into the roots of cabbage, lettuce and wheat. His report of the spread of the pathogen through the cortex is similar to that of the present author. However, he did not report the entry of the pathogen via wounds caused by the emerging side roots. Chi and Childers (1963) found that Pellicularia filamentosa, after penetrating the epidermis of alfalfa and clover roots, spread through the cortex, endodermis, phloem and xylem by means of inter- and intra-cellular hyphae. At the completion of invasion, they noted sclerotia were formed in the cortex and in the pith.

In the infection of the hypocotyls of radish, the present author did not detect any lignitubers, callosities or calluses (Akai 1959) in the colonized tissues. Even when the whole of the cortex was colonized, no obvious structural changes were evident with the exception that the intracellular hyphae in the lesion directly beneath the infection cushion became dark in colour. Sections of intercellularly colonized hypocotyl tissues show a striking resemblance to sections of some ectotrophic mycorrhizal rootlets of plants. This is

interesting because some of the perfect states of Rhizoctonias from orchids have been identified as T. cucumeris (Warcup & Talbot 1967). However, the penetration of the endodermis and the stele caused the cytoplasm of the phloem to become granular and withdrawn from their walls. This eventually led to the collapse of the whole hypocotyl.

When roots of wheat and radish are inoculated with either a stem or root strain of T. cucumeris, an intense dark host reaction was seen at the site of host-pathogen contact. Cells within the colonized tissue have granular cytoplasm which was withdrawn from the cell walls. Besides granulation and withdrawal of the cytoplasm from the cell wall little or no other obvious symptoms were noticed by other authors in newly colonized tissues (Flentje 1957, Christou 1962, Khadga et al. 1963, Chi & Childers 1964).

It is apparent that the mechanisms of penetration and subsequent spread of the hyphae of T. cucumeris within plants are variable and complex. This may reflect the pathogen's enormous flexibility in causing a large number of plant diseases and its ability to survive under a range of ecological environments.

PART III: PHYSIOLOGICAL ASPECTS OF THE HOST-PATHOGEN RELATIONSHIP.

Introduction.

There is ample evidence to show that when pathogens enter and colonize hosts alterations of the host physiology occur. These changes have interested plant pathologists and plant physiologists alike for some time since knowledge of the regulation of the metabolism of diseased hosts may lead to an understanding of the mechanisms of susceptibility and resistance of hosts to fungal attacks.

The physiology of the interactions between plants and T. cucumeris can be conveniently divided into two stages: before and after host penetration. Before penetration the greatest apparent effect is on the pathogen. After host penetration the most dramatic apparent effect is on the host.

Before host penetration.

This part of the host-pathogen relationship has been reviewed by Flentje (1959, 1965) and Wood (1967). The influence of plant exudates on soil-borne fungi has been reviewed by Börner (1960), Woods (1960), Schroth and Hildebrand (1964) and Rovira (1965a;b). Evidence has

been presented (Kerr 1956, Kerr & Flentje 1957, Flentje et al. 1963a, Dodman 1965) that the formation of infection cushions by T. cucumeris on susceptible hosts was controlled by chemicals in the plant exudates. Flentje (1957) showed that different strains of Pellicularia filamentosa (T. cucumeris) react differently on different plant surfaces. This has been confirmed by the present author in part two of this dissertation. Flentje (1957) also showed that a crucifer strain formed infection cushions on the stems of crucifer hosts whereas the same strain failed to react on solanaceous seedlings. Similarly, a solanaceous strain formed cushions on solanaceous hosts but not on crucifers. Later, Flentje et al. (1963a) showed that infection cushions were formed by T. cucumeris on collodion membranes overlying either host tissue or agar blocks containing exudates from hosts. In radish seedlings, exudates diffusing out of the roots, hypocotyls and cotyledons induced infection cushion formation. Whether or not differences exist between exudates from different parts of the plant are unknown. Dodman (1965) made preliminary studies of the properties of the exudates from radish seedlings but was unable to identify the chemical nature of the active principle. Further evidence that substances in plant exudates may influence the formation of infection structures by soil-borne fungi was presented by Hussain and

McKeen (1963) who worked with Rhizoctonia fragariae. They reported that various amino acids in the root exudate induced the formation of fungal pads. Guttation fluid is also found to stimulate the formation of infection structures of Helminthosporium sorkinianum (Endo & Amacher 1964, Endo & Oertli 1964). Similarly, wheat root exudate has been shown to stimulate the formation of pre-penetration structures by H. sativum (Ullah & Preece 1966).

However, the theory that infection cushions are formed in response to specific substances exuding from susceptible but not from resistant hosts was challenged by de Silva and Wood (1964). They worked with a lettuce-attacking and a cabbage-attacking strain of T. cucumeris. They found no correlation between the stimulation of the two T. cucumeris isolates on "cellophane" in vitro and the source of exudate causing stimulation. Infection cushions formed on epidermal strips from lettuce and cabbage regardless of the source of exudate added to the strips. They found that the isolates tended to form infection cushions on the epidermal strips to which they were pathogenic and they contended that the nature of the epidermis was important in the formation of infection cushions.

Although it is clear that the formation of infection

cushions of T. cucumeris is stimulated by exudates from susceptible hosts, further evidence is required before it can be concluded that specificity is governed by the exudates, the nature of the host surfaces or both. However, the present author has demonstrated, in part two of this dissertation, that successful penetration of the radish host beneath an infection cushion is intimately related to the topography of the host surface.

Although infection cushions of T. cucumeris are known to be structures stimulated to form by exudates of susceptible host plants, nothing is known of the biochemical changes induced in the fungal hyphae that accompany the stages in the development of infection structures. Such a study together with the chemical analysis of the plant exudates may lend to the identification of the active principles involved in the stages of morphogenesis of T. cucumeris infection cushions.

After infection.

One of the symptoms of plant disease is the striking change in the rate of respiration of infected tissues. Respiratory changes in diseased plants have attracted much attention since such changes indicate changes in host metabolism. A number of reviews on respiratory metabolism have appeared during the last ten years (Allen

1959, Uritani & Akazawa 1959, Rubin & Artsikhovskaya 1963, Shaw 1963, Sadasivan 1964, Goodman et al. 1967, Wood 1967, Yarwood 1967). Usually, an intensification of respiratory metabolism begins to take place from the moment of infection, becoming most pronounced at the time of rapid proliferation of the pathogen, followed by a fall in respiratory activity of infected tissues. This reaction is not specific since chemical and mechanical injuries as well as pathogens can increase the rate of plant respiration. The fall in respiratory activity may be due to necrosis of the infected tissue leading to the death of the affected organ or the whole plant or in the case of resistant plants it may be an indication that infection has been overcome and that oxidative metabolism has returned to its initial rate.

There are many factors that contribute to the changes in the respiratory activities of the host upon infection. Increased respiratory activities of hosts has been attributed to secretions by pathogens within the hosts. In part two of this dissertation, the present author showed that upon entry into the host, the hyphae of the pathogen colonized the first few layers of host cells, directly beneath the infection cushion, intracellularly and then rapidly spread through the cortex intercellularly. On completion of invasion, the whole of the hypocotyl

collapsed. This is a phenomenon common to many Rhizoctonia diseases and many factors may contribute to such a collapse. Since the parenchymatous cell walls are composed mainly of pectic substances and cellulose, the production of cell wall degrading enzymes by T. cucumeris apparently occurs.

The structure of the plant cell wall and the chemical composition of the different components in relation to various plant diseases have been treated in some details in two recent books (Goodman et al. 1967, Wood 1967). Although many studies have been done on cellulose and pectic substances during the last decade, details of the inter-relationship of the cellulose and the pectic substances with other substances in the native state within the cell wall are still debatable. This may account, in part, for the fragmentary knowledge of the mechanism of the cell wall degrading enzymes secreted by pathogenic organisms within hosts.

The roles of cellulolytic and pectinolytic enzymes in the degradations of plant tissues have been reviewed recently (Husain & Kelman 1959, Wood 1960, Reese 1963, Brown 1965, Mandels & Reese 1965, Rateman & Millar 1966, Goodman et al. 1967, Norkrans 1967, Wood 1967). Almost all of what is known about the breakdown of cell walls by plant parasites comes from the study of soft rots

(Wood 1967).

In spite of the intensive study by many workers the mode of action of cellulases is not known with certainty. One view is that there is a single enzyme which by random cleavage converts the cellulose to glucose (Whitaker 1957). Another view is that a second enzyme, cellobiase, is required for the conversion of cellobiose to glucose (Aitken et al. 1956) whereas Gascoigne and Gascoigne (1960) and Mandels and Reese (1965) consider that cellulose is degraded completely only by successive action of a number of enzymes, the first designated C_1, converts native cellulose into soluble straight chains of anhydroglucose residues which are then degraded by a series of enzymes, Cx, which hydrolyse the β1, 4 glucosidic linkages to produce cellobiose, this then being converted to glucose by the action of a β-glucosidase or cellobiase. However, Thomas and Whitaker (1958) suggested that the apparent multiplicity of cellulases is an artifact which results from the formation of complexes between a single enzyme and various degradation products of cellulose. This is supported by the observations of Eriksson and Patterson (1968). So far, a completely carbohydrate-free Cx enzyme system has not been isolated (Norkrans 1967). However, the multi-enzymic hypothesis is currently the most acceptable in explaining the breakdown of

cellulose (Norkrans 1967, Wood 1967).

Most micro-organisms produce cellulases *in vitro* much more freely when the main, or only energy source is cellulose or a cellulose derivative (Wood 1967). Therefore, most cellulases are adaptive or inducible enzymes and their formation or activation is repressed by the presence of sugars (Mandels & Reese 1965). Wood (1967) noted that few attempts have been made to detect cellulases in rotted tissues or to extract them from diseased plants. Probably, this is because the demonstration of cellulase in a host plant is difficult (Mandels & Reese 1965). Since *T. cucumeris*, as shown in part two of this dissertation, is capable of penetrating the cell walls of its hosts especially during intracellular penetration and causing the collapse of the hosts, it appears that *T. cucumeris* hyphae are able to destroy native cellulose in the cell wall.

Although much attention has been devoted to the study of pectinolytic enzymes and their roles in plant diseases, much confusion exists in the literature since most of the investigations were done with ill-defined pectic substances and impure pectic enzymes. In a recent review, Bateman and Millar (1966), divided the pectinolytic enzymes into two main groups, the pectin methylesterases and pectic glycosidases and lyases.

Pectin methylesterases (PME) are hydrolases and are common among higher plants and micro-organisms. They remove the methoxyl groups of pectin or pectinic acid to yield pectic acid. Their activity is little affected by chain length of the substrate. PME are considered unimportant in the maceration of plant tissues. Pectic glycosidases and lyases effect the cleavage of the α, 1-4 glycosidic bond between adjacent uronic acid monomers in pectic substances by either a hydrolytic (hydrolases) or a trans-eliminative (lyases) mechanism. Enzymes that have a substrate "preference" for pectic acid are polygalacturonases (PG) or polygalacturonate-trans eliminase (PGTE) while enzymes that attack pectin preferentially are named polymethylgalacturonase (PMG) or pectin methyl-trans-eliminase (PMTE). The prefix "endo" or "exo" are used to designate, respectively, a random or terminal cleavage.

Maceration of plant tissue as a result of pathogenic attack by micro-organisms has been associated with a single enzyme, protopectinase. However, there is evidence that maceration is due to a number of enzymes and both Bateman and Millar (1966) and Wood (1967) suggested that the non specific term "macerating enzyme" be used instead of protopectinase.

McClendon (1964) and Bateman (1967) have presented

evidence that endo-PG and endo-PGTE are involved in tissue degradation. However, according to Bateman (1967) endo-PG is of major significance in diseased tissue. Wood (1967) has presented evidence that maceration of tissue may involve enzymes other than the polygalacturonase type. However, the evidence for these other mechanisms is scanty. Some of these macerating enzymes are of host origin (Tani 1967) and some, although of fungal origin, are not pectic enzymes. For example, Kůc (1962) demonstrated the importance of proteolytic enzymes of Cladosporium acting in synergistic action with pectinolytic enzyme in tissue maceration. In part two of this dissertation, the present author showed that although lesions beneath infection cushions initially contained intracellular hyphae, subsequent spread of the pathogen through the cortex was rapid and this involved intercellular hyphae. Ultra thin sections of radish cell walls with intercellular hyphae in situ showed signs of cell wall degradation next to the hyphae.

Induced resistance to infection.

(i) The effect of cations.

Bean hypocotyl tissues accumulate calcium on maturity in the form of calcium pectate which has been correlated with resistance to polygalacturonase activity of R. solani

(Bateman 1964a, Bateman & Lumsden 1965). Bateman (1964a) is of the opinion that the formation of calcium pectate prevents intercellular spread of R. solani thereby limiting the size of the lesion. In part two of this dissertation, the present writer showed that on radish hypocotyls, T. cucumeris after the formation of the lesion, spreads rapidly through the cortex intercellularly. It would be interesting to find out if the addition of different cations would affect the susceptibility of radish hypocotyls to attacks by T. cucumeris.

(ii) The effect of ethylene.

Ethylene is a natural product from all plant tissues (Burg 1962), a common metabolic product of many fungi (Ilag & Curtis 1968) and some bacteria (Freebairn & Buddenhagen 1964, Goodman et al. 1967). It's concentration is highest in the youngest plant parts where the natural auxin level is also greatest. An application of auxins to plants increase ethylene production in a short time (Morgan & Hall 1962). A homeostatic regulation by auxin and ethylene exist in plants (Burg & Burg 1968). Therefore, an excess of auxin tend to maintain a leaf in a non-senescent state and an excess of ethylene to enhence senescence (Nichols 1966, Galliard et al. 1968). It is biologically active in very small amounts and is involved in leaf abscission (Abeles & Rubinstein

1964) and in fruit ripening (Burg & Burg 1965). It is produced by injured and diseased plant tissues (Dimond & Waggoner 1953, Smith *et al*. 1964, Stahmann *et al*. 1966). It is the cause of epinastic symptoms and curly top symptoms of virus diseases (Goodman *et al*. 1967, Gold & Faccioli 1968) and the cause of epinastic symptoms in *Fusarium* wilt of tomato (Dimond & Waggoner 1953). The symptoms of plant tissue senescence as well as many of the enzyme changes that occur within the infected tissue are similar to those induced by ethylene (Clare *et al*. 1966, Stahmann *et al*. 1966, Wood 1967, Imaseki *et al*. 1968). It has been shown to be able to induce disease resistance against black rot of sweet potato (Clare *et al*. 1966, Stahmann 1967). The effect of ethylene on the host-pathogen relation of *Rhizoctonia* disease of radish seedlings is unknown. A direct and an indirect method were used, in the experiments described below, to test the effect of ethylene on the susceptibility of radish seedlings to attacks by *T. cucumeris*. A new class of plant growth regulators, 2 haloethane phosphonic acid, has recently been reported, which on entry into plants, were degraded with the liberation of ethylene (Cooke & Randell 1968). The effect of 2 chloroethane phosphonic acid ("Ethrel ACP 68-64") on the susceptibility of radish seedlings was tested.

Materials and Methods.

A. Respiratory changes of radish seedlings as a result of infection by T. cucumeris.

(1) Carbon dioxide production measured using an infra-red CO_2 analyzer.

Two week old radish seedlings, raised in U.C. mixture in a growth cabinet, were washed clean of soil. The seedlings were divided into four batches. Each batch consisted of three replicates of ten seedlings each. One batch was used as control. The rest were inoculated with a stem strain of T. cucumeris using the glass slide method. The time of inoculation was adjusted so that on the day of measurement, the fungus had been in contact with the hypocotyls of the three sets of seedlings for one, two and four days respectively. Incubation was done in a growth cabinet. Seedlings were kept in the dark in pint-sized respiratory chambers for twelve hours before measurements were made. Carbon dioxide from healthy plants and fungal colonies was also measured.

The respiratory chamber was made air-tight except for three outlets which were connected to a pump, an infra-red gas analyzer (Type S.L., The Infra Red Dev. Co., Welwyn Garden City, England) via a silica gel tower and a soda lime tower (Fig. 52) to form two closed circuit

gas-flow systems. By the manipulation of three valves, only one system operates at a time. The air flow through the system was adjusted to a constant rate. The air was first passed through the soda lime tower to remove most of the carbon dioxide and through the silica gel to remove moisture. For calibration of the infra-red gas analyzer and measurement of the rate of respiration, the soda lime tower was by passed. Calibration was done with air, containing 0.035% carbon dioxide, from a cylinder. Respiratory rates of inoculated plants were expressed as percentage increases above that of the uninoculated plants on a fresh weight basis.

(ii) Oxygen uptake measured using a Warburg respirometer.

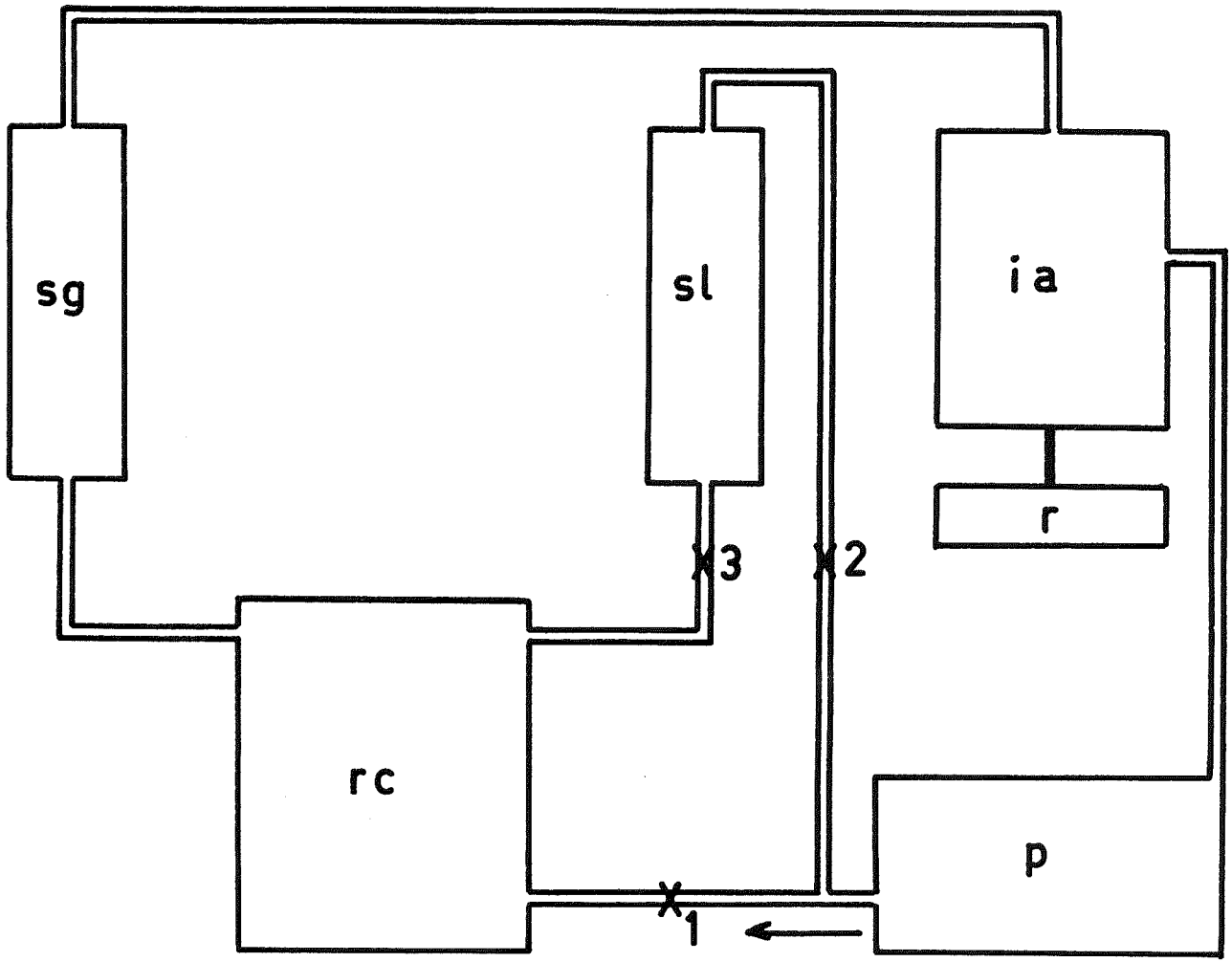
Radish seedlings were grown in U.C. mixture. Two methods of inoculation were used. Seedlings were inoculated by spraying them with a suspension of a stem strain of T. cucumeris in 0.1% water agar. Hypocotyl tissues were collected at different times after inoculation and their respiratory activities determined. Controls were done with uninoculated seedlings.

Other seedlings were inoculated by pouring a suspension of a stem strain of T. cucumeris on top of the soil. After incubation for three days, lesions

Fig. 52: A close-circuit gas-flow system for measuring carbon dioxide exchange by radish seedlings using an infra-red gas analyzer. The respiratory chamber is detachable. The arrow shows the direction of the air flow through the pump.

1, 2, 3 = valves
ia = infra-red gas analyzer
p = pump
r = recorder
rc = respiratory chamber
sg = silica gel tower
sl = soda lime tower

Fig. 52



developed at soil level. Infected hypocotyls were harvested and dissected into three tissue portions, portions from lesions, portions which extended from the lesion edge to one centimetre from the lesion, and portions which extended from 1cm to 2cm from the lesion. Controls consisted of representative portions of hypocotyl tissues from uninoculated plants.

Respiratory rates were determined with a Warburg Constant Respirometer at 30°C (Umbreit et al. 1964). The reaction vessels contained 3.0 ml of 0.1M KH_2PO_4, 0.2g to 0.4g of tissue and 0.2 ml of 2N NaOH. Vessels were equilibrated for 30 minutes in the dark before the start of each experiment. Measurements were done every ten minutes over a period of 90 minutes. Each experiment was done in triplicate and two flasks were used as thermobarometers. Respiratory rates were expressed as percentage increases above that of the uninoculated plants on a fresh weight basis or as μ litres oxygen uptake per gram fresh weight per hour.

B. The production of cell-wall degrading enzymes by *T. cucumeris* *in vitro* and *in vivo*.

(i) *In vitro* enzymes.

(a) Degradation of cellulose membranes by *T. cucumeris*.

RM2 agar medium without sucrose was amended separately with 0%, 0.01%, 0.1% and 1.0% glucose and dispensed into sterile petri-dishes. Squares (3cm x 3cm) were cut from cellulose acetate dialysis tubings. These were boiled in several changes of distilled water to remove soluble contaminants. The cellulose membranes were inter-leaved between moist filter paper in petri-dishes and then sterilized by exposure to propylene oxide gas overnight. A single piece of cellulose membrane was placed on top of the agar medium in each petri-dish and inoculated with a 7 mm diameter agar plug bearing hyphae of a strain of T. cucumeris grown on distilled water agar. Plates without cellulose membranes were also inoculated to determine the effect of the different glucose concentrations on the growth of the fungi. Inoculated plates were incubated in the dark at 25°C for three days to determine fungal growth or for four weeks before the degradation of the cellulose membranes were tested. The degree of degradation was determined by gripping a corner of the cellulose membrane with broad tip forceps and then lifting to see how much of the cellulose membrane remained on the agar surface.

(b) In vitro production of pectolytic enzymes.

RM2 nutrient solution minus sucrose was supplemented with 1.0% pectin (w/v). 200 ml of the solution was

dispensed into each 500 ml Erlenmyer flask and autoclaved at 15 lb per sq. in. for 20 minutes. Each flask was inoculated with a strain of T. cucumeris and incubated at 25°C on a rotary shaker. After five days of growth, the mycelium was filtered off. The culture filtrate was used as an enzyme source and frozen at -4°C, under toluene, until required.

(ii) In vivo enzymes.

(a) Preparation of crude enzymes.

Radish seedlings, 7-10 days old, were grown in U.C. mixture in a glass house (approximately 25°C) or a growth cabinet (day length 16 hr., day temp. 20°C, night temp. 18°C). A stem strain of T. cucumeris was grown in RM2 liquid medium for four days on a rotary shaker at 25°C. The mycelial mats were harvested, washed twice with distilled water and dispersed in 0.1% water agar using a Waring blender. The mycelial suspension was then sprayed uniformly onto the hypocotyls of the radish seedlings. Inoculated plants were kept at high humidity. After about four days incubation, the attacked hypocotyls were collected and the fresh weights determined. Diseased tissue was blended with an equal volume of 0.5M NaCl or 0.1M phosphate buffer, pH 7.5 at 4°C. The suspensions were filtered through two layers of cheese-cloth.

Residues were ground with medium-fine acid-washed sand together with a little of the filtrate and again filtered through two layers of cheese-cloth. Filtrates were bulked, centrifuged at 20,000 x g for 10 minutes at 4°C. Supernatant liquids were used as enzyme sources or stored frozen at -4°C, under toluene, until required. Uninoculated seedlings were similarly treated and used as controls.

(b) Viscosity determination of enzymatic activities.

A viscosity method was chosen because it is rapid and precise and is extremely sensitive. Viscosity changes in a soluble substrate due to the actions of enzymes were observed in size 300 Fenske-Ostwald viscometers at 30 ± 0.5°C over a range of pH values. The buffers used were 0.2M citrate (pH 3-6) and 0.2M Tris-HCl (pH 7-9). All apparatus and reagents were warmed to 30°C before use. The reaction mixture was made by adding the substances in the following order with thorough mixing by bubbling air through the solutions: 4 ml of 2.5% (w/v) substrate, 5 ml buffer and 1 ml of enzyme solution. The viscosity was read after an incubation of twenty minutes. Controls were similarly done but with autoclaved preparations or extracts from uninoculated seedlings. Relative viscosity is recorded in arbitrary units. The flow time of the control mixture is the time (in seconds)

required for a standard volume of the mixture to pass through the capillary of the viscometer. The flow time of the control mixture is taken as equal to one hundred units of relative viscosity. The substrates were carboxyl-methylcellulose for the presence of cellulases and pectin (apple 250 grade) or sodium polypectate for pectolytic enzymes.

(c) "Macerating enzyme" activity.

Cores of potato tissue were cut with a 7 mm diameter cork borer and then sliced into discs about 1.5 mm thick. The discs were washed with distilled water. Six potato discs, placed at equal distances from each other on a tooth-pick were submerged in the crude enzyme preparation in a test-tube. Radish hypocotyls, bearing lesions caused by T. cucumeris isolate 69, were extracted in 0.2M phosphate buffer (pH 6.0) and the supernatant used as an enzyme source. At different time intervals, discs were removed and then assessed for maceration on a 0-5 scale. Maceration was assessed, using a dissecting microscope, on the loss of coherence of tissue. Macerating properties were also assessed using 1 mm transverse sections of radish hypocotyls. The epidermis of the sections were cut through at several places to allow better contact with the enzyme solution. All experiments were done at 25°C. A drop of toluene was

added to prevent bacterial contamination. A boiled enzyme control was included.

C. Changes in pH of host tissues as a result of infection.

Radish seedlings, ten day old, grown in U.C. mixture in a growth cabinet were inoculated with a mycelial suspension of a stem strain of T. cucumeris by spraying uniformly onto the hypocotyls. Materials were ready for use after three days of incubation. Controls consisted of uninoculated plants. Two methods were used to assess the pH of the infected and healthy hypocotyl tissues.

(i) pH meter with glass electrodes.

Portions of radish hypocotyls bearing lesions were macerated with or without 0.5M NaCl (1:1/w:v). The slurry was passed through two layers of cheese-cloth and the pH of the filtrate was determined using a pH meter (Radiometer, Copenhagen). Three replicates were done. Controls were extracts of uninoculated radish hypocotyls.

(ii) Indicators.

Eight indicators with narrow pH ranges were chosen. They were made into 0.04% solutions with double glass distilled water except methyl red which was prepared in 0.02% solution according to the recommendations of Small

(1956). Each indicator solution, just before use, was brought into its change zone by titration with N/20 HCl or N/20 NaOH. It's pH was determined with a pH meter. A drop of the solution was placed on a glass slide (cleaned with alcohol and distilled water) and its colour noted (Table 18) when viewed through a low-power microscope. Transverse sections of radish hypocotyls bearing lesions, caused by a stem strain of T. cucumeris, were cut and washed briefly in double glass-distilled water. Each section was transferred to a glass slide and a drop of indicator solution was added. After five minutes, the sections were viewed under a low-power microscope without a cover glass. Each indicator was used to define the upper or lower limits of a range of pH within which the pH of the material must lie. Controls consisted of sections of uninoculated plants.

D. The water content of diseased tissue.

Radish seedlings, ten day old, grown in U.C. mixture in a growth cabinet, were inoculated with a stem strain of T. cucumeris using the glass slide method. Two or three days after inoculation, diseased areas showing water-soaked appearance were dissected out and placed in a previously weighed crucible. Representative portions of hypocotyl tissues from uninoculated seedlings were

Table 18: Calibration of indicators at their change zone using a low power microscope.

Indicators (B.D.H.)	pH at change zone	colour at change zone
Bromophenol blue	2.7 - 2.9 - 3.9	yellowish green-green-purple
Bromocresol green	3.5 - 3.7 - 5.45	orange-green-blue
Methyl red	4.2 - 6.5 - 7.4	pink-light yellow-yellow
Bromocresol purple	4.8 - 5.2 - 6.7	yellowish green-blue-purple
Bromothymol blue	5.4 - 6.0 - 7.4	straw-yellowish green-blue
Phenol red	5.7 - 7.0 - 7.4	straw-yellowish red-red
Cresol red	5.2 - 7.9 - 9.4	straw-pink-purple
Thymol blue	7.0 - 8.0 - 9.1	straw-green-blue

placed in another crucible. The fresh weights of the tissues were determined. They were then dried in an oven at 90°C to constant weight, cooled in a dessicator before determining the dried weight.

E. Induced resistance to infection.

(i) The effect of cations.

Radish seedlings were grown in U.C. mixture in a growth cabinet. At the age of ten days, the roots were removed. The cut ends of the seedlings were placed in 0.02M solution of either calcium, magnesium, potassium or sodium chloride for three hours at 21°C. Excess or unbound ions were removed by soaking the seedlings in distilled water for half an hour. Controls consisted of seedlings treated with distilled water for three and a half hours. Seedlings were inoculated with a stem strain of T. cucumeris using the glass slide method. Inoculated plants were kept in humid chambers inside a growth cabinet. Six plants per treatment were used. Seedlings were incubated for seven days.

(ii) The effect of ethylene.

(a) Direct method.

Ethylene gas from a pressure cylinder was allowed to displace a pre-determined amount (approximately 20 ml) of

cold (approximately 5°C) distilled water in a 100 ml glass cylinder. The gas was shaken up vigorously with the remaining volume of water in the cylinder. The volume of ethylene (approximately 10% solution) dissolved was noted after adjusting to normal atmospheric pressures. A pre-determined volume of the cold ethylene solution was applied to a piece of filter paper in a chamber containing specimens for treatment. The chamber was then sealed hermetically. The volume of ethylene solution used depended on the volume of the container and the concentration of the ethylene gas required. An assumption was made that the whole of the ethylene in solution would evaporate.

(1) Effect of ethylene on the growth of T. cucumeris.

Petri-dishes, 7.5 cm in diameter, each containing a layer of 0.3 cm thick RM2 nutrient agar medium were inoculated with a 7 mm agar plug bearing actively growing mycelium of a stem strain of T. cucumeris. The covers of the petri-dishes were removed under sterile conditions. Inoculated agar plates with agar surface downwards, each separated by a piece of stainless wire gauze, were stacked in fours. Each stack was placed inside a 500 ml glass jar with a screw top. The gas inside the jars was adjusted to 0, 10, 50 and 100 parts per million of

ethylene by transferring a pre-determined amount of ethylene solution onto a piece of filter paper held by each of the lids. The jars were sealed and incubated at 25°C in the dark for 2 to 3 days. The effect of ethylene on the pathogen was measured by linear growth of the treated against the control.

(2) Effect of ethylene on disease development.

Ten day old radish seedlings were grown in U.C. mixture. Seedlings were either treated first with ethylene for two days followed by inoculation or the treatment with ethylene and inoculation were done simultaneously. Treatments with 0, 10, 50 and 100 parts per million of ethylene were done inside 900 ml screw-top glass jars, each containing six seedlings. High humidity inside the jars was maintained by moist filter paper. Incubation was either done in a glass house or in a growth cabinet. The period of incubation was adjusted as required. Treatments were renewed daily. At the end of the experiment, infected plants were stained with acetic trypan blue. Superficial mycelium was removed, the plants restained and the amount of damage to the hypocotyls was determined using a disease index on a 0-8 scale similar to that used in part one of this dissertation.

(3) Effect of ethylene on the respiration of radish seedlings.

Radish seedlings, grown in U.C. mixture, were treated with 0, 10, 50 and 100 parts per million of ethylene as above. After treatment for two days, the oxygen uptake by the radish hypocotyls was measured using a Warburg respirometer.

(b) Indirect method.

Radish seedlings, two week old, grown in U.C. mixture, had their roots and cotyledons removed. The hypocotyls were treated for half an hour with various concentrations of 2-chloroethane phosphonic acid and then inoculated with a stem strain of T. cucumeris using the glass slide method. The damage to the hypocotyls was assessed using a disease index on a 0-8 scale after staining with acetic trypan blue as described in part one of this dissertation.

Results.

A. Respiratory changes of radish seedlings as a result of infection by T. cucumeris.

(i) Carbon dioxide production by infected seedlings.

An increase in carbon dioxide output by seedlings inoculated for one, two and four days with T. cucumeris (Fig. 53) was seen. The greatest increase was two days after inoculation. By the fourth day after inoculation, respiratory activities of inoculated plants fell rapidly. The fungal inoculum, isolate 69, had a higher rate of carbon dioxide output than uninoculated radish seedlings on a fresh weight basis.

(ii) Oxygen uptake by healthy and diseased hypocotyl radish tissue.

The respiratory activity of radish hypocotyls decreased rapidly after emergence. Respiratory activity was lowest from the seventh to the tenth day after sowing. After the eleventh day, a slight rise in respiratory activity corresponded to the increase in girth of the lower parts of the hypocotyl which was due to cell divisions in the formation of storage tissues. Extensions of the hypocotyl did not increase respiratory activity (Fig. 54).

Fig. 53: Percentage increase in carbon dioxide production of radish seedlings infected with T. cucumeris, isolate 69, over uninoculated seedlings. Measurements done in a closed-circuit gas-flow system using an infra-red gas analyzer. A and B are two separate experiments. F is the respiratory activity of the fungal inoculum.

Fig. 53

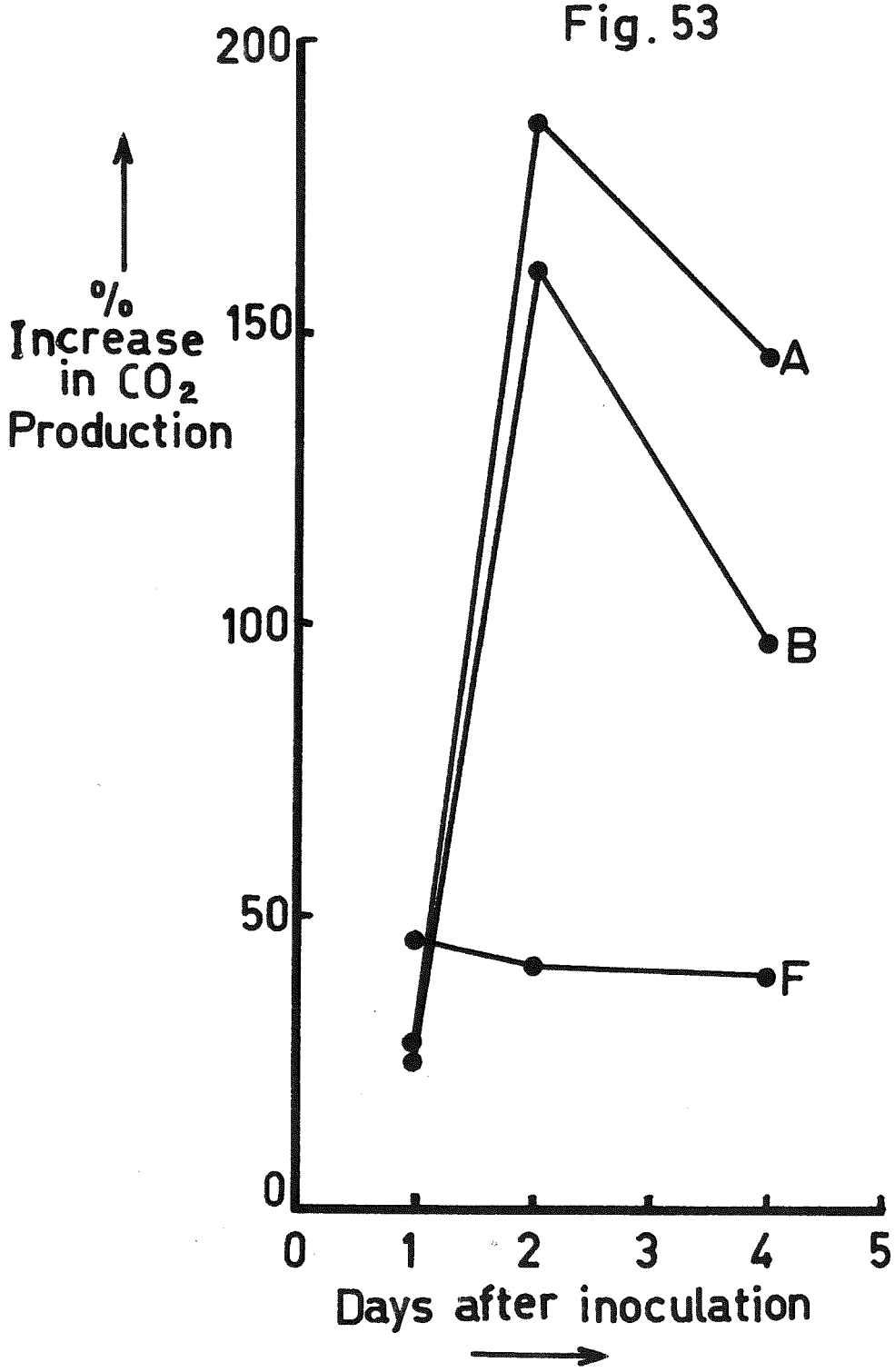
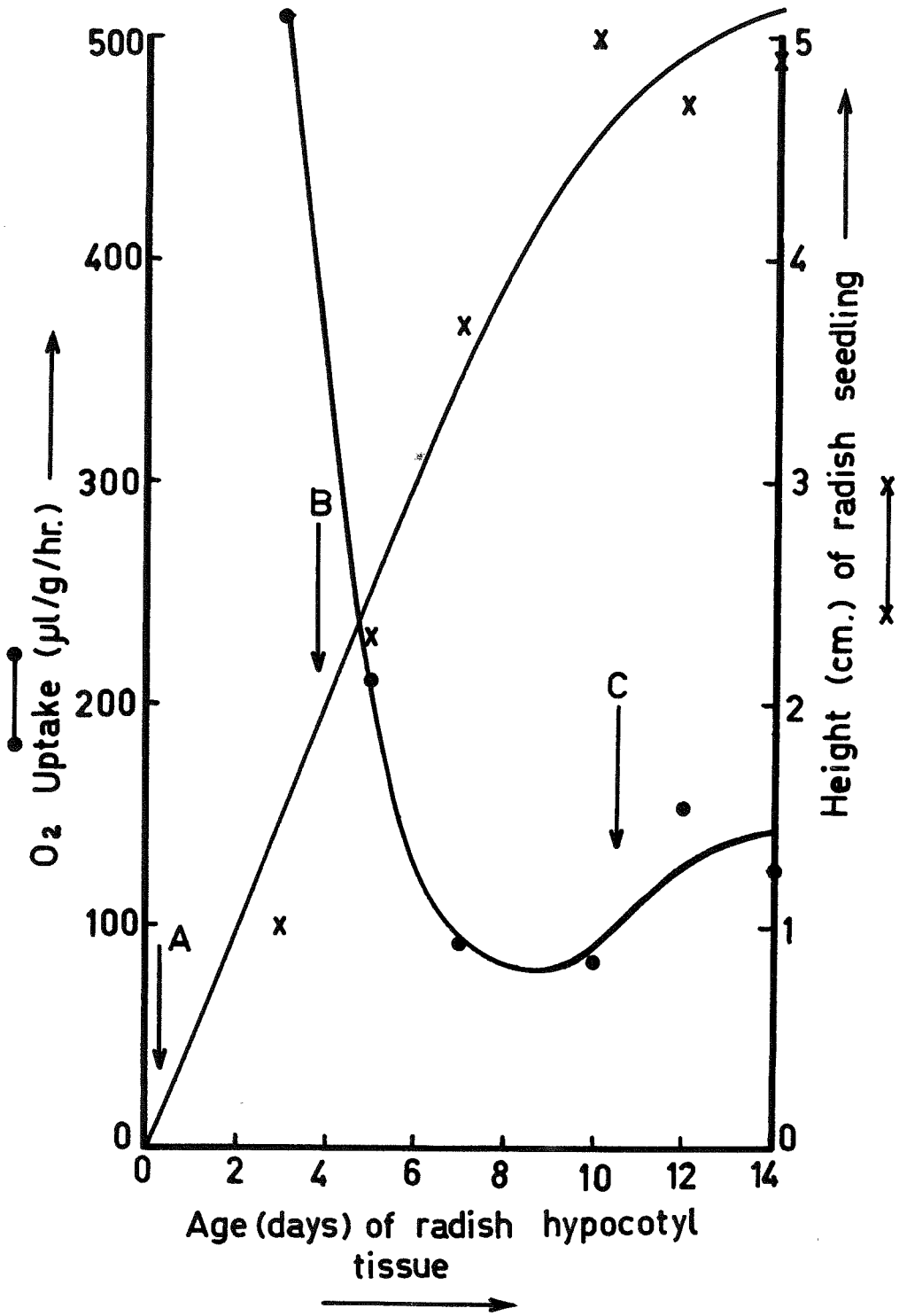

200
150
100
50
0
% Increase in CO_2 Production
A
B
F
0
1
2
3
4
5
Days after inoculation

Fig. 54: The relation between oxygen uptake by radish hypocotyl tissue, height and age of radish seedlings.

Arrows A, B and C show the age when germination, emergence and formation of storage tissue, respectively, took place.

Fig. 54


500
400
300
200
100
0
O₂ Uptake (µl/g/hr.)
A
B
C
0
2
4
6
8
10
12
14
Age (days) of radish hypocotyl tissue
15
4
3
2
1
0
Height (cm.) of radish seedling

After inoculation, a steady increase in oxygen uptake by inoculated hypocotyls was seen (Fig. 55). The most rapid increase in oxygen uptake was from 24 to 48 hours after inoculation. The respiratory rate of radish hypocotyls 48 hours after inoculation was roughly six times that of the uninoculated plants. When inoculated hypocotyls were separated into different portions, increase in oxygen uptake was mainly confined to the lesion (Fig. 56) where respiratory activity was 5 to 7 times that of uninoculated tissue. A rate of 1.2 times that in uninoculated tissue was recorded in hypocotyl tissue adjacent to the lesions. No increase was recorded in tissue 1 - 2 cm from the lesion.

B. The production of cell wall degrading enzymes.

(i) Effect of glucose on growth characteristics and cellulase production by T. cucumeris.

There was no significant difference between the linear growth of different strains of T. cucumeris on RM2 media containing different concentrations of glucose (Table 19). However, in all cases, the production of aerial mycelium was poor on media with 0% or 0.01% glucose, fair on 0.1% glucose and good on 1.0% glucose.

The stem strains of T. cucumeris were able to degrade totally cellulose membranes in the presence of up to 0.1%

Fig. 55: Percentage increase in oxygen uptake by one-week old radish hypocotyls inoculated with isolate 69 for periods up to 48 hours.

Fig. 55

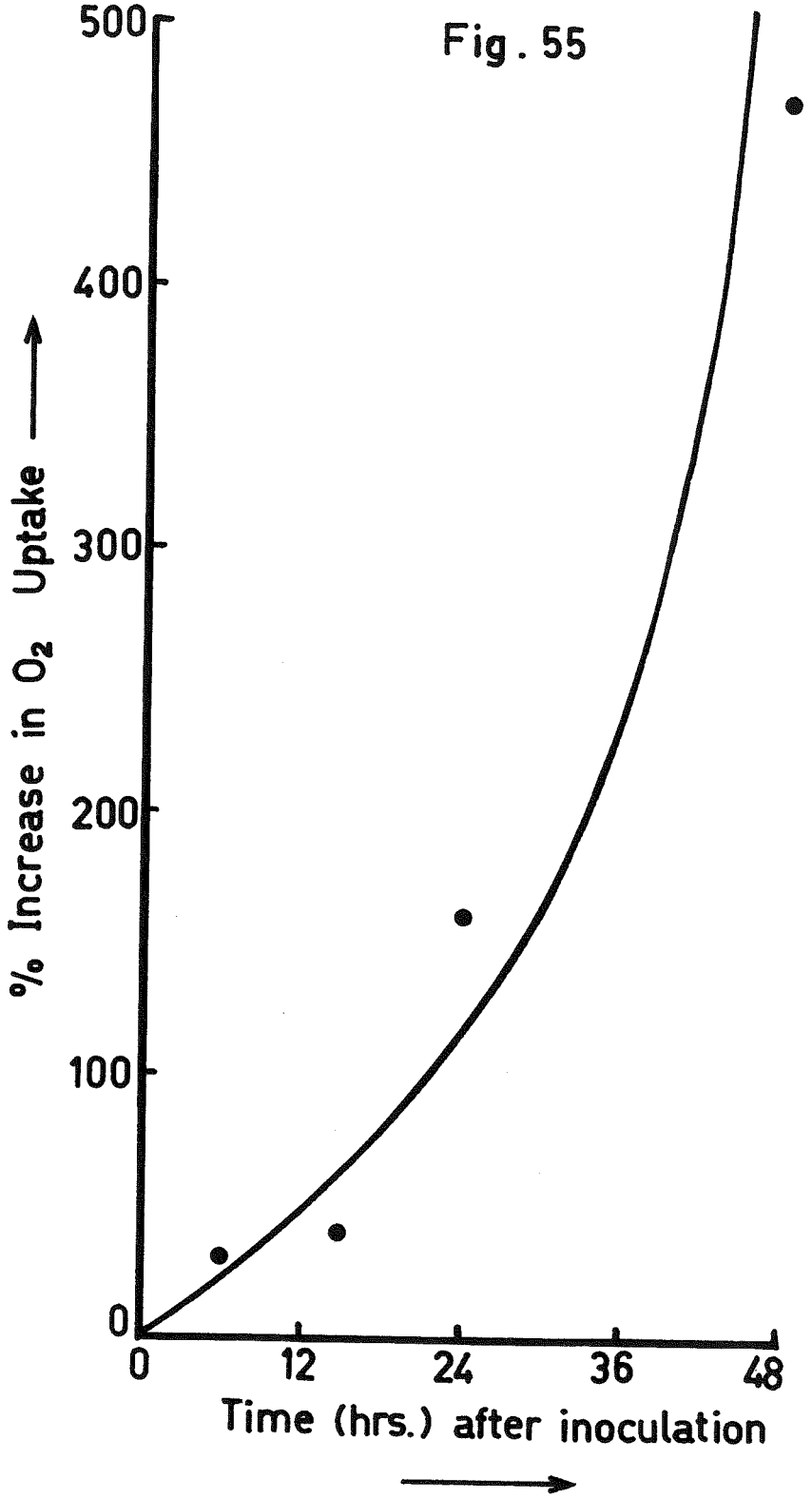

500
400
300
200
100
0
% Increase in O_2 Uptake
0
12
24
36
48
Time (hrs.) after inoculation

Fig. 56: Oxygen uptake of different parts of 13-day old radish hypocotyls infected with T. cucumeris, isolate 69, for three days.

l = lesion
nl = next to lesion (edge of lesion to 1cm from lesion)
al = awey from lesion (1 cm to 2 cm from lesion)
c = control (lower portions of hypocotyls of uninoculated seedlings).

Fig. 56

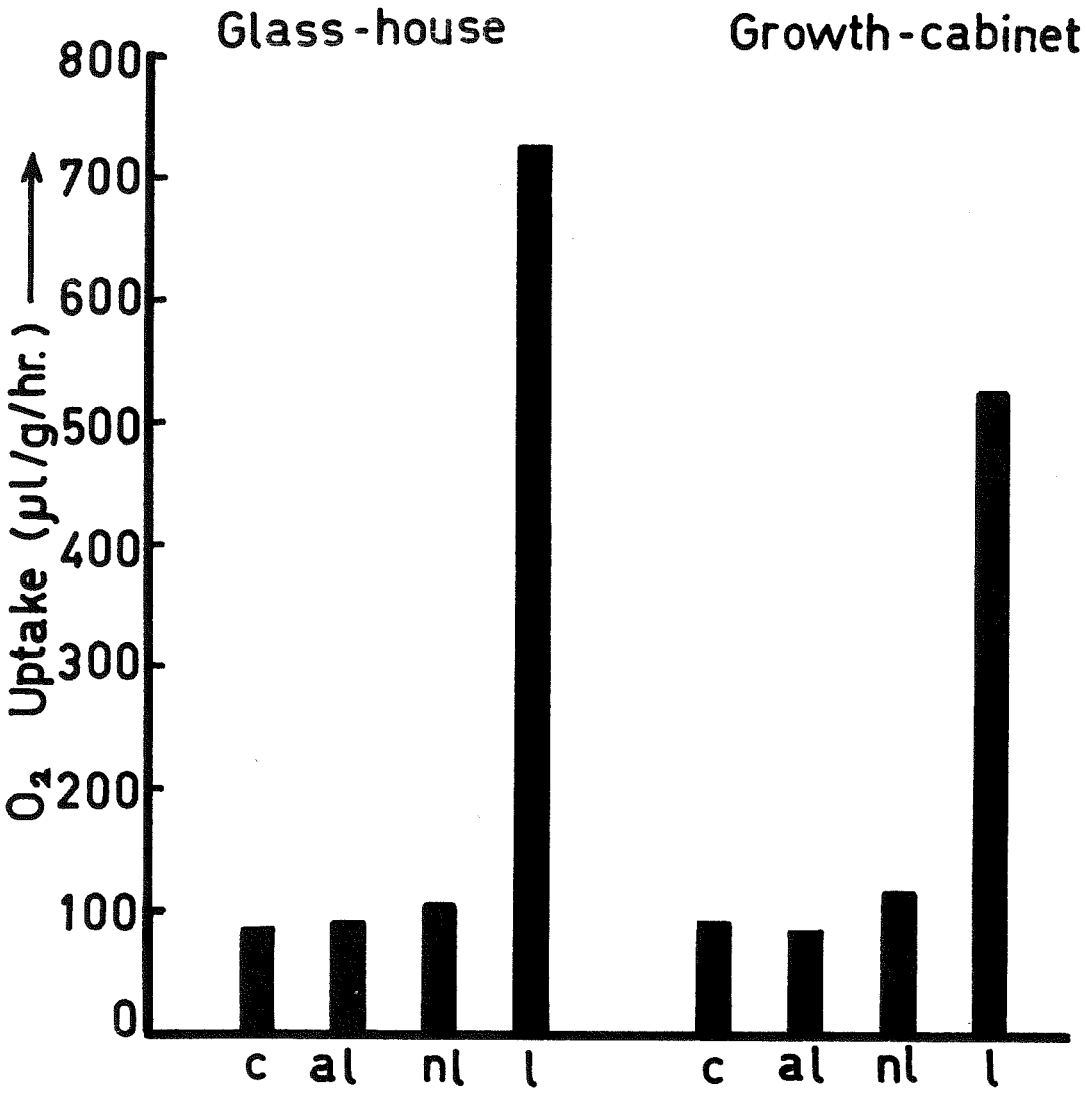

Glass-house
Growth-cabinet
800
700
600
500
400
300
200
100
0
O2 Uptake (µl/g/hr.)
c
al
nl
l
c
al
nl
l

Table 19: The effect of glucose concentration on the linear growth (in cm.)* of T. cucumeris isolates** after incubation for three days at 25°C in the dark.

Glucose concentration of RM2 media minus sucrose.

Isolate	0%	0.01%	0.1%	1%
48	4.2	4.4	3.9	4.4
68	4.5	4.6	4.5	4.2
69	4.6	4.4	5.1	4.6
R10	4.5	4.3	5.2	5.6

* Average of three replicates, each an average of two measurements of the colony diameter done at right angles.

** Diameter of inoculum = 0.8 cm.

glucose after an incubation period of four weeks (Table 20). The root strain, R10, destroyed the cellulose membrane completely in the absence of glucose and partially up to a concentration of 0.1%. The cellulose membranes were intact on agar medium containing 1% glucose when in contact with isolates 68 and R10. Degradation of cellulose membranes is indicative of the production of cellulases by isolates of T. cucumeris.

(ii) In vivo production of cellulase.

The presence of cellulase, Cx, activity was tested using extracts of radish seedlings infected with a stem strain of T. cucumeris. The reduction in viscosity of a standard concentration of carboxyl methylcellulose (CMC) is taken as indicative of cellulase, Cx, activity. Cx activity was found to be active from pH3 to 9. The maximum activity was around pH5 (Fig. 57).

(iii) Production of pectolytic enzymes.

When grown on a pectin liquid medium, the three stem strains and the root strain of T. cucumeris were capable of producing pectolytic enzymes which reduced the viscosities of aqueous pectin and sodium polypectate at pH4 to 8 (Fig. 58, 59). The maximum pectolytic activity using aqueous pectin as a substrate was around pH 5 and that with aqueous sodium polypectate was

Table 20: The effect of glucose concentration on the degradation* of cellulose membranes (3 cm x 3 cm) by T. cucumeris isolates** after incubation for four weeks at 25°C in the dark.

Glucose concentration of RM2 minus sucrose media.

Isolate	0%	0.01%	0.1%	1%
48	++	++	++	+
68	++	++	++	-
69	++	++	++	+
R10	++	+	+	-

* Assessing the loss of coherence of cellulose membrane by gripping the corners with broad tip forceps and lifting.

** Diameter of inoculum = 0.8 cm.

++ = total loss of coherence, no fractions could be lifted.

\+ = small fractions could be lifted.

\- = whole piece could be lifted.

Fig. 57: Relative viscosity of 1% aqueous carboxylmethylcellulose at different pH levels incubated with extracts of radish hypocotyls infected with T. cucumeris isolate 69.

Fig. 57

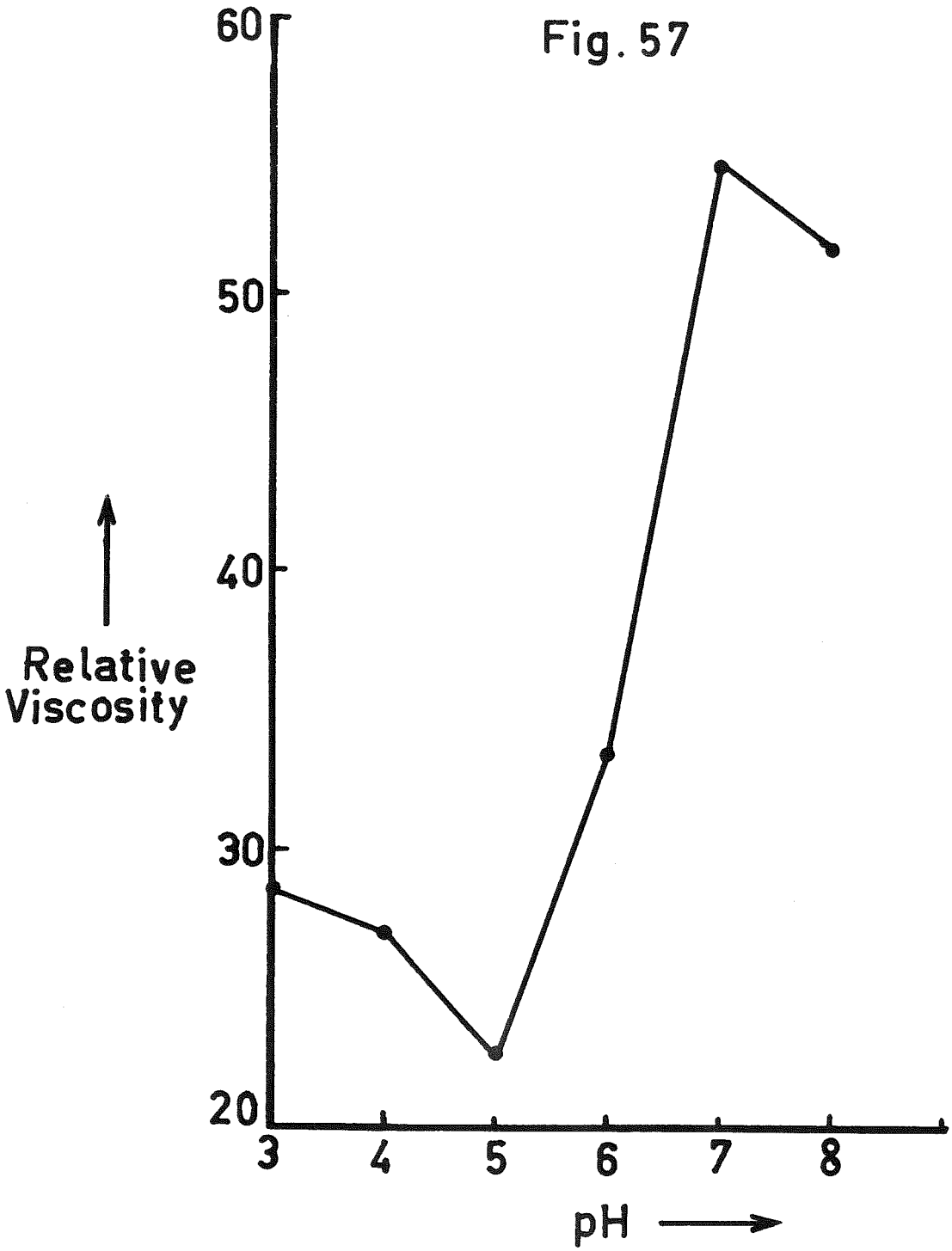
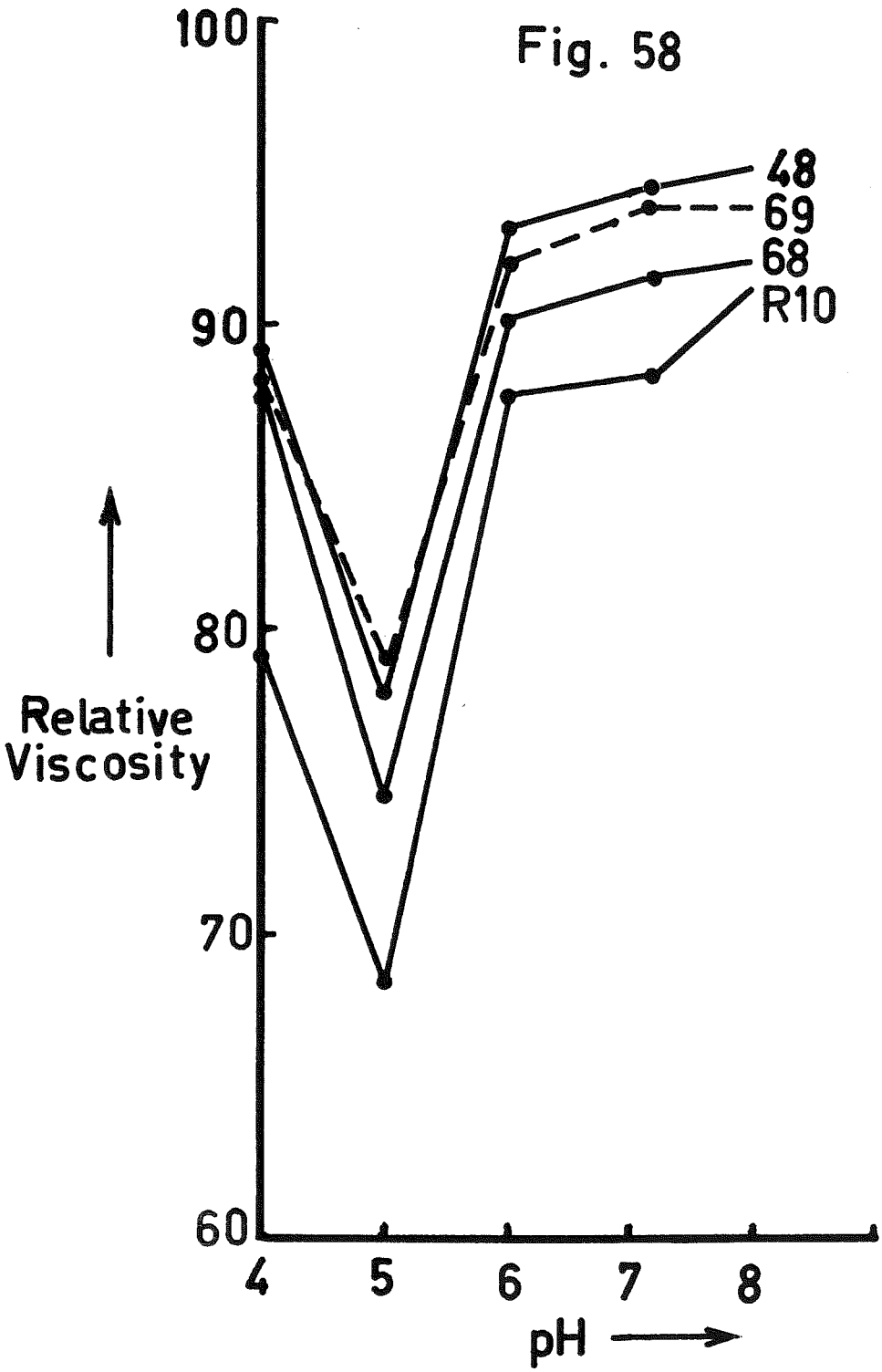


Fig. 58: Relative viscosity of 1% aqueous pectin, at different pH levels, incubated with culture filtrates of T. cucumeris, isolates 48, 68, 69 and R10, grown in a liquid pectin medium.

Fig. 58



between pH 5 and 6. Extracts of radish hypocotyls inoculated with isolate 69 also showed pectolytic activities when pectin and sodium polypectate were used as substrates but maximum activity in both cases was around pH 5 (Fig. 60). When sodium polypectate was used as a substrate, a second peak was evident for culture filtrates of isolates 68 and 69 and extracts from radish hypocotyls infected with isolate 69 (Fig. 59, 60). This peak was around pH 8. Culture filtrates of isolate 68 and R10 were most active in pectolytic activities. No correlation was found between the production of pectolytic enzymes and pathogenicity of the isolates of T. cucumeris.

(iv) "Macerating enzyme" activity.

Extracts of hypocotyl tissues bearing lesions caused by isolate 69 were found to contain "macerating enzyme" activities (Table 21). Radish hypocotyl sections were completely macerated after 18 hours at 25°C while potato discswere not completely macerated even after 24 hours in the extracts.

C. Changes in pH of the host tissue as a result of infection.

Expressed sap of healthy and diseased radish hypocotyls were both acidic (Table 22) but extracts of diseased

Fig. 59: Relative viscosity of 1% aqueous sodium polypectate, at different pH levels, incubated with culture filtrates of T. cucumeris isolates 48, 68, 69 and R10 grown in a liquid pectin medium.

Fig. 59

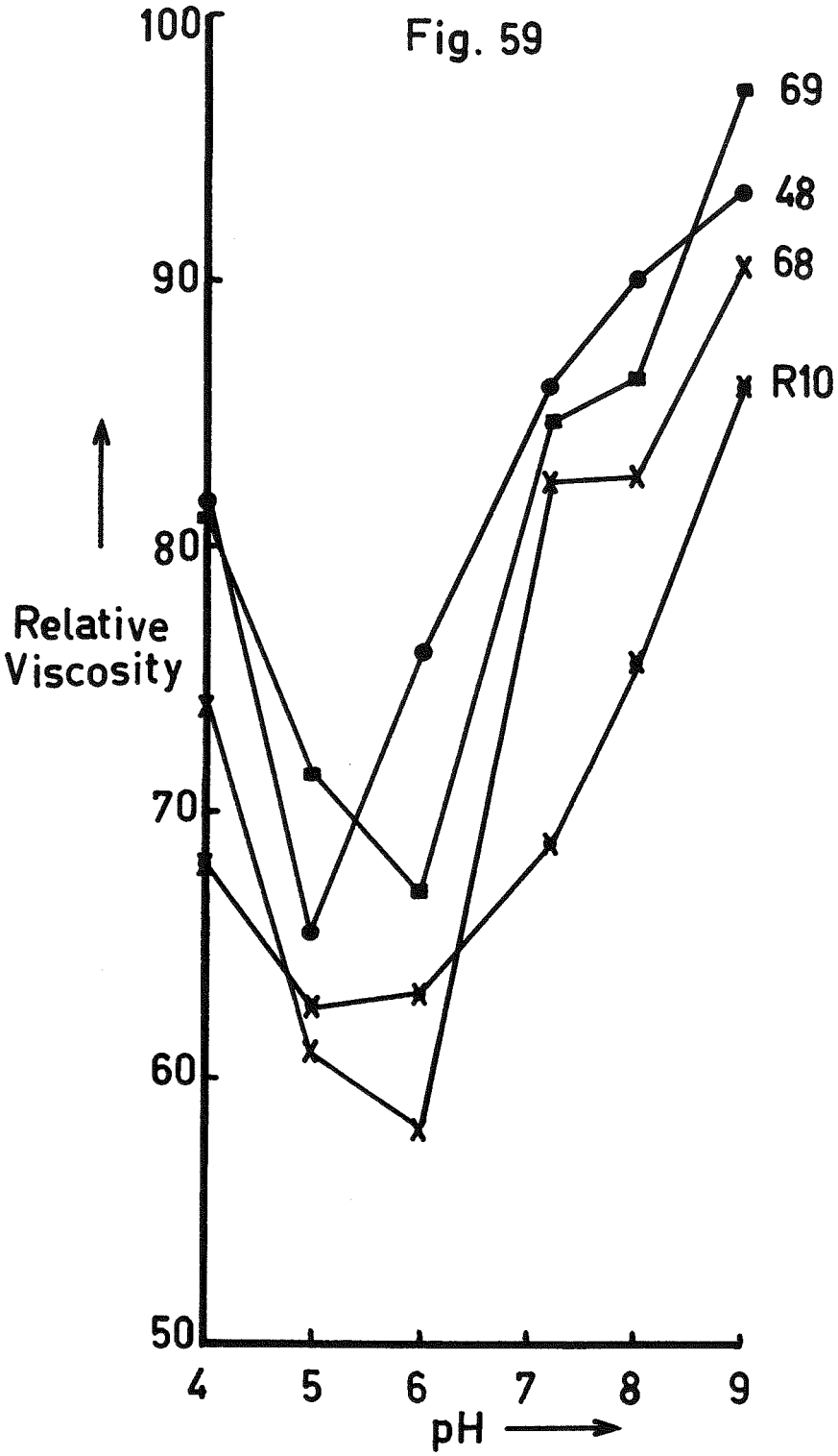


Fig. 60: The effect of extracts of radish hypocotyls infected with T. cucumeris isolate 69 on the relative viscosity of 1% aqueous pectin and 1% sodium polypectate at different pH levels.

Fig. 60

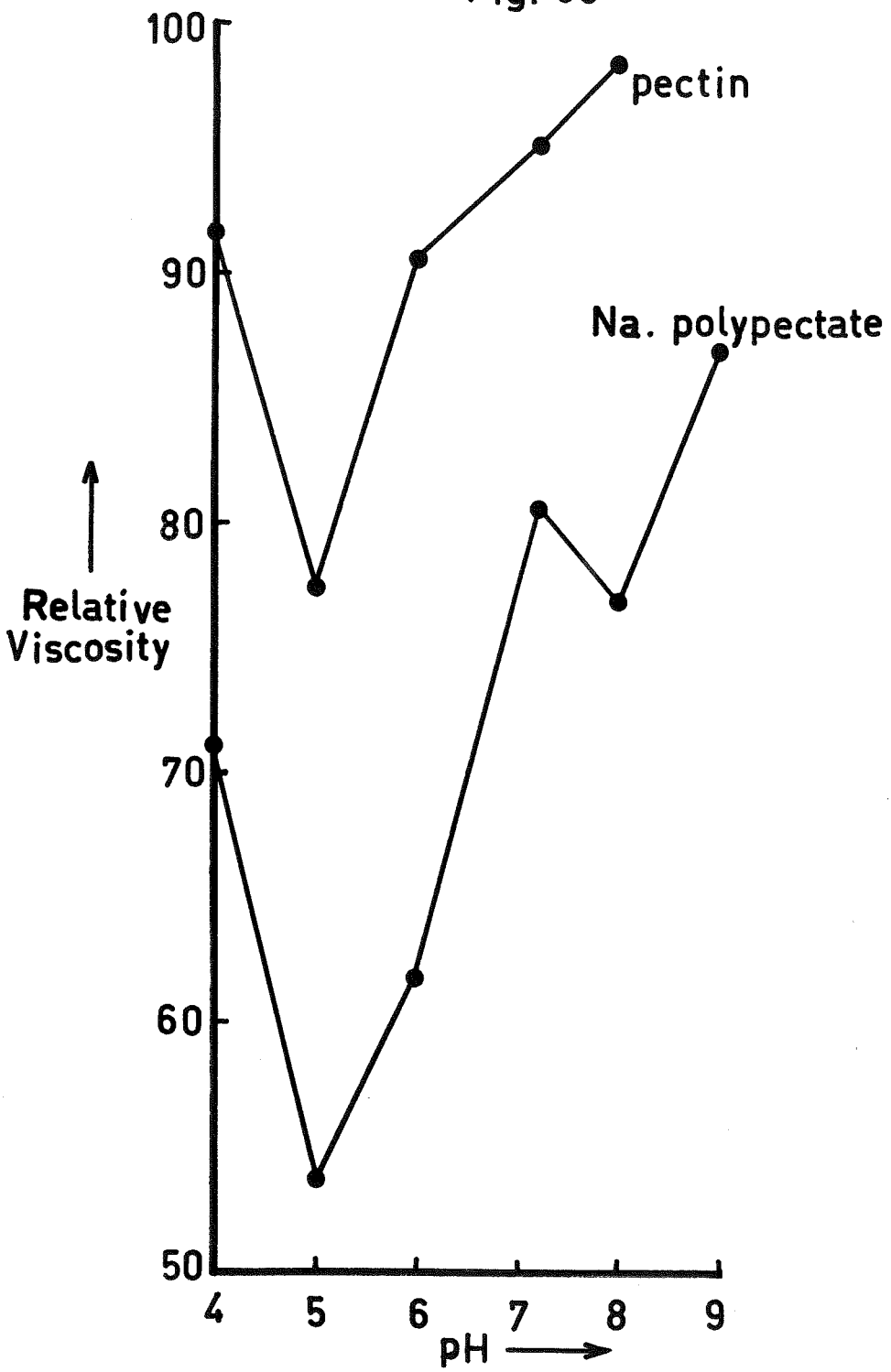

100
90
80
70
60
50
pectin
Na. polypectate
Relative Viscosity
4
5
6
7
8
9
pH

Table 21: Maceration* of potato tuber discs and radish hypocotyl sections by extracts of diseased radish hypocotyls.

	Time (Hour) of immersion in extracts.				
	3	6	9	18	24
Potato discs	0	1	2	3	4
Hypocotyl sections	0	2	4	5	-

* Maceration assessed using a dissecting microscope on a 0 - 5 scale.

0 = no maceration, comparable to control

5 = complete loss of coherence of tissue.

Table 22: Change in pH of host hypocotyl tissue as a result of infection by isolate 69.

(a) Using glass electrodes.

	pH
Extract of healthy tissue	5.57
Extract of diseased tissue	6.40

(b) Using indicators.

	pH
Healthy hypocotyl sections	
Epidermis	5.4 - 5.7
Cortex	5.4 - 5.7
Phloem	5.7 - 6.5
Xylem	3.5 - 4.2
Diseased hypocotyl sections	
Middle of lesion	7.0 - 7.4
Edge of lesion	5.2 - 5.7
Surrounding tissue	5.2 - 5.7

tissues had the higher pH. Indicators showed that the middle of the lesion was slightly alkaline while the edge of the lesion and surrounding tissues were acidic.

D. The water content of healthy and diseased tissues.

There was no significant difference between the water content of healthy and diseased tissues (Table 23). Therefore, the water-soaked appearance around lesions was not due to an accumulation of water.

E. Induced resistance to infection.

(i) The effect of cations.

When radish hypocotyls were treated with either calcium, magnesium, sodium or potassium chloride and then inoculated with T. cucumeris isolates only those that were treated with calcium chloride showed a reduction in the amount of collapsed tissue (Fig. 61).

(ii) The effect of ethylene.

When T. cucumeris isolates 68 and 69 were treated with different concentrations of ethylene for two days, little or no change in the linear growth was noted (Table 24). However, when isolate 68 was treated with ethylene for three days, the colonies showed obvious sectoring (Table 24). Five colonies showing the most

Table 23: Water content of healthy and diseased* radish hypocotyl tissue.

	Wt. in gm	
	1	2
Fresh healthy tissue	0.54	0.21
Oven dried healthy tissue	0.017	0.007
Water lost	0.52	0.20
% water/fresh weight	97	97
Fresh diseased tissue	0.46	0.16
Oven dried diseased tissue	0.017	0.008
Water lost	0.44	0.15
% water/fresh weight	96	96

* inoculated with isolate 69.

Fig. 61: Excised hypocotyls of radish seedlings, 10 day old, treated for 3 hours with (L - R) H_2O, Ca^{++}, Mg^{++}, Na^{+}, K^{+} (supplied as chlorides), then incubated for 7 days in humid chambers.

Top row: Uninoculated
Middle row: Inoculated with isolate 69
Bottom row: Inoculated with isolate 48.

Fig. 61

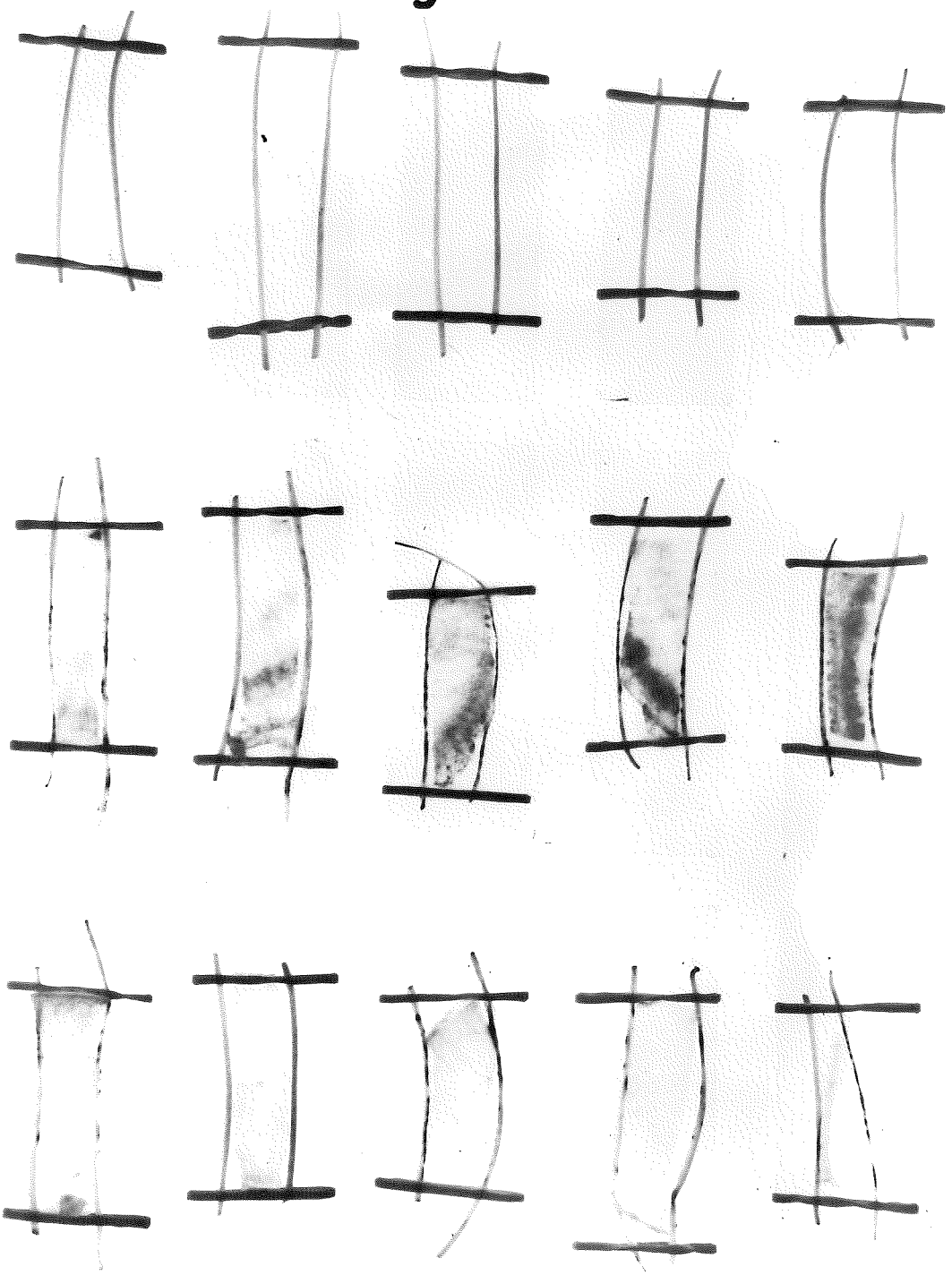


Table 24: Diameter of *T. cucumeris*[*1] colonies treated with ethylene.

Concentration of ethylene (ppm)	Age of cultures[*2]			
	Isolate 69		Isolate 68	
	2½ days[*3]	3 days[*3]	3 days[*3]	3½ days[*4]
0	5.4 cm	6.5 cm	5.5 cm	5.5 cm
10	-	6.6 cm	5.5 cm	5.6 cm
50	-	6.4 cm	5.2 cm	6.1 cm
100	5.5 cm	6.5 cm	5.1 cm	4.7 cm
200	5.5 cm	-	5.4 cm	4.7 cm
500	5.6 cm	-	5.5 cm	4.7 cm
1000	5.6 cm	-	5.5 cm	4.7 cm

- = experiment not done.

*1 = inoculum consisted of 0.7 cm diameter agar plug bearing mycelium.

*2 = on the day of measurement.

*3 = treated with ethylene for two days.

*4 = treated with ethylene for three days, colonies showed sectors.

distinct differences in slow and fast growing sectors were chosen. However, after two transfers on RM2 medium, the growth rates of fungal colonies from the fast and slow growing sectors were found to be nearly the same but slightly greater than that of the parent isolate (Table 25). No significant difference was seen between the pathogenicity of fungal colonies isolated from ethylene induced fast and slow growing sectors and the parent isolate (Table 26).

Preliminary results using a few radish seedlings showed the most effective concentration of ethylene was 50 parts per million when treatment with ethylene and inoculation with isolate 69 were done simultaneously. This treatment induced a slight decrease in the number of radish hypocotyls grossly attacked by T. cucumeris, isolate 69 (Table 27). The oxygen uptake by radish seedlings treated with ethylene for two days was found to increase slightly (Table 28). No significant differences were found between the disease indices of plants treated with 2-chloroethane phosphonic acid and the controls (Table 29).

Table 25: Diameter* (in cm.) of ethylene-sensitive isolates from strain 68 after three days of growth on RM2 nutrient agar after two transfers.

Isolate	Average Diameter	Isolate	Average Diameter
68	6.3	-	-
68-1F	6.9	68-1S	6.7
68-2F	6.6	68-2S	7.2
68-6F	7.0	68-6S	6.3
68-7F	7.2	68-7S	6.9
68-9F	7.1	68-9S	7.0

* = Average of five replicates

F = isolated from fast growing sectors

S = isolated from slow growing sectors

Table 26: Disease index of radish hypocotyls*1 inoculated with mycelia from fast and slow growing sectors*2 of isolate 68 induced by treatment with ethylene.

Disease rating*3	Hypocotyls inoculated*4 with										
	68	-1S	-2S	-6S	-7S	-9S	-1F	-2F	-6F	-7F	-9F
0	-	-	1	1	1	2	1	-	-	-	-
1	-	2	1	1	2	-	1	-	-	-	-
2	1	1	-	2	4	-	2	1	-	-	2
3	1	2	1	2	2	3	1	2	-	-	-
4	2	5	5	3	-	2	2	4	1	-	-
5	5	1	2	1	1	2	2	3	1	1	4
6	3	1	2	2	2	2	3	2	7	3	5
7	-	-	-	-	-	1	-	-	3	5	1
8	-	-	-	-	-	-	-	-	-	3	-

*1 = seven day old radish seedlings.

*2 = after three transfers on RM2 nutrient medium.

*3 = scored after inoculation for two days, incubated in a growth cabinet, see table 13 (Part I).

*4 = using the glass-slide method.

Table 27: Number of radish hypocotyls having similar disease index after treatment with ethylene and inoculation with T. cucumeris.

	Ethylene Concentration (ppm)					
	Treatment A*				Treatment B*	
	isolate 68		isolate 69		isolate 69	
Disease Rating**	0	50	0	50	0	50
0	-	-	-	-	-	-
1	-	-	-	-	-	-
2	-	3	1	-	-	-
3	1	-	-	1	-	-
4	3	3	-	2	-	-
5	6	3	1	8	1	3
6	2	3	9	7	6	4
7	-	-	6	-	11	10
8	-	-	1	-	-	1

* Treatment A = treatment with ethylene and inoculation were done similtaneously for two days.

Treatment B = treated with ethylene for two days followed by inoculation.

** See Table 13 (Part I).

Table 28: Respiratory rates of twelve day old* radish seedlings treated with ethylene** for two days.

Ethylene concentration (ppm)	Oxygen uptake (µl/g/hr.)
0	93.5
10	101.2
50	115.1
100	107.8

* on the day of the experiment

** in a growth cabinet

Table 29: Number of radish hypocotyls having similar disease rating after treatment with 2 chloroethane phosphonic acid and then inoculated* with T. cucumeris, isolate 69.

2 chloroethane phosphonic acid (ppm)	Disease Rating** 5	6	7	8
0	-	1	2	3
5	-	2	1	3
10	-	2	1	3
50	-	-	1	5
100	-	-	2	4
500	-	1	-	5
1000	-	-	1	5
2000	2	-	2	2

* inoculated for 40 hours in a growth cabinet.

** see Table 13 (Part I).

Discussion.

Infected whole seedlings showed two and a half to three-fold increase in the output of carbon dioxide over controls two days after inoculation. This corresponds to the active intercellular colonization of the radish hypocotyls as shown in part two of this dissertation. A fall in respiratory rate then follows due to the death of infected seedlings. However, a steady increase in uptake of oxygen over a forty-eight hour period by inoculated hypocotyls was found. A six-fold increase was found forty-eight hours after inoculation. Bateman (1964a) found that R. solani infected bean tissue had approximately three-fold increase over the control. However, in a later publication (Bateman & Daly 1967) a two-fold increase was reported for bean hypocotyl tissue bearing mature lesions. This increase in respiratory rate was maintained for a nine-day period (Bateman 1964a).

One of the difficulties in respiratory studies is to determine the contribution of the host and the parasite to total respiration. Cytological studies in part two of this dissertation showed that spread of the pathogen from the lesion is faster towards the centres of hypocotyls than up and down longitudinally. Therefore, most of the fungus is confined to the lesion and the tissue immediately below it and relatively little is found in the tissue

adjacent to the lesion. The present writer dissected radish hypocotyls infected at soil level into different portions of tissue at different distances from the lesions. The oxygen uptake by these portions were determined. A five to seven-fold increase in oxygen uptake by the lesion portion over the control was found. Very slight increases were recorded in the tissue adjacent to the lesion. No increase was recorded for tissues 1 - 2 cm away from the lesion. Therefore, the marked increase in respiratory rate in the lesion was probably due mostly to the pathogen and any contaminants active in the moribund tissue in the lesions. A slight increase in respiratory activities in the tissue adjacent to the lesion indicates a mild response to infection by T. cucumeris on the part of the host. This may explain the absence of morphological defense mechanism to invading hyphae, as shown in part two of this dissertation, and hence the "spreading-type" of lesion. Bateman and Daly (1967) found the respiratory rate of Rhizoctonia lesions on bean hypocotyl and the tissue below the lesions to average between 2.3 to 2.9 and 1.5 to 1.7 times respectively greater than the control. They attributed the increase in respiratory rates at the lesions and the tissue below the lesions mainly to the pathogen and the host respectively. The differences between the respiratory rates of lesions on radish and on bean may be due to

the different types of lesions involved, the "spreading-type" on radish and the discrete-type on bean. High respiratory rates have been reported for lesions caused by some aerial fungal pathogens. Samborski and Shaw (1956) found a seven-fold increase in respiration at the centre of vigorously sporulating rust pustules but only a 1.5 fold increase in adjacent host tissue. Similarly, Bushnell and Allen (1962) found a 7 to 10 fold increase at the centre of mildew colonies but only a 2 to 3 fold increase in tissue adjacent to a colony.

Increases in respiratory rates as a result of infection indicate changes in host metabolism. Wood (1967) in discussing the mechanism by which the host is damaged by a fungal pathogen noted that a pathogen rarely damages its host by its physical presence and that most plant parasites cause disease because they produce substances that interfere with the metabolism of the host. One of the symptoms of Rhizoctonia disease is the breakdown of host cell walls as a result of pathogenesis. The breakdown of cell walls is widely accepted as due to the production of cellulolytic and pectolytic enzymes by the pathogen.

Cellulolytic activity has been found in culture filtrates of R. solani (Barker & Walker 1962, Bateman 1963b, 1964b, van Etten et al. 1967) and in extracts of

diseased tissue (Bateman 1963b, 1964b, van Etten et al. 1967). Similar activities were demonstrated by the present author using the stem and root strains of T. cucumeris in culture and in infected hosts. Since on radish hypocotyls, the formation of lesions by T. cucumeris involves intracellular colonization immediately on penetration, cellulases at the early stages of pathogenesis may be of importance. However, Bateman (1963b, 1964b) maintains that cellulases are of secondary importance in tissue colonization in the Rhizoctonia disease of bean although the lesions on bean hypocotyls consist mainly of intracellular hyphae (Christou 1962, van Etten et al. 1967). The mechanism of cellulase activities in vivo is not clear and the present writer is of the opinion that failure to extract a significant amount of the enzyme from diseased tissue should not be taken as a criterion for its insignificance in the early stages of pathogenesis. In part two of this dissertation, it was shown that intracellular penetration of the cortex and the phloem preceded cellular collapse of the infected tissue. Cellulases are likely to be important in the collapse of infected tissues. Similar conclusions were also made by Bateman (1964b).

The pectolytic activity of R. solani has been studied extensively in culture (Elarosi 1958, Deshpande

1960 a,b,c, Barker & Walker 1962, Bateman 1963 a,b, Papavizas & Ayers 1965, Ayers *et al.* 1966, Sherwood 1966) and in diseased tissue infected by *R. solani* (Bateman 1963 a,b, 1964a, Ayers *et al.* 1966, Sherwood 1966, van Etten *et al.* 1967). Production of pectolytic enzymes by the stem and root strains of *T. cucumeris* *in vitro* and by a stem strain *in vivo* have been demonstrated in the present study. Barker and Walker (1962) found a high correlation between polygalacturonase but not pectin methylesterase and cellulase activities of various isolates of *Pellicularia filamentosa* (*T. cucumeris*) and their pathogenicity. In the present study, no correlation was found between the pectolytic activities of culture filtrate of the stem and root strains of *T. cucumeris* and their pathogenicity. Perombelon and Hadley (1965) compared the production of pectic enzymes of a symbiotic strain and two parasitic strains of *R. solani* and found no correlation between pectic enzyme production and their symbiotic or parasitic habits. Similarly, Papavizas and Ayers (1965) found no correlation between pectinolytic activities of thirty randomly selected single-basidiospore isolates of *R. solani* *in vitro* and their virulence, nor between pectinolytic activities *in vivo* or abilities of culture filtrate to macerate potato discs and virulence on bean hypocotyls. The ability to elaborate

cellulolytic and pectinolytic enzymes is widely distributed amongst micro-organisms and this ability, therefore, does not indicate pathogenic capabilities. As pointed out by Brown (1965) the macerative activity, as a result of secretion of extracellular enzymes, is significant in pathogenesis if it is able to operate in the face of defensive mechanisms of the host. The present author has shown that extracts from radish hypocotyls infected with T. cucumeris contained "macerating enzyme" activity which macerated radish hypocotyl tissue faster than potato tuber tissue. Similar activity has been demonstrated from culture filtrate of R. solani and from bean hypocotyls infected with R. solani (Bateman 1963b). The production of pectin-degrading enzymes within the infected tissue undoubtedly aids the pathogen to spread within the host tissues especially when intercellular penetration is involved. The ease with which pectolytic enzymes were isolated from diseased tissues showed that a significant amount was secreted by the pathogen. In part two of this dissertation, it was shown that after the formation of a lesion, subsequent spread of the pathogen was rapid and mainly intercellular. This together with ultrathin sections showing changes in the cell wall next to intercellular hyphae, indicate the importance of pectin degrading enzymes in intercellular spread of the pathogen within the cortical tissues.

Ayers et al. (1966) and Sherwood (1966) found that R. solani is capable of producing polygalacturonase and polygalacturonate trans-eliminase (PGTE) in vitro and in vivo. Calcium ions stimulate PGTE but were inhibitory to PG. Sherwood (1966) found that the pH of healthy alfalfa sap was 5.8-6.0 and that of the diseased plant sap was 6.4-7.0. Since PGTE is produced in culture filtrate above pH6 and that the pH of the diseased plant sap is near the maximum of PGTE activity, Sherwood (1966) suggested that PGTE could be important in the maceration of host tissue. The pH of both the extracts of healthy and Rhizoctonia infected bean hypocotyl tissue is approximately 6 (Bateman 1967). The trans-eliminative cleavage also occurred under acid conditions, although this type of activity was at a maximum between pH8 and 9 (Bateman 1967). However, trans-eliminative activities of extracts of lesions were weak (Bateman 1967) and that endo- PG is considered the dominant enzyme capable of degrading pectic substances in diseased tissue. The present writer has shown that T. cucumeris is able to produce pectolytic enzymes in vitro and in vivo. Two peaks of pectolytic activities were found when the substrate sodium polypectate was mixed with extracts of diseased radish hypocotyl tissues. The first at pH5 and the second at pH8. The pH of the healthy and the diseased tissues is important in determining the relative

importance of the different enzymes secreted by the pathogen. The present author found that the pH of the sap of healthy tissue is around 5.75 and that of diseased tissue is 6.40. However, expressed sap probably shows the average pH of the tissue. In diseased tissues, the pH of the sites of host-pathogen interactions at the cellular level are of utmost importance. Using indicators, the present writer has shown that the middle of the lesion is slightly alkaline and the pH of the edge of the lesion and surrounding tissue is 5.2-5.7. This finding indicates that pectolytic enzymes and cellulases are reacting at their maximum rates during the early parts of pathogenesis and that when the tissue becomes moribund a rise in pH enables a second peak of activity around pH8. Bateman (1967) also found high PGTE activities in alkaline pH in the filtrates of 18 day old cultures of R. solani.

In part one of this dissertation, the present writer showed that T. cucumeris is capable of parasitism over a wide range of environmental conditions. It's ability to secrete cellulolytic and pectolytic enzymes having activities in both acidic and alkaline medium testify to its adaptability. It's ability to grow through soil (Blair 1942) and competitively colonize and decompose cellulose in soil (Garrett 1956) indicate that

cellulolytic and pectinolytic enzymes are involved in the parasitic and saprophytic survival of T. cucumeris.

A water-soaked halo around the Rhizoctonia lesions of bean (van Etten et al. 1967) and radish may be due to a number of factors. The present writer found that there is no selective accumulation of water around the lesion. Such appearance may be due to the actions of cell-wall degrading enzymes secreted by the pathogen.

It is interesting to note that bean hypocotyls become resistant to attacks by R. solani within a few days after being attacked (Christou 1962). However, Bateman and Lumsden (1965) showed that bean hypocotyls are highly susceptible during the first two weeks of plant growth, moderately resistant during the third and resistant thereafter. The cortex of radish hypocotyls remains susceptible up to six weeks of growth (Flentje et al. 1963a). Although radish seedlings are attacked, they are not killed after the age of four weeks due to the development of a resistant endodermis. Cortical tissues are shed as the radish plant matures. The extreme susceptibility of the radish seedlings to attacks by T. cucumeris during the first few weeks of growth is probably due to the fact that no known resistant mechanism is developed in the cortex before the endodermis becomes an effective barrier to fungal penetration.

Rhizoctonia lesions on bean hypocotyls on becoming mature exhibit a reduced rate of enlargement (Christou 1962, van Etten et al. 1967) although very active preparation of pectolytic enzymes and cellulases could be extracted from these lesions (Bateman 1963a,b, van Etten et al. 1967). Bateman (1964a) examined the cause for the limitation of lesion growth and postulated that an induced accumulation of bivalent ions especially calcium in advance of the pathogen resulted in the formation of pectate salts which were resistant to hydrolysis by the fungal polygalacturonases. However, in a later publication, Bateman and Lumsden (1965) suggested that the resistance of bean hypocotyl tissue to attack by R. solani is associated with the normal conversion of pectin to calcium pectates during the process of hypocotyl maturation rather than an induction mechanism. The Rhizoctonia lesions on radish hypocotyls are of the "spreading-type", the hyphae are not confined to a definite zone within the hypocotyl. Since calcium pectate is formed only in the middle lamina, such a deposit will be effective against intercellular hyphae. Histological examinations by van Etten et al. (1967) showed that R. solani in bean hypocotyl developed both intercellular and intracellular hyphae at all stages of lesion maturation. Bateman and his associates have not examined the relative importance of inter- and intra-

cellular hyphae in the spread of R. solani within the host. No explanation was given for the confinement of intracellular spread of the pathogen. The present writer tested a number of cations on the susceptibility of radish hypocotyls to attacks by T. cucumeris and found that all the treated hypocotyls were susceptible. However, those hypocotyls that were treated with calcium ions showed a significant reduction in hypocotyl collapse up to a week after inoculation. Total collapse of the calcium treated hypocotyls was obtained when incubation was extended for a week possibly due to the breakdown of calcium pectate by PGTE. The above observations indicate the importance of polygalacturonases and polygalacturonate trans-eliminases in the maceration of radish tissues.

It has been reported that low concentrations of ethylene induced an increase in peroxidase and polyphenol oxidase activities and resistance of sweet potato tissue against black rot (Clare et al. 1966, Stahmann et al. 1966). The present writer studied the effect of ethylene on two stem strains of T. cucumeris and on the host-pathogen relationship of radish and T. cucumeris. When prolonged treatment of isolate 68 with ethylene was done, sectors of fast and slow growing mycelia were induced. However, after two transfers on nutrient agar,

fungal isolates from these sectors reverted to a growth rate comparable or slightly faster than the parent isolate 68. No change in pathogenicity was found. Ethylene had very little effect in reducing the susceptibility of radish seedlings to attacks by T. cucumeris although it induced a slight increase in respiratory rates of healthy seedlings. This increase in respiratory activities is approximately the same as that at the edges of lesions and ethylene from damaged tissue in lesions might be responsible for the increase in respiration of tissues surrounding lesions. Little or no effects on susceptibility to attacks by T. cucumeris were obtained when radish seedlings were treated with 2 chloroethane phosphonic acid which on absorption is known to produce ethylene within host tissues. Stahmann et al. (1966) have reported that ethylene had no apparent effect upon the peroxidase or polyphenol oxidase activity of carrot, radish or turnip root tissues.

The results of the present investigations on the parasitism of T. cucumeris provide background knowledge for a more fundamental approach to the studies of the resistance mechanisms of fungal diseases of higher plants. Radish seedlings are easily grown and within a week of sowing, they grow to a size which is easy to handle both for testing the pathogenicity of T. cucumeris isolates

and for biochemical investigations involving *in vivo* interactions between host and pathogen. The present study illustrates these two points. In many studies of host-pathogen interactions, chemical compounds forming in susceptible hosts are often discounted as functioning in resistance mechanisms. However, the interaction of a host and a pathogen is a competition between the aggressive mechanisms of the parasite and the defensive mechanisms of the host. The reactions of a susceptible host to invasion by a pathogen may be part of an unsuccessful mechanism.

Considering the extreme economic importance of *T. cucumeris*, surprisingly little is known of the genetical and biochemical aspects of its pathogenicity. The development of mutants of *T. cucumeris* with various pathogenic capabilities together with studies on the host-pathogen interactions can provide fruitful means of elucidating the manner in which the aggressive mechanism of *T. cucumeris* operates successfully against the defensive mechanisms of hosts. Such information may lead to the formulation of effective control methods against *T. cucumeris*.

APPENDIX

Culture media

(a) Potato "Marmite" Dextrose Agar (PMDA)

Materials:

Mashed potato	22 g.
Dextrose	20 g.
Con. yeast extract ("Marmite", Sanitarium Health Food Co., Aust.)	1 g.
Agar	15 g.
Distilled water	1 litre

Method:

The potato was extracted in 500 ml of distilled water by autoclaving at a pressure of 15 lb per square inch for ten minutes and filtering through macerated filter paper using a Buchner funnel. The agar was heated in the filtrate until melted. After addition of dextrose and concentrated yeast extract, the volume was made up to 1 litre using warm distilled water, dispensed into bottles and autoclaved at a pressure of 15 lb per square inch for twenty minutes.

(b) Potato dextrose agar (PDA)

Materials:

Mashed potato	22 g.
Dextrose	20 g.
Agar	15 g.
Distilled water	1 litre

Method:

Similar to that described for PMDA except that no concentrated yeast extract was used.

(c) Rhizoctonia medium No. 2 (RM2)

Materials:

KH_2PO_4	0.68 g
K_2HPO_4	0.87 g
$MgSO_4 \cdot 7H_2O$	0.12 g
Sucrose	17.10 g
Bacto-peptone	7.50 g
Distilled water	1 litre

Method:

The ingredients were dissolved in a litre of distilled water. For culturing R. solani on a rotary shaker the solution was dispensed into 200 ml lots per 500 ml Erlenmyer flask. For solid nutrient medium, 15 g of agar was melted in the solution and dispensed into bottles. Media were autoclaved at a pressure of 15 lb per square inch for 20 minutes before use.

Fixatives and preservatives for plant materials

(a) Formo-acetic alcohol (F.A.A.)

The mixture consists of:

Formalin	13 ml
Acetic acid (glacial)	5 ml
Ethyl alcohol (50 percent)	200 ml

(b) Newcomer's fluid (Newcomer 1953)

The mixture consists of:

Isopropyl alcohol	6 ml
Propionic acid	3 ml
Petroleum ether	1 ml
Acetone	1 ml
Dioxan (Diethylene dioxide)	1 ml

Stains

(a) Feulgen or Schiff's Reagent

Materials:

Basic fuchsin	1 g
Distilled water	100 g
Sodium metabisulphite ($Na_2S_2O_5$)	2 g
N HCl	20 ml
Activated charcoal	300 mg

Method:

The basic fuchsin was dissolved in 100 ml of hot distilled water (90° - 95°C). The solution was filtered at 50° - 60°C. On cooling to room temperature (approx. 30°C), sodium metabisulphite and hydrochloric acid were added to the filtrate in a bottle. The mixture was stoppered tightly and stored overnight in the dark. Finely powdered activated charcoal was added and the solution was shaken for a minute and filtered. The straw coloured filtrate was used directly or stored in a brown bottle at 5°C. The solution was

discarded when pink in colour.

(b) Reynold's lead citrate (Kay 1965).

Materials:

$Pb(NO_3)_2$

$Na_3(C_6H_5O_7).2H_2O$ (sodium citrate)

N NaOH

Method:

Lead citrate was prepared by mixing 1.33 g of lead nitrate, 1.76g sodium citrate and 30 ml distilled water in a 50 ml volumetric flask. The resultant suspension was shaken vigorously for one minute and then allowed to stand for 30 minutes with intermittent shaking to ensure complete conversion of lead nitrate to lead citrate. 8.0 ml of N NaOH was then added, the suspension was diluted to 50 ml with distilled water and mixed by inversion. The lead citrate dissolved. The clear solution was used immediately to stain ultrathin sections or stored under oil until required.

Fixatives for Electron Microscopy.

(a) Caulfield's buffered osmium tetroxide fixative with sucrose (Kay 1965).

The fixative consists of:

2% OsO_4 solution	4.0 ml
Veronal acetate buffer*	1.0 ml
Distilled water	2.0 ml
0.1 N.HCl	1.0 ml

The fixative was adjusted to pH 7.4 and 0.015g sucrose per millilitre of fixative were added.

* Veronal-acetate buffer.

Sodium veronal (baritone sodium)	2.89 g
Sodium acetate (anhydrous)	1.15 g
Distilled water	100 ml

(b) Millonig's phosphate buffered osmium tetroxide fixative (Kay 1965).

Solution A: 2.26% $NaH_2PO_4.H_2O$ in water
Solution B: 2.52% NaOH in water
Solution C: 5.40% glucose in water
Solution D (isotonic disodium phosphate) was prepared by mixing 41.5 ml. of solution A with 8.5 ml. of solution B. The pH was adjusted to 7.4 with solution B.

The fixative consists of:

OsO_4	0.5 g
Solution C	5.0 ml
Solution D	45.0 ml

(c) Luft's buffered permanganate fixative (Kay 1965).

The fixative consists of:

1.2% $KMnO_4$	12.5 ml
Veronal-acetate buffer	5.0 ml
Distilled water	2.5 ml
0.1N.HCl	5.0 ml

The pH of the fixative was adjusted to 7.4 with the HCl.

(d) Phosphate buffered paraformaldehyde fixative (Kay 1965).

Paraformaldehyde was prepared by dissolving 40 g of paraformaldehyde powder in 80 ml. of double distilled water at 60-65°C. A few drops of 40% NaOH were added until the solution became clear. 7.5g of sucrose was added and the solution was then buffered at pH 7.4 with 0.067M phosphate. The final solution was made up to 100 ml. with double distilled water.

U.C. Mixture (Baker 1957)

Physical Ingredients:

50% fine sand
50% peat moss

For each cubic yard of physical ingredients, the following chemical ingredients were added:

Potassium nitrate	4 oz
Potassium sulphate	4 oz
Magnesite	8 oz
Blood meal	3 lb
Super phosphate	3 lb
Lime	4 lb
Plaster	2 lb

The mixture was steamed at a pressure of 140 lb per square inch for half an hour and then cooled before use.

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