

A COMPARATIVE STUDY OF THE PATHOGENICITY OF THREE STRAINS
OF RHIZOCTONIA SOLANI KÜHN.

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by

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ABSTRACT

The pathogenicity of two strains of the fungus Rhizoctonia solani Kuhn, and a strain of a related fungus, Corticium praticola Kotila, to three hosts, was studied, to determine which factors affect the specificity of parasitism. One R. solani strain parasitized lettuce, the other, cabbage, and the third fungus C. praticola parasitized both these hosts. Wheat was not severely parasitized by any of these fungi.

Pathogenicity was unrelated to the stimulation of fungus growth by host exudates, which also had little effect on the formation of infection cushions, by which these fungi normally penetrate their hosts. The surface configuration of the host, and the contact stimulus were important in penetration, and attachment of the fungi. Adhesive structures were formed on non-living surfaces by each fungus, but not on damaged host surfaces.

Each fungus penetrated only some tissues of each host. Pathogenicity was found to be unrelated to the inhibitory effect of host tissue on fungus growth, but was correlated to the speed of invasion and direction of advance of the hyphae inside the host tissues. A hypersensitive reaction took place, but this could not account for the specificity of parasitism.

Pectolytic and cellulolytic enzymes were produced in culture by each fungus when grown on synthetic substrates. Enzyme activity was unrelated to the growth of the fungi, and to pathogenicity. Extracts of diseased plants contained pectolytic and cellulolytic enzymes, but their activity was unrelated to that of culture filtrates from synthetic media, and also unrelated to pathogenicity. These extracts were toxic to young seedlings, but toxicity too, was unrelated to the specificity of parasitism. The toxic principle was thermolabile and may have been enzymic, and was able to macerate young seedling tissues.

INTRODUCTION

Most higher plants are parasitized by one fungus or another, and each fungal parasite has its own limited host range. The great majority of parasitic fungi, however, ~~are~~ ^{do} not parasitize the great majority of higher plant species. In other words, parasitism must be regarded as exceptional, and resistance the rule.

In spite of much work on the subject, the basis of resistance of higher plants to attack by fungi is still largely unknown. The associated problem of why a fungus selectively parasitizes one plant species instead of another closely related ~~one~~, has not been satisfactorily answered except in one or two isolated cases. In attempting to investigate the problem of the specificity of parasitism, plant pathologists have approached ~~it~~ in many different ways. One of these has been to select convenient host-parasite combinations, which enable the ready comparison of one system with another. This approach has been adopted in the present work.

Three fungi have been selected in this investigation. They are closely related in some features, two of them being different strains of the same species, Rhizoctonia solani Kuhn. The third fungus, Corticium praticola Kotila, has been considered by some workers to be sufficiently closely related to R. solani, to justify its inclusion in the same species. Differences in the pathogenicity of these three fungi towards two selected host species,

cabbage and lettuce, were, however, quite distinct. One fungus (R.solani strain (S)), parasitized cabbage, but not lettuce, whereas another (R.solani strain (L)), parasitized lettuce, but did not parasitize cabbage. The third fungus (C.praticola), parasitized both these host species. A third host, wheat, which was parasitized only to a very limited extent by each fungus, was studied for the purpose of comparison.

The earlier part of the present work is concerned with the possible effects of plant exudates on the fungi before they come in contact with the plant. Attention has also been paid to the behaviour of the fungi on the plant surfaces. The latter part of the work deals with the entry of the pathogens into the hosts, and the consequences. At each stage, differences between fungi, and between the hosts have been studied, in an attempt to determine whether the observed divergences could account for the specificity of parasitism.

HISTORICAL SURVEY

It has been known for a long time that certain species of the genus Rhizoctonia are pathogenic towards higher plants. The literature is rich in accounts of many aspects of diseases caused by these fungi, and can be traced as far back as 1728, when a species, (the description of which corresponded with later ones of R. crocorum) was recorded by Du Hamel, in a report published in Paris in 1758. Since then, much research has been done with Rhizoctonia spp., and in the course of it, a number of difficulties have arisen. The first of these involves the nomenclature and taxonomic position of these fungi, and this is perhaps best clarified at the outset.

The first report of the species Rhizoctonia solani was published in 1858 by Kühn in Berlin. He described the potato isolate as a mycelial form with no known perfect stage, and distinct from R. crocorum. Since 1858, many workers have described fungi as being identical, or closely related to R. solani, and some of them have been given different names. In the present work, three isolates were used, of which only one has a known perfect stage - Corticium praticola Kotila. The genus Corticium has appeared in the literature for nearly a century. Rolfs (1903) connected this genus with Rhizoctonia and his isolate was described as C. vagum B. & C. Kotila (1929)

named his isolates C. praticola , having decided that they were distinct from R. solani because they had a perfect stage, and differed in certain cell dimensions from C. vagum.

Patouillard (1891) described a fungus Hypochnus filamentosa, which parasitized leaves of Dianthus and Amaryllis, and a few months later Prillieux and Delacroix (1891) described a perfect fungus which they called Hypochnus solani. Rogers (1943) renamed the perfect stage of H. solani, Pollicularia filamentosa (Pat.) Rogers, and stated that Rhizoctonia was an imperfect form of this fungus. Cunningham (1953) regarded Kotila's C. praticola as synonymous with P. filamentosa, whereas Flentje (1956) accepts Kotila's distinctions, but renamed C. praticola as Pollicularia praticola.

In this text, the name Rhizoctonia solani Kühn is used for the two imperfect isolates, and the perfect isolate is called Corticium praticola Kotila. No attempt is made here to review completely, the literature pertaining to R. solani and related species. Selected extracts will be quoted where relevant.

Occurrence and Host Range

R. solani and related species are remarkably widespread in their distribution, being found in each of the five continents. As a group, these fungi have a wide host range. Braun(1930),reported that 230 species of higher plants, representing over 66 families were parasitized.

Disease symptoms

There is much variation in the symptoms of disease, which these fungi are known to cause. Some form of soft rotting is usually present in diseased tissues. Roots, hypocotyls, coleoptiles, stems, leaves and leaf petioles, cotyledons, and storage organs may all be parasitized. One of the most important and best known types of disease is the "damping-off" of seedlings, which is the type investigated in the present work, and upon which there is an abundant literature.

Strain Variation

It is now well established that R. solani and related species exist in the form of different strains. These differ from each other in cultural, physiological and pathogenic characters. Studies on cultural and physiological variation in strains have been comprehensively reviewed by Kernkamp et al. (1952).

It is also well established that strains differ remarkably in their pathogenicity towards various host plants. (Storey, 1941; Le Clerg, 1941; Blair, 1942; Thorpe, 1951; Richter & Schneider, 1953; Kerr, 1956; Flontje & Saksona, 1957; Flontje, 1957; Shephard, 1957.) Some strains have a wide host range, others are restricted to hosts belonging to a single family, while still others are restricted to a single variety of potato. (Kornkamp et al., 1952).

A number of attempts have been made, (Houston, 1945) none of them wholly successful, to separate these strains on characters developing in pure culture on various substrates, as well as on pathogenicity towards various host plants. Flontje (1950) showed that cultural characters and pathogenicity are not closely linked, and observed that isolates which show similar growth and cultural characteristics might differ in their pathogenicity, whereas those which differ in their cultural features may have the same pathogenicity.

Host resistance

Much research has been done on the various aspects of the process of parasitism by R. solani, of different host plants, with a view to finding out why some strains attack particular host plant species and not others. The answers to this question are still incomplete. Some of the more important researches are discussed below.

Resistance by definition is a matter of degree, as it can be evaluated for a particular host-parasite combination only in terms of other such combinations. True resistance on the part of a plant, to parasitism by a particular pathogen depends on the genotypes of the host and of the parasite, and also, the condition of the host plant, both before and after it has been inoculated with the parasite.

In studying the process of parasitism, it is convenient to divide it into pre-penetration, penetration and post-penetration stages, as Brown,(1936); Ward,(1905); and other workers did when they investigated it. Further sub-division of these stages may be necessary. In resistant hosts the process of parasitism could be interrupted at any of these stages. The pre-penetration stage can now be sub-divided further into the pre-contact and contact phases.

Pre-contact phase

In this phase there is the possible influence of substances of host or pathogen origin, which can influence the process of parasitism, before pathogen and host come into intimate contact with each other. Hiltner (1904) used the expression "rhizosphere" for the zone around plant roots influenced by the roots themselves. It is recognized that the "rhizosphere effect" has a chemical base. Substances of pathogen origin may influence the host plant at all stages of the parasitic process, and will be discussed

later. Those of host origin are considered under this heading.

Three possibilities can be envisaged, whereby substances of host origin, can influence the process of parasitism.

- a) A general stimulation of growth, affecting the majority of fungi in the same way
- b) A general reduction or inhibition of growth
- c) Different effects on different fungi. When this occurs, it may be related to specificity of parasitism, so that parasitic fungi are stimulated by exudates from roots of susceptible plants, whereas non-parasitic fungi are either unaffected, or even inhibited, by exudates from the roots of non-susceptible plants.

There are many instances of general effects, but these will not be considered here as they have little bearing on the specificity of parasitism. The literature on the differential stimulation of fungi by host root exudates has been reviewed by Flentje, (1959); who concludes that there are indications that plant exudates affect soil borne pathogens in both general and specific ways, citing the work of Noble, (1924); West & Lockhead, (1940); Rovira, (1956); and Buxton, (1957), that amino acids and other growth factors released by living roots affect spore germination and hyphal growth of fungi. Flentje further points out that these substances may affect not only general pathogenicity, but may also be a

contributory factor in strain or race specialization. Kerr, (1956), working with strains of R. solani, suggested the possibility that "root excretions may affect the ability of a fungus to 'find' a suitable host."

Contact phase

After certain pathogens and their hosts come into contact, penetration of the host tissue takes place from appressorial hyphae. The structure and development of appressoria differ greatly in different fungi. Kühn (1858) observed a clumping effect of the hyphae of R. solani on the surface of infected potato tissue. Prillieux (1891), described structures which he called "corps miliaires" on the surface of beet plants infected by R. crocorum. Both Kühn and Prillieux suggested that these structures were the initial stages in the establishment of infections. Duggar (1915), however, stated that the adhesive structures of R. crocorum which he observed on infected plant surfaces were not essential for infection, and that R. solani did not form these structures.

Many later workers have described adhesive structures formed on several plant surfaces by R. solani and related species. (Abdel Salam, 1933; Ullstrup, 1936; Schaal, 1939; Nakayama, 1940; Schlatter, 1943; Thorpe, 1951; Townsend & Willots, 1954; Flentje, 1957; Kerr & Flentje, 1957; Shophard, 1957; Christou, 1962; Gonzalez & Owen, 1963.)

These structures have been described as multiple appressoria or "infection cushions". The question arises as to what causes these fungi to produce these structures. The classical researches of Blackman and Wellsford (1916), and Brown and Harvey (1927), have lead to the hypothesis proposed by Brown (1936), still widely accepted, that the stimulus which causes the hyphae to penetrate plant surfaces is physical and non-specific in nature, rather than one specific to substances of host origin. According to this, infection cushion formation is a reaction to a contact stimulus.

Kerr (1956), buried radish, lettuce and tomato seeds in cellophane bags in soil inoculated with R. solani. He found aggregations of hyphae on the surface of the cellophane opposite the roots of radish and lettuce seedlings, but not opposite the tomato roots or the hypocotyls of any of the three types of seedlings. Radish and lettuce are susceptible to attack by R. solani, but tomato was resistant. So it was concluded that diffusible substances from the roots of radish and lettuce seedlings stimulated the aggregation of the hyphae, and so influenced the behaviour of the fungus. However, the isolate used attacks hypocotyls and not roots, so the significance of substances diffusing from roots is problematical. Flentjo (1957), did not observe any characteristic attachment of several isolates of R. solani to non-living surfaces, including glass, collodion, cellophane and cotton. He cites this, together

with the selective response to different hosts, as evidence against a simple contact stimulus hypothesis, and put forward an alternative hypothesis that a chemical stimulus alone, or in conjunction with a contact stimulus, might be operative.

Kerr and Flentje (1957), state that "the hyphae of a pathogenic strain (of R. solani), become attached to the cuticle of a susceptible host above the junction of epidermal cells, grow in a characteristic manner along the stem, following the lines of junction of these cells, and form complex appressoria or infection cushions. Infection takes place only from these cushions. Strains not pathogenic to the host, fail to become attached to, or organize on the stems." Kerr and Flentje state that there is a close correlation between the susceptibility of the seedlings to the pathogens, and the stimulation of the pathogens by the seedlings. They suggest that the differential stimulation was caused by a root excretion. They observed structures resembling infection cushions on cellophane moistened with radish root exudate, and inoculated with the radish strain of R. solani. Known nutrients failed to produce these characteristic effects. Strains not parasitic to radish did not respond to the radish root exudate.

In further experiments, Kerr and Flentje grew R. solani strains on epidermal strips from hypocotyls, ~~and~~ moistened with root exudate. In response to the radish root exudate,

the radish strain produced infection cushions and penetrated radish hypocotyl epidermal strips. When no exudate was present, there was no cushion formation and penetration, though the hyphae attached themselves to, and grew along the lines of junction of the epidermal cells. Two other strains not parasitic on radish tested in this way with radish root exudate, failed to produce infection cushions and did not penetrate the epidermal strips of radish, or those of the hosts to which they were pathogenic. They conclude that " attachment to, organization on, and penetration of a host by the radish strain of P. filamentosa (R. solani), is controlled by the nature of the cuticle surface, and the presence of a diffusible material which is excreted onto the cuticle surface from the underlying cells. Specificity of reaction of the different strains to different hosts is probably due to variation from host to host, of the nature of the cuticle surface and the diffusible excretion."

As evidence that the cuticle surface is important in penetration, Kerr and Flentje cite the results of their experiments, where penetration of the cellophane never took place in response to root exudates. Further, they state that the radish strain attacks stems and not roots, and as the surfaces of these organs differ, the surface structure would be important in cushion formation. Finally in their experiments with damaged cuticles, they found that infection cushions were never produced on exposed cortical cells, but were formed only on the intact surface of the cuticle, through which penetration occurred.

Shephard (1957) found that crucifer and lettuce strains of R. solani are not stimulated by host excretions, and suggests that cuticular structure could play an important role in the organization of the hyphae on the surface of host hypocotyls before penetration. She cites the observations of Mueller, Carr and Loomis, (1954), who found much variation in the sub-microscopic structure of cuticles in different plant species. Further, Shephard observed adhesive structures formed by her crucifer strain of R. solani on non-living surfaces like cellophane.

Flentje (1959) reports that infection cushions vary in their detailed structure for different pathogenic strains of R. solani. A composite strain formed appressoria freely on the stems of cruciferous hosts, but the appressoria were usually very loosely attached and no penetration pegs appeared to develop from the majority of them. Gentle irrigation of the stems with water caused most of the appressoria to float off the stems, whereas on lettuce, they could not be dislodged. These observations are in conflict with the earlier observations of Kerr and Flentje (1957), who stated that "strains not pathogenic to the host, fail to become attached to, or organize on the stem."

Flentje (1959), puts forward the following hypothesis regarding appressorial formation. R. solani shows two distinct reactions before appressorial formation, one being the reaction to the surface which directs the growth of the main hyphae, and the other,

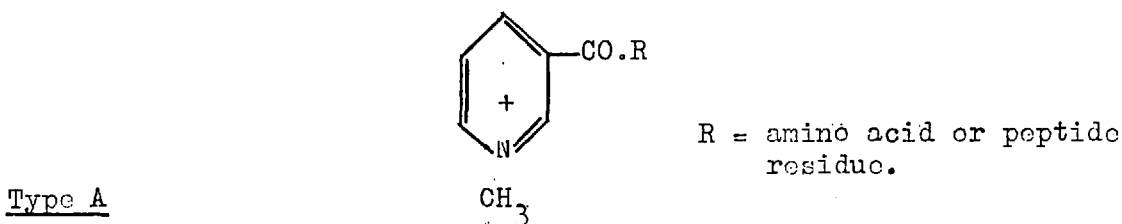
the reaction to diffusible substances, which causes arrested growth and multiple branching of the laterals, resulting in appressorial formation. He states however, that there is no real evidence yet, of the nature of the active materials responsible for these stimuli.

Cannon and Corbett (1962) fractionated radish root exudates and tested the action of these fractions on the growth of an isolate of R. solani which had a wide host range, including radish. They found that all fractions were active and thought that stimulation might have been caused by the amino acids which they detected in all fractions. Aqueous extracts of radish seeds also stimulated the growth of the fungus strongly.

Corbett (1962), suspected that "the general stimulation" of fungal growth by root exudates might be due to simple nutrients such as amino acids and sugars, but when glycine, ammonium glutamate and the ammonium salt of L-aspartic acid were tested, she found that they produced only a slight stimulation compared to that obtained when radish root exudate was used.

Dodman and Atkinson (1962), state that alkaline hydrolysis of radish root exudate produces a substance that causes the hyphae of R. solani to become highly branched. They identified this substance as trigonelline, which they state, is a product of hydrolysis of substances having the structure of Type A,

which they detected in radish root exudates.



Cannon and Corbett, (1962), and Dodman and Atkinson (1962), have observed that nicotinamide is converted in vivo into derivatives of Type A by R. solani, or alternatively, derivatives of Type A could be converted into nicotinamide by this fungus.

Wood (1960) has emphasized that responses leading to cushion formation, and therefore to infection, may differ for different host parasite combinations, even for a single species of pathogen.

Cuticular structure of plants.

Two features of plant cuticles are relevant to the problem of host penetration by fungal pathogens. These are, the permeability of the cuticles, and the possible variation in their surface texture. De Bary (1871), reported variation in the cuticular wax structures found on leaves, and attempted to classify them. This was the first evidence that there is great variation in cuticular structure. Martens (1934, 1940,) stated that folds could

be formed on the waxy cuticular surface, and that these folds often show some relation to the direction of growth (cell elongation), or the growth during a previous stage of development.

Juniper (1959), found that in many plants there are minute projections from the surfaces of cuticles. These can take many different forms, and may be characteristic of the species, genus, or even of the family. For instance, the surface structure of the leaf of Chrysanthemum segetum, looks unlike that of any other species of Chrysanthemum. He states that in cabbage however, leaf surfaces look alike in all forms of cabbage.

Rooelofsen (1959), stated that cuticular surfaces could be granular or plicate. He reports that from observations of cuticular surfaces reported so far, it appears that surfaces do not show special structures, apart from an uniform granulation that varied from fine to coarse in different objects. This could also differ on the two sides of a single leaf.

Permeability of cuticles

Dous (1927), stated that cuticles are permeable, and claimed to have observed pores in some of them. He assumed that movement of substances could take place through these pores. More recent work however, did not confirm these findings. (Weber, 1942; Bange & Rooelofsen, 1950; Volz, 1952; Sitte, 1954; Skoss, 1955; Bock, 1955.)

Bango and Roelofsen (1950), experimented with cotyledon gibbiflora in which Dous claimed to have seen very distinct pores. Bango and Roelofsen found that under the light microscope, the surface appeared to be marked with light dots, but electron micrographs revealed that it had an uniform surface, with possibly, some thinner spots. These findings are supported by those of Scott, Hamner, Baker and Bowler, (1957), and Mueller, Carr and Loomis, (1954). Scott et al. (1957) found no pores in onion cuticles, or in any of several other plant cuticles which they examined. Mueller et al. (1954), suggest that the pore patterns found on cabbage leaves might be filled with wax. These show no change in size with age.

Some interesting results on cuticular diffusion have been published, as a result of work done on the path of entry of chemicals applied as sprays, on plant surfaces. Some reports claim that diffusion through the cuticles was responsible for the penetration of the chemicals. (Crofts, 1933; Weaver & de Rose, 1946; Roberts, Southwick and Palmiter, 1948; Crofts and Harvey, 1949; Norman, Minarik and Weintraub, 1950.)

Skoss (1955), takes exception to this view on the grounds that injury caused by the penetration of foliar sprays is localized in the immediate vicinity of the stomata before it occurs elsewhere. This is supported by Adicott, 1944; Aslander,

1927; Rohrbaugh, 1934; Knight and Cleveland, 1934; Ebeling, 1938. Skoss (1955), also cites his own results, where he showed that the cuticle in an in vitro system is impermeable to the passage of certain organic and inorganic salts in solution. Further circumstantial evidence is provided by the fact that herbicidal penetration corresponds with stomatal penetration.

Juniper (1959), states that the view that everything external to the epidermal cells is "static" can no longer be held, and believes that there is some evidence to indicate the passage of substances through the cuticular layers.

Penetration and Post-penetration stages

Most Rhizoctonia spp. which parasitize young seedlings are hypocotyl or stem attacking strains. Some isolates however, are known to parasitize leaves, (Boosalis, 1950), and roots. (Samuel & Garrett, 1932; Hynes, 1937; Cherewick, 1940; Blair, 1942; Boosalis, 1950; Kernkamp et al., 1952; Flentje & Saksona, 1957; Flentje, 1957.)

Penetration of the host tissue can take place through natural openings, like stomata (Ullstrup, 1936; Nakayama, 1940; Christou, 1962); through wounds or cracks, (Ullstrup, 1936; Nakayama, 1940); or directly through the intact surface by means of haustoria, from infection cushions. (Kernkamp et al., 1952;

Flentje, 1957; Shephard, 1957; Christou, 1962.) The latter type of penetration proceeds through the cuticle and either directly through underlying epidermal cell walls into the cell lumen, or in some cases through the cuticle and in between the epidermal cells. (Christou, 1962.)

The evidence presented by Kerr and Flentje (1957), that no penetration of cortical tissues takes place when the epidermal cells are removed or damaged, indicates that the fungus is especially equipped for penetration through the intact host surface, and that the infection cushion is of great importance in this respect. However, Flentje (1957), reports that in some cases, penetration can take place in the absence of infection cushions.

There has been much discussion in the literature regarding the actual mode of penetration of host tissues by plant pathogens. This literature has been recently reviewed by Dickinson, (1960), and Wood, (1960). From these reviews it emerges that penetration could be (a) a purely mechanical process, (b) a chemically based process, or (c) a combination of mechanical pressure and chemical activity. Dickinson (1960), has pointed out that under natural conditions it is most difficult to prove whether or not barriers have been altered by chemical action in advance of what appears to be mechanical penetration.

Shophard (1957), states that there was no evidence to indicate that a cutinase was involved in the penetration of cauliflower and lettuce hypocotyls by R. solani. She regarded penetration as a mechanical process where the infection cushion provides the anchorage which withstands the pressures needed to enable the infection tube to penetrate the cuticle. She states that further evidence for mechanical penetration is provided by the fact that the hyphae constrict at the point of penetration, and by the fanning out of the hyphae both inside and outside a bottle neck at points of penetration of masses of hyphae through the cuticle.

Action of macerating and cell wall degrading enzymes.

Researches by Brown, (1915, 1917,) and other workers have shown that pectolytic enzymes produced by plant pathogens play some part in the degradation of plant cell walls. The pectolytic and cellulolytic enzyme systems are now considered to be the most important ones involved in the rotting of non-woody tissues, and have been the subjects of intensive studies, which have been reviewed by Kortesz, (1951), and Wood, (1955, 1958, 1960a).

Many workers have investigated the ability of R. solani to produce pectolytic and cellulolytic enzymes in culture. (Holler, 1919; Mannozi, 1932; Storcy, 1941; Blair, 1943; Siu, 1951; Doshpando, 1956; 1960; Elarosi, 1958; Barker & Walker, 1962; Gonsalez & Owen, 1963; Bateman, 1963.).

Storey (1941), did not find any correlation between host specificity of isolates of R. solani and their ability to produce pectolytic enzymes in culture. More recently, however, Barker and Walker (1962), investigated the production of pectin polygalacturonase, pectin methylesterase and cellulase by several isolates of R. solani grown in culture on synthetic media. Under these conditions, they found that cultures of foliage strains showed a higher polygalacturonase and pectin methylesterase activity than did those of root and stem isolates. They also concluded that there was a high correlation between polygalacturonase activity and pathogenicity, but that there was no correlation between cellulase activity and pathogenicity. Gonzalez and Owen (1963), did not observe any adverse effects of culture filtrates of two isolates of R. solani on tomatoes.

Khan (1952), reported that deficiencies of calcium and magnesium predisposed pea and soybean seedlings to attack by R. solani. Wood (1958), has pointed out that the composition of the middle lamella of cells could be variable, and if the middle lamella consists of pectates of calcium and magnesium, availability of these metals may have profound effects on the properties of the middle lamella.

Bateman (1963), has pointed out that in the majority of studies the source of pectolytic enzymes produced

by plant pathogens, has been culture filtrates of the organisms grown on synthetic media, and implications of enzyme action in the host tissue have often been made without a demonstration of these enzymes in the host tissues. He further points out that where the presence of pectolytic enzymes in host tissues have been demonstrated, few attempts have been made to determine whether the enzymes produced by a pathogen in vitro and those found in vivo are similar or different. Bateman (1963), found that there was no correlation between the growth of R. solani on various media and enzyme production, and concludes that both pectin methylesterase and polygalacturonase were adaptive rather than constitutive enzyme systems for Rhizoctonia. He found great variation in the liberation of reducing groups, by preparations of culture produced polygalacturonase, and that from diseased tissues. of comparable viscosity reducing power. He also found that polygalacturonase from diseased tissues converted sodium polypectate to three lower molecular weight polymers, but not to galacturonic acid, whereas culture produced polygalacturonase converted this substrate into galacturonic acid. The preparations also differed in their points of thermal inactivation.

Toxins

Gäumann (1954), stated that micro-organisms are pathogenic only if they are toxigenic. This concept, however, has been modified to accommodate special cases which have been studied later. According to some workers, (Ludwig, 1960), enzymes cannot be considered as toxins unless they themselves are toxic. If enzymes are excluded in the following considerations, there appear to be two types of phyto-toxins, the vivo-toxins (sensu Dimond & Waggoner, 1953), and the toxins acting outside the plant, which condition it for subsequent penetration by the pathogen. The latter type has been cited for R. solani by Müller (1924), and Kerr (1956).

Some instances where toxins have been responsible for resistance to disease have been cited in the literature. (Link & Walker, 1933; Virtanen & Hietala, 1955; Litzenberger, 1940; Meehan & Murphy, 1947.) Many workers have reported phytotoxic effects on plants caused by R. solani. (Ramsey, 1917; Müller, 1924; Cappolletti, 1931; Mannozi, 1932; Nowton & Mayers, 1935; Boosalis, 1947, 1950; Kernkamp et al., 1952; Kerr, 1956.) Some of these descriptions, however, have been concerned with the toxic nature of culture filtrates, and therefore, enzyme action cannot be excluded.

Boosalis (1950), and Nowton and Mayers (1935), found that the toxicity of culture filtrates had some relation to pathogenicity. Shephard (1957), stated that no toxins were involved in the attack of cauliflower and lettuce plants before penetration by R. solani .

Hypersensitive reaction

Hypersensitive reactions were originally considered to be reactions peculiar to obligate parasites. It is now well known that facultative parasites can induce hypersensitive reactions in various plants. (Nusbaum & Keitt, 1938; Muller & Berger, 1940; Meyer, 1940; Ort, 1947; Muller, 1950, 1953, 1955; Paddock, 1953; Priston & Gallegly, 1954; Tomiyama, 1955, 1956; Hooker, 1956; Flentje, 1957; Takakuwa & Tomiyama, 1957.)

Flentje (1957), reports that after the penetration of lettuce seedlings by R. solani, progressive invasion failed, and there were all the indications of a hypersensitive reaction where invaded cells and their immediate neighbours were killed. He concluded that the hyphae too were killed as they did not grow when dissected out. In seedlings treated with narcotics Flentje found that the hypersensitive reaction did not take place, but instead, progressive invasion followed. Further, at low light intensities, the sensitivity of the reaction was reduced. Hasselbrauk (1940), however, observed that the opposite took place, where high light intensity reduced the sensitivity of the reaction of wheat to Puccinia triticina. Flentje supports the findings of Müller and Behr (1949), that hypersensitive areas contain fungicidal or fungistatic substances. Müller (1960), points out that hypersensitivity is reckoned in terms of degree, the resistant plants being more sensitive than the susceptible ones.

MATERIALS AND METHODS

The Fungi

The following fungi were used.

1. Rhizoctonia solani Kühn Strain S, isolated from diseased cabbage seedlings.
2. Rhizoctonia solani Kühn Strain L, isolated from diseased lettuce seedlings.
3. Corticium praticola Kotila obtained from Dr. P.H.Williams, Glasshouse Crops Research Station, Rustington, Sussex, and descended from an isolate supplied by Dr. N.T.Flentje (see Flentje, 1956). Cultures are now in the stock culture collection of the Department of Botany and Plant Technology, Imperial College of Science and Technology, Prince Consort Road, London S.W. 7.

Type cultures were grown from single hyphal tips on V-8 juice agar (see page 28), and maintained at 4°C., and at -20°C., and also under mineral oil at room temperature.(c. 18°C.) Stock cultures were maintained on V-8 juice agar at 25°C., and sub-cultured every three months. The type cultures retained their viability and pathogenicity towards the host plants used in this work.

Table 1 and plate 1 show the cultural features of the isolates.

Table 1

Cultural features of the isolates.

Isolate	Mycelium				Sclerotia	
	Young		Old		Colour	Appearance
<u>R.solani</u> (S)	off white	fluffy	brown	fluffy, zoned	off white	woolly, tufted
<u>R.solani</u> (L)	white	dense mat	off white	papery sheet	black deep brown	hard incrustations exuding black liquid
<u>C.praticola</u>	off white	finely woven mat	off white	papery sheet	brown	small, tending to be radially arranged

Plate 1 Appearance of the colonies of the fungi in culture(S-R.solani S, L- R.solani L, C- C.praticola.)

Nutrient media for the growth of the fungi

All chemicals used, unless otherwise stated were of the Analytical Reagent grade.

Agar media

All the agar media were steamed for 30 minutes in a steam bath before autoclave sterilization. The autoclaving was done for 15 minutes at 15 p.s.i. pressure unless otherwise stated. The pH was adjusted to 5.6 with 0.1 N. NaOH or 0.1 N. HCl unless otherwise stated.

1. V-8 juice agar

The contents of one 12 1/2 oz. can of V-8 vegetable juices (obtained from Campbell's soups Ltd., King's Lynn, Norfolk.) were diluted to 1.5 l. with tap water.

Agar 30 g.

2. Potato dextrose agar

200 g. fresh potato tissue were boiled in 500 ml. water for 30 - 45 minutes. The liquid was filtered through muslin, and the pH adjusted to 5.6. 10g. dextrose and 15g. agar were added and the solution made up to 1000ml.

3. Distilled water agar

15g. agar, washed in distilled water and dried for 24 hours at 70°C. 1000ml. water.

4. Hypocotyl extract agar

Cabbage or lettuce hypocotyls were rinsed in water and air dried. 100g. fresh weight of hypocotyls were boiled in 500ml. water for 20 minutes and the liquid strained through muslin.

20ml. agar and distilled water were added to make the solution up to 1000ml.

5. Coleoptile extract agar

Wheat coleoptiles were rinsed in water and air dried. 60g. fresh weight of coleoptiles were boiled in water for 20 minutes and the liquid strained through muslin. 20g. of agar and distilled water were added to make the solution up to 1000ml.

6. Czapek - Dox Agar

NaNO_3	2g.
K_2HPO_4	1g.
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5g.
KCl	0.5g.
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.01g.
Sucrose	30.0g.
Agar	15.0g.
Distilled water	1000ml.

Sand/Oatmeal medium

250g. washed and dried coarse sand.

12.5g. finely ground rolled oats (obtained from Quaker Oats Ltd.,
Southall, Middlesex.)

80ml. tap water. Autoclaved in 500ml. conical flasks at 15 p.s.i. pressure for 45 minutes.

Liquid MediaMicro-nutrient solution

Table 2 shows the composition of the micro-nutrient solution, used where stated.

Table 2

Composition of micro-nutrient solution used in fungal growth media

Compound	Wt. of compound in stock solution	Essential element	p.p.m. element in stock solution
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	6.9 g.	Fe	556.5
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.06 g.	Cu	61.4
$\text{ZnSO}_4 \cdot 4\text{H}_2\text{O}$	0.07 g.	Zn	63.4
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	0.56 g.	Mn	552.4
H_3BO_3	0.52 g.	B	120.8
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.01 g.	Mo	25.9
Water	250 ml.	-	-

1 ml. of the stock micro-nutrient solution was added per litre of macro-nutrient, to make the micro-nutrient content of the final solution as follows.

Fe 0.55 p.p.m.; Cu 0.06 p.p.m.; Zn 0.06 p.p.m.; Mn 0.55 p.p.m.;
B 0.12 p.p.m.; Mo 0.02 p.p.m.

Macro-nutrient liquid media

(a) Sucrose	15 g.	KCl	0.5 g.
NaNO_3	2 g.	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5 g.
KH_2PO_4	1 g.	Yeastrol	0.2 g.

Yeastrol was obtained from the Brewer's Food Supply Co. Ltd.,
Edinburgh 3, Scotland.

Trace element stock solution 1 ml. made up to 1000 ml., and dispensed
in 50 ml. portions in 10 oz. medicine flats and autoclaved at 15 p.s.i.
pressure for 15 minutes.

(b) Bacto vitamin-free caseamino acids (casein hydrolysate), obtained
from Difco Laboratories Inc., Detroit, Michigan, 4.0 g.

KH_2PO_4	1.0 g.
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.5 g.
Glucose	0.5 g.
Sucrose	15.0 g.

Trace element stock solution 1 ml. made up to 1000ml. with water,
dispensed in 50 ml portions in 10 oz. medicine flats, and autoclaved
at 15 p.s.i. pressure for 15 minutes.

(Bacto vitamin-free caseamino acids contains the following:
total nitrogen - 7 % ; NaCl - 38 % ; Ash - 41 %.)

(c) Host hypocotyl or coleoptile decoctions.

10 g. of washed and air dried hypocotyl or coleoptile tissue, were
autoclaved for 15 minutes with 50 ml. distilled water at 15 p.s.i.
pressure in 10 oz. medicine flats.

The Host Plants

The following host plant species were used.

1. Cabbage (Brassica oleracea L.) variety "Enfield Market"
2. Lettuce (Lactuca sativa L.) variety "Trocadero Improved"
3. Wheat (Triticum vulgare L.) variety "Capolla Desprez"

Cabbage and lettuce seed were obtained from Carters Ltd., London.

Wheat seed was obtained from the Imperial College of Science and Technology Field Station, Silwood Park, Sunninghill, Berks. (1960 harvest)

The seed was stored in dry polythene containers at 4°C. and -20°C.

Soil medium for plant growth

The host seedlings were grown in a greenhouse either in plastic pots (upper diameter 13 cm., lower diameter 7.5 cm.) , or in bitumen coated tins 7.5 cm. tall and 7.5 cm. in diameter. Equal volumes of washed and dried fine sand and peat were mixed thoroughly with the following fertilizer mixture.

<u>Fertilizer</u>	<u>Quantities per cu.ft. of peat/sand mixture</u>
Hoof and horn meal	42.0 g.
(Obtained from W.Unwins Ltd., London S.E.25.)	
KNO ₃	4.2 g.
K ₂ SO ₄	4.2 g.
Magnesium limestone	126.0 g.
CaCO ₃	42.0 g.

The mixture was brought to a suitable moisture content before the seeds were sown.

Illumination

In winter illumination was provided for 13 hours each day, by batteries of four 80 watt daylight type fluorescent tubes spaced 5 cm. apart, and suspended 24 cm. from the surface of the soil.

Temperature

The temperature in the greenhouse did not fall below 16°C., and exceeded this only for relatively short periods, if at all.

Moisture

The pots and tins were well drained but the soil was always kept moist.

Host plant liquid nutrient

The following nutrient solution was used for host plants , where stated , when the plants were grown in vitro. The figures are in p.p.m.

KNO ₃	202	(NH ₄) ₆ Mo ₇ O ₂₄ ·4H ₂ O	0.035
Ca(NO ₃) ₂	656	MnSO ₄ ·4H ₂ O	2.23
NaH ₂ PO ₄ ·2H ₂ O	208	CuSO ₄ ·5H ₂ O	0.24
MgSO ₄ ·7H ₂ O	369	ZnSO ₄	0.29
Ferric citrate	24.3	H ₃ BO ₃	1.86

Method of growing seedlings in vitro

Plastic boxes(with lids) 16 cm. x 11.5 cm. x 6 cm. were cleaned with alcohol to make sterile, and Petri dish bottoms, previously autoclaved, were placed in them. Seeds were sown after surface sterilization on sterile filter paper pads immersed in nutrient solution (10 ml.per dish). Illumination was provided by 80 watt daylight type fluorescent tubes spaced 5 cm. apart and suspended 25 cm. from the

tops of the boxes. The light intensity at the tops of the boxes was 800 to 850 lumens per sq. ft. The temperature was 18 - 20°C.

Methods of surface sterilization of seed

In all the methods described for the surface sterilization of the seed, sterile distilled water was used for all the washings.

Mercuric chloride

0.1 % HgCl_2 with 2 drops of Tween "80" per 250 ml. solution for either 2, 4, 5, or 6 minutes with several washings afterwards.

Tween was obtained from Honeywill & Stein Ltd., London W.1.

Mercuric chloride /Silver nitrate

0.1 % HgCl_2 with 2 drops of Tween "80" per 250 ml. of solution with several washings afterwards, and then 0.1 % AgNO_3 with 2 drops of Tween "80" per 250 ml. solution. Several washings were done, one of which was with 0.2 M. NaCl which precipitated any remaining AgNO_3 .

Times of immersion - HgCl_2 Either 2, 3, or 4 minutes

AgNO_3 Either 2, or 3 minutes.

Chlorine

0.1 % available chlorine from sodium hypochlorite for either 5, 10, or 15 minutes with several washings afterwards.

Propylene oxide

The seeds were placed in a gas-tight 500 ml. flask in which was placed a small tube containing 0.5 ml. of propylene oxide, for 30, or 45 minutes. The cotton wool plugs were loosened and the seeds left to aerate for 48 hours in a fume cupboard. The seeds were then washed.

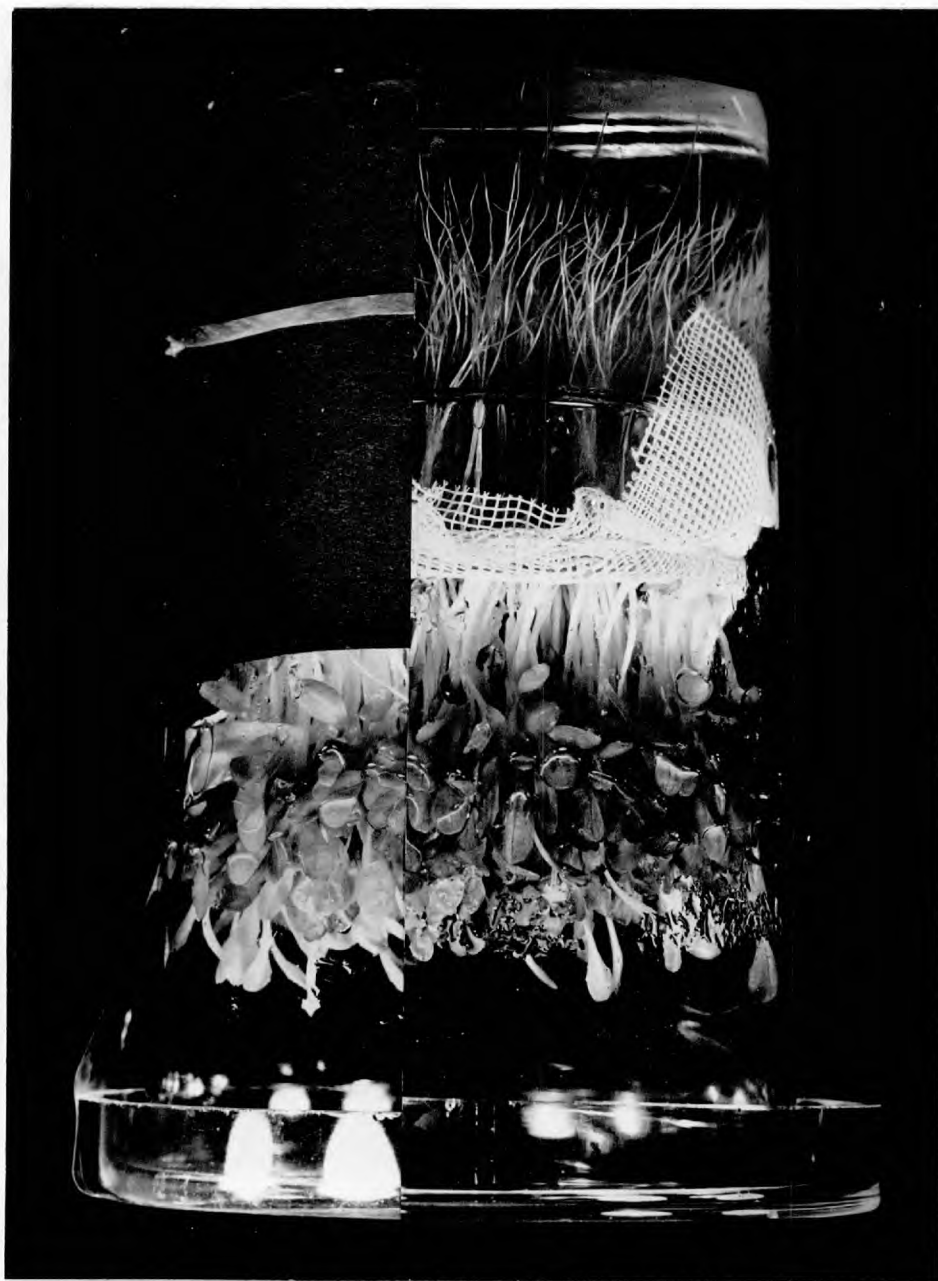
Technique used for growing seedlings under sterile conditions

A simple apparatus was devised (see Plate 2,) for the growth of seedlings under sterile conditions. A "Pyrex" glass cylinder 7 cm. in diameter and 3.5 cm. tall, was placed in an 800 ml. glass beaker (without spout) of slightly greater diameter. Two mm. mesh nylon net, previously boiled for 30 minutes in distilled water, and washed in distilled water, was stretched over the cylinder and secured by a rubber band which had been boiled in distilled water for 30 minutes, and leached for several days afterwards in distilled water to remove water-soluble contaminants.

Glass-distilled water or a nutrient solution, was put in the beaker to within 2 mm. of the nylon net. The beaker was covered with a close fitting Petri dish lid, held in place by adhesive cellophane, and sterilized by autoclaving for 25 minutes at 15 p.s.i. pressure.

Seeds, surface sterilized by the methods described above, were placed on the nylon net and the beakers were kept at room temperature (about 18 - 20°C.) under light from 80 watt daylight type fluorescent tubes, spaced 5 cm. apart and suspended 25 cm. from the tops of the beakers. The light intensity at the top of the beakers was approximately 250 lumens per sq. ft. The base of each beaker was covered with black paper during the experiments, up to the level of the nylon net. Contaminated beakers were discarded. This apparatus was used for obtaining root exudates from seedlings, and also for growing seedlings as a substrate for fungus growth.

Plate 2 Apparatus used for growing seedlings under sterile conditions.



A modification of this technique was used in other experiments where the effect of smaller quantities of liquid on the growth of seedlings was being investigated. A glass cylinder 2.5 cm. in diameter and 4.5 cm. long was used in a "Pyrex" glass boiling tube 3 cm. in diameter, and 20 cm. long. The nylon net was stretched over the glass cylinder by means of a rubber band as before, and the test tube was plugged with cotton wool and covered with aluminium foil over the plug before autoclaving.

Up to 20 seedlings were grown in each tube. The tubes were covered with black paper up to the level of the nylon net as before. The same illumination was used, except that the fluorescent tubes were 12 cm. above the tops of the aluminium foil caps. The light intensity at the tops of the tubes was 3000 lumens per sq. ft.

Inoculation techniques

A - For soil experiments

1) With sand/oatmeal inoculum

Cultures were grown in the sand/oatmeal medium (see p.29) at 25°C. for 3 weeks, after which they were made homogeneous by passing through a 3 mm. sieve. One part by weight of this inoculum mixed with 19 parts of the peat/sand/fertilizer mixture, was placed in the top half of each tin, the bottom half of which contained the uncontaminated peat/sand/fertilizer mixture.

2) With agar inoculum

Disks 5 mm. in diameter, or rings of outer diameter 10 mm. and inner diameter 7 mm., cut from the edge of a ~~growing~~ colony of the fungus growing on potato dextrose agar on Petri dishes, were placed near, (or around, in the case of the rings) the seedling hypocotyls at soil level. In some cases pots containing the inoculated plants were kept in polythene frames to ensure a high humidity.

B - For experiments where seedlings were grown in vitro

a) Agar inocula

Agar disks were used as described above for soil inoculation.

b) Liquid inocula

Liquid cultures were grown on various media for 7 days, homogenized by shaking thoroughly with sterile glass beads, and when an uniform mycelial suspension was obtained, 1 ml. of the inoculum was introduced by means of a sterile pipette, into the vessels containing the seedlings,

Techniques for the bio-assay of root exudates

The exudates were concentrated in a rotary evaporator under reduced pressure so that 1 g. of the dried root material corresponded to 15 ml. of the final solution. Disks of sterile Whatman No. 1 filter paper 5 mm. in diameter, were set on islands of agar 5 mm. in diameter, and surrounded by a space 5 mm. wide, in agar in Petri dishes. Each dish contained 7 ml. of distilled water agar, and each dish was 9 cm. in diameter. To the filter paper disks was added 0.005 ml. of the concentrated exudate. The agar had been washed well

before preparing as a 1.5 % gel with glass-distilled water. The three fungi were put in different dishes. In each dish there were six islands spaced equidistantly from each other. Alternatively, these carried filter paper moistened with root exudate or with water.

The entire agar surface in each plate was covered with a single disk of cellophane (porous - Grade PT 300, obtained from British Cellophane Ltd., London W.1.), which previously, had been repeatedly boiled, and leached in water before sterilizing by autoclaving. After the fungus had grown on the cellophane and reached the edge of the dish, the cellophane surface was flooded with a 0.1 % solution of cotton blue in lactic acid to stain the hyphae.

All the hyphae in a field of view above the centre of the island of agar were then drawn by camera lucida at constant magnification. The total length of the hyphae in the standard area was measured with a map reading device. The ratios of these figures for an area with exudate, to an adjacent area without exudate, were calculated.

Microtechnique

1. Techniques used for staining hyphae growing on agar, cellophane, glass, plastic, and host epidermal peels

a) The hyphae were flooded with 0.1 % aqueous cotton blue, and the excess stain was washed away in water. The material was cleared in dilute lacto-phenol, made up as follows:
glycerine, 2parts; aqueous/saturated phenol, concentrated lactic acid,

and distilled water, 1 part each. The preparations were mounted in lacto-phenol. Whenever it was necessary to keep the preparations for some time, the cover glasses were ringed with a sealing compound which was obtained from Harland and son Ltd., London S.W.19.

b) 0.1 % cotton blue in lactic acid, cleared and mounted in lacto-phenol were used for some preparations.

2. Techniques for sectioning infected seedling tissue.

Fixation

a) Formalin/acetic acid/alcohol fixative (FAA)

50 % ethyl alcohol 90 %

Glacial acetic acid 5 %

Formalin (35 - 40 %) 5 %

The material was injected with the fixative for 10 - 15 minutes under suction, left in fresh fixative for 24 hours, and later rinsed thoroughly in 50 % alcohol to remove the fixative.

b) Craf fixative

A - Chromic acid 1 g.

Glacial acetic acid 7 ml.

Distilled water 92 ml.

B - Neutral formalin 30 ml.

Distilled water 20 ml.

Equal quantities of solutions A and B were mixed to prepare the fixative. The material was injected with the fixative for 24 hours in all, during the first 10 - 15 minutes of this period it was injected under suction.

The material was rinsed in 2 - 3 changes of distilled water and transferred to 25 % alcohol.

Dehydration and embedding

The material was taken through a series of mixtures of tertiary butyl alcohol, ethyl alcohol and water for dehydrating before embedding.

Mixture	Water	95 % ethyl alcohol	Absolute alcohol	100 % TBA	Approx. total alcohol conc.	Time (hours)
1	50	40	-	10	50 %	2
2	30	50	-	20	70 %	24
3	15	50	-	35	85 %	2
4	-	45	-	55	95 %	2
5	-	-	25	75	100 %	2
6	-	-	-	100	100 %	3 changes, 1 of 24 hr. 2 of 2 hr. each

Mixture No. 5 contained a few crystals of dry erythrosin, to make the material visible so as to facilitate orientation during embedding. From the last change of TBA in mixture 6, the material was transferred to a mixture of liquid paraffin and TBA in the proportion of 3 : 1. Embedding in paraffin wax (melting point 56°C.) was carried out according to the method described by Johansen (1940), pp. 134 - 5.

Staining techniques

Microtome sections were cut ~~at~~ 12 - 18 μ thick, and fixed on slides with Haupt's adhesive mixture, (see Johansen, 1940), and floated on water at 50 °C. to spread and then air dried for 24 hours.

The following procedure was then adopted to bring the sections to water. Placed in xylol 5 minutes

1:1 v/v xylol/absolute ethanol 3 minutes

Absolute ethanol 3 minutes

Absolute ethanol (fresh change) 5 minutes

95 % ethanol 5 minutes

& then placed for 3 minutes each in the following ethanol water mixtures; 80 %, 70 %, 50 %, 35 %, 25 %, 15 %, & 5 %.
placed in water for staining.

1. Safranin/aniline blue

Sections in water

Stained in 1 % aqueous safranin for 5 - 10 minutes.

Washed off excess stain in water

placed for 3 minutes in each of the following ethanol water mixtures; 5 %, 25 %, 50 %, 70 %, 70 %,

differentiated in 70 % ethanol containing 0.1 % HCl until the intensity of the safranin was a little stronger than that finally required. Excess acid was washed off in 70 % ethanol.

Placed in 95 % ethanol for 3 minutes

Counter stained in 1 % aniline blue in 90 % ethanol for 3 minutes.

95 % Ethanol 3 minutes

Absolute ethanol 2 changes of 3 minutes each

Xylol 3 minutes

Cleared in clove oil

Mounted in Canada Balsam.

2. Cotton blue

The sections were brought to water and stained by the method described earlier for hyphae.

3. Neutral red

Sections were infiltrated with water under suction for 10 - 15 min.

They were then placed in a mixture containing 1 ml. 0.1 % aqueous neutral red, 0.5 ml. 0.2 M phosphate buffer at pH 7.5, and 8.5 ml. 0.5 M mannitol for 30 minutes, before examination.

Histochemical techniques for testing for pectic substances

1. Ruthenium red

The sections were cut with a freezing microtome 12 - 15 μ thick, and stained with freshly prepared 0.1 % aqueous ruthenium red by the method described by Johansen (1940), pp. 200 - 210.

2. Hydroxylamine/ferric chloride

Reagents:- 13.9 g. hydroxylamine dissolved in 100 ml. water.

14.0 g. NaOH dissolved in 100 ml. water.

1 volume conc. HCl (Sp. gr. 1.18) diluted with 2 volumes of water.

10 g. $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ dissolved in 100 ml. 0.1 N HCl.

To about 10 mg. of plant tissue in 2 ml. of water, were added 2 ml. of the hydroxylamine reagent, and then 2 ml. of the NaOH solution. The reactants were allowed to stand for 2 minutes. Then, 2 ml. of the diluted HCl were added, followed by 2 ml. of the FeCl_3 reagent. (A red precipitate is produced in positive tests.) As a control, the test substances were added to the reactants after the addition of the HCl.

Sections of cabbage and lettuce hypocotyls and wheat coleoptiles were cut at 15 - 20 μ with a freezing microtome. They were placed in water containing 300 p.p.m. SO_2 . The containers were swirled after the addition of each reagent to ensure good mixing. The colour was developed within one minute. The sections were washed in water to remove excess FeCl_3 and examined after vacuum infiltration with water.

ELECTRON MICROSCOPY

Preparation of material for examination under the electron microscope

Several epidermal strips from 7 days old cabbage and lettuce hypocotyls and wheat coleoptiles were stretched on slides, at different angles to each other, taking care to see that they were never damaged or distorted. The slides were then allowed to dry in air, rinsed in distilled water to remove any plant juices, and prepared by different methods for examination.

Cabbage - The epidermal strips were shadowed with a gold/palladium mixture at an angle of tangent 1:3 to the slide, and covered with a

film of carbon in a shadow caster. The slides were then flooded with a 5 % formvar solution in chloroform, and the solvent allowed to evaporate in a draught free atmosphere. The metal-carbon-formvar replica was then stripped from the slide with gummed paper. The replica was floated off the gummed paper by placing the latter (replica facing upwards) , on water. The replica was picked up on a copper grid and washed with chloroform to remove the formvar, and was then examined.

Lettuce - The epidermal strips were shadowed with gold/palladium and covered with carbon as for cabbage. The slides were then flooded with a 2 % formvar solution in chloroform. The chloroform was allowed to evaporate as before. The slide was then immersed in a 1 % solution of collodion in acetone. The acetone was allowed to evaporate in a draught free atmosphere. The replicas were stripped off from the slides using adhesive cellophane, and then placed in acetone to remove the collodion. The replicas were picked up on copper grids and then washed with chloroform to remove the formvar, leaving a metal shadowed carbon replica for examination.

Wheat - The epidermal strips were shadowed with gold/palladium and covered with carbon as before. The slides were then soaked in strong potassium hydroxide solution to dissolve and remove the epidermal strip, and separate it from the metal - carbon replica, which was then picked up on a copper grid for examination.

Preparation of replicas for the growth of the fungi

Epidermal strips were placed on glass slides as before, and rinsed in distilled water. After the slides were dry they were immersed in a 1 % collodion solution, made up in a mixture of diethyl ether and absolute ethanol in the proportion of 3 : 1. The solvent was allowed to dry in a draught free atmosphere. The collodion replicas were stripped off the slides with gummed paper, leaving the epidermal strip behind on the slide. The gummed papers were floated on water, and the replicas were picked up on glass slides. They were then shadowed with carbon.

The slides were next immersed in 5 % formvar in chloroform, and the solvent allowed to evaporate in a draught free atmosphere. The whole film was then stripped off with gummed paper which was floated on water. The film was picked up on glass slides, with the collodion layer uppermost and air dried. The collodion was dissolved away in amyl acetate, added drop-wise from a small pipette, leaving a positive carbon replica on a formvar base on the slide.

Method used for growing fungi on carbon/formvar replicas

The slides containing the replicas were rinsed in distilled water, to remove any traces of impurities present on the replicas. Czapek Dox agar was poured into 13 cm. diameter Petri dishes. The slides containing the replicas were cleaned with a piece of cotton wool moistened with alcohol taking care not to

touch the replica. The inoculum was a 3 mm. diameter disk, cut from the edge of a young colony growing on potato dextrose agar. The inoculum was placed at the periphery of the Petri dish. The fungus grew over the agar, onto the slide, and over the replica.

Preparation of homogenates of host seedling tissues

Surface sterilized seeds were sown in beakers containing the liquid nutrient described on page 33. Hypocotyls of cabbage and lettuce seedlings, and coleoptiles of wheat seedlings were air dried ^{to remove} excess moisture. 30 g. fresh weight of the tissues were added to 100 ml. distilled water at 4 °C., and the tissue macerated in a Moulinex blender for 5 minutes. The coarse material in wheat was squeezed through muslin. The homogenates were spun at 2°C. in a refrigerated centrifuge at 16,000 g. for 20 minutes.

The super-natant liquid was stored in 20 ml. portions at -20°C. Some of the homogenates were sterilized by bringing to 4°C. and filtering through an Oxoid membrane filter 120 μ thick, the pores of which ranged in size from 0.5 to 1.0 μ in the upper layers and 3 to 5 μ in the lower layers. The filtration was done under suction and the receiving flask was surrounded in an ice bath.

Techniques used in enzyme and toxin experimentsEnzymes investigated

1. Pectin methylesterase (PME), which catalyses the hydrolysis of methyl ester groups of pectinic acids to yield methyl alcohol and pectinic acids of lower methoxyl content.
2. Pectin polygalacturonase (PG), which breaks the polygalacturonide chains of pectic acids at the α 1, 4 linkages to produce shorter chains with the liberation of reducing groups.
3. Cellulase, which hydrolyses the α 1, 4 glucosidic linkages of cellulose chains, to produce shorter chains with the liberation of reducing groups.

Chemical materials

1. Sodium polypectate (NaPP), the sodium ammonium salt of polygalacturonic acid. (Referred to in the text as pectate)
2. Pectin (P). Both NaPP and P were obtained from Sunkist Growers Inc., Ontario, California, U.S.A.
3. Carboxymethyl cellulose (CMC), high chain length polymers with a degree of substitution of 0.4, obtained from the Hercules Powder Co.Inc., Wilmington, Delaware, U.S.A.
4. Wood cellulose (WdC), Solka Floc, (containing 99.5 % cellulose) obtained from Brown Co., Boston, Mass., U.S.A.

Each of these substances were used as substrates for the growth of the fungi, and NaPP, P, and CMC were also used as substrates in the estimations of enzyme activities.

Chemicals used in the measurement of reducing groups1. Copper reagentSolution A

Na_2CO_3 (anhydrous)	25 g.
Rochelle salt	25 g.
NaHCO_3	20 g.
Na_2SO_4 (anhydrous)	200 g.

These substances were dissolved in about 800 ml. of water by warming to 30°C . It was then diluted to make 1 litre, and stored at 25°C .

Solution B

15 % CuSO_4 containing 2 drops of H_2SO_4 (conc.) per 100 ml.

Solutions A and B were mixed immediately before use in the proportion of 25 : 1, to make up the copper reagent.

2. Developing reagent

$(\text{NH}_4)_6\text{MoO}_7 \cdot 4\text{H}_2\text{O}$ - 25 g. dissolved in 450 ml. distilled water. 21 ml. of H_2SO_4 (conc.) were added to this solution. 3 g. of Sodium arsenate were dissolved in 25 ml. of water and added to the ammonium molybdate sulphuric acid mixture. The mixture was stirred, incubated at 37°C . for 48 hours, and stored in a dark glass-stoppered bottle at room temperature.

Technique for obtaining culture filtrates of the fungi, grown on synthetic substrates

Basal medium for liquid cultures

Bacto vitamin-free caseamino acids	0.4 %
KH_2PO_4	0.1 %
Glucose	0.05 %
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.05 %
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	0.56 p.p.m. Fe
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.06 p.p.m. Cu
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.06 p.p.m. Zn
$\text{MnSO}_4 \cdot 5\text{H}_2\text{O}$	0.55 p.p.m. Mn
H_3BO_3	0.12 p.p.m. B
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.26 p.p.m. Mo

4 different carbon sources were used with the basal medium in separate containers. These were :-

1 % pectin, 1 % NaPP, 1 % CMC, and 1 % WdC.

The pH of the media was adjusted to 5.7 with 0.1 N NaOH and maintained with 0.2 M phosphate buffer. The media were dispensed in 50 ml. portions in medicine flats and autoclaved for 20 minutes at 15 p.s.i. pressure. The media were inoculated with 1 ml. of a homogeneous liquid inoculum obtained as described on page 38, by growing the fungi on medium B for 10 days at 25°C. After inoculation the flats were incubated at 25°C. for 7 days. The culture filtrates were harvested by filtering off the fungus under suction through Whatman No. 541 filter paper. Salt

extracts of the fungi with 50 ml. of 2M NaCl for each lot of mycelium were also taken. The mycelium was washed with distilled water and transferred to weighed aluminium foil cups, dried until no further change in weight was observed, and the dry weight determined. (The weighings were done after the cups containing the dry mycelium had cooled to room temperature in a desiccator over calcium chloride.)

The culture filtrates were centrifuged at 10,000 g for 20 minutes to make them cell-free, and stored in 20 ml. portions at -20°C . until required.

Technique for obtaining culture filtrates of the fungi growing on the host seedlings

Host seedlings were grown under sterile conditions by the method described on page 35. 500 cabbage or lettuce seedlings were surface sterilized with mercuric chloride (see page 34) and placed in each of the beakers. The seedlings were grown for 8 days at $20 - 25^{\circ}\text{C}$. Sterile distilled water was used in the beakers. A 13 hour day was provided as before with artificial light. When the seedlings were 8 days old, 1 ml. of a standard liquid inoculum was applied as described on page 30. The fungus for the inoculum was grown on medium A, but without yeastrel. (see page 30). After inoculation the beakers were transferred to an illuminated constant temperature room maintained at 25°C . and kept for 10 days. The beakers were swirled as often as possible each day so that the liquid bathed the fungus and the seedlings.

The culture filtrates were harvested ten days after inoculation with the fungi as follows; The glass cylinders and rubber bands were removed from the beakers. The pieces of nylon net with the seedlings growing through them were cut into smaller pieces with a pair of scissors, and the whole mass agitated for 2 hours on a magnetic stirrer, and then strained through cheese cloth. Salt extracts were taken using 300 ml. 0.2 M NaCl per the contents of each beaker. These were added to the liquid which had been strained through the cheese cloth earlier. The extracts were then centrifuged at 16,000 g. for 20 minutes to make cell-free, and stored in 20 ml. portions at -20°C. until required for use.

Standardization and dialysis of the culture filtrates

The culture filtrates were standardized to make the different filtrates comparable. The culture filtrates of the fungi grown on synthetic substrates, as well as the extracts of fungus-infected host seedlings were dialysed against several changes of distilled water at 4°C. for 24 hours. They were then concentrated in a rotary evaporator under reduced pressure. The culture filtrates of the fungi grown on synthetic media were concentrated so that 100 ml. of the filtrate corresponded with 0.5g. dry weight of fungus. The extracts of diseased seedlings were standardized so that 100 ml. of the extract was obtained by the growth of the fungi on 1000 seedlings. This was done as the actual amount of fungus could not be measured.

Methods of estimation of enzyme activity1. Pectin methylesterase (PME)A. Detection of acid liberated from pectin using methyl red indicator

PME removes the methyl ester groups from methylated anhydro-galacturonic acids. The activity of the enzyme is estimated in terms of the carboxyl groups produced. 5 ml. of the enzyme preparation, adjusted to pH 5.5 was added to a mixture of 15 ml. 1 % pectin, and 1 ml. 2 M NaCl, with sufficient 0.1 N NaOH to bring the pH of the mixture to 5.5, and distilled water to bring the total volume to 20 ml. A few drops of methyl red indicator and toluene were added, and the mixtures incubated at 25°C. for 48 hours. Active enzyme preparations changed the colour of the indicator from beige to pink.

B. Estimation of carboxyl groups liberated from pectin by titration

PME activity of the enzyme preparations was estimated by titrating the free carboxyl groups liberated during the enzymic deesterification of high methoxyl pectin against 0.01 N NaOH (Kertesz, 1951, p. 362). This was carried out as follows:-

Pectin was first washed to purify by suspending 25 g. in 150 ml. of 70 % ethanol containing HCl sufficient to make the final solution 0.05 N. The suspension was agitated for one hour on a magnetic stirrer. It was then passed through Whatman No. 541 filter paper under suction and the solid residue was washed several times in 70 % ethanol until no further chloride could be

detected in the washings when tested with AgNO_3 solution. The solid then washed once with 95 % ethanol and dried at 70°C . for 48 hours. It was allowed to equilibrate with air at room temperature for 48 hours. The moisture content of the pectin was determined at this stage by drying at 70°C . for 24 hours, cooling it in a desiccator over calcium chloride to room temperature and weighing.

For the estimation of PME activity, 20 ml. of a 1 % w/v of washed pectin in distilled water, adjusted to pH 5.7 with 0.1 N NaOH, together with 5 ml. of M NaCl was used as the substrate in 100 ml. beakers. At time 0, 5 ml. of the enzyme preparation was added, and the pH adjusted to 5.7 within 2 minutes. The reaction mixture was stirred continually with a magnetic stirrer. The carboxyl groups liberated were titrated with 0.01 N NaOH to a constant pH of 5.7 using a Pye Cambridge pH meter. The pH was not permitted to change more than 0.5 unit from the stated pH value during the experiments.

The quantities of NaOH added and the times of addition were recorded, hence the μg . equivalents of carboxyl groups, and the degree of deesterification were obtained. The activity was expressed as the percentage hydrolysis of methyl ester groups.

$$\% \text{ hydrolysis } = \frac{\mu\text{g. COOH liberated}}{\mu\text{g. COOH liberated by complete deesterification}}$$

2. Pectin polygalacturonase (PG)

A Estimation of PG activity by the reduction in viscosity of P & NaPP

The ability of enzyme preparations to reduce the viscosity of solutions of pectin and pectate, has been used by many workers to estimate PG activity. Enzyme activity estimated in this way is referred to in the text as PG(viscosity) activity. Pectin and pectate were washed as described for pectin on pages 53 - 4, before use.

Five size 200 Cannon-Fenske viscometers were used in a water bath maintained at 25°C. and controlled within 1/4 °C. by means of a mercury toluene thermostat. The water bath was stirred mechanically. The flow times for 10 ml. of distilled water were determined for each viscometer.

The enzyme/substrate reaction mixtures consisted of 5 ml. of substrate (pectin or pectate 1 % w/v solution adjusted to pH 5.7 with 0.1 N NaOH), 2 ml. of 0.2 M phosphate buffer, 2 ml. distilled water and 1 ml. of the enzyme preparation. The enzyme preparation was brought to 25°C. and was added to the reaction mixture in the viscometers at time 0. Mixing of the reaction mixtures was done by bubbling, and the flow times were recorded at times 1,5,10,15,20, 25, and 30 minutes from zero time. As controls the flow times for similar mixtures using enzyme preparations autoclaved for 15 minutes at 15 p.s.i. pressure and cooled to 25°C., were recorded. Relative viscosities were obtained by dividing the flow times by those for water for the same viscometer. Curves were drawn, plotting relative viscosity

against time. The times taken for a 25 % or a 50 % reduction in viscosity of the maximum possible were calculated from the curves. Activity is expressed as follows:-

$$\text{PG (viscosity) activity} = \frac{100}{\text{time for 25\% (or 50\%) reduction in viscosity}}$$

B. Estimation of PG activity by the measurement of the reducing groups produced from pectin or NAPP

The reducing groups were estimated by the micro-Somogyi method as modified by Nelson (1944). The reaction mixtures consisted of the following:-

10 ml. of a 1 % solution of pectin or pectate adjusted to pH 5.7 with 0.1 N NaOH; 4 ml. of 0.2 M phosphate buffer at pH 5.7; 4 ml. distilled water and 2 ml. of the enzyme preparation.

At time 0, the enzyme preparation was added to the reaction mixture, in Erlenmeyer flasks, and shaken. At various times up to 24 hours, 1 ml. aliquots were taken from the mixture and added to a tube containing 1 ml. of the copper reagent. (In some cases 0.5 ml. of the reaction mixture was used with 0.5 ml. of water.) This was boiled in a water bath at 100°C. for exactly 20 minutes, and cooled immediately after to 20°C. When cooled, 1 ml. of the developing reagent was added and allowed to stand until the reaction had stopped, as observed by the stoppage of frothing. The contents of each tube was then made up to 25 ml. with distilled

water, and read on an E.E.L. colorimeter using a red filter (No. 205). Water was used instead of the reaction mixture as the control standard for colorimetry. The colorimeter readings were calibrated with standard glucose solutions (5 - 50/ug. per ml.).

The reducing powers of the mixtures were estimated in terms of /ug. equivalents of α -galacturonic acid. PG (reducing groups) activity was expressed as the percentage hydrolysis of the 1, 4 glycosidic linkages in the α -galacturonic acid polymer.

3. Cellulase

A. Estimation of cellulase activity by the reduction in viscosity of CMC

The method is based on the ability of the enzyme preparation to reduce the viscosity of solutions of CMC, and was similar to the method used for the estimation of PG (viscosity) activity, except that the reaction mixtures contained a 1 % w/v solution of CMC instead of P or NaPP. Cellulase (viscosity) activity was expressed as for PG as follows:-

$$\text{Cellulase (viscosity) activity} = \frac{100}{\text{time for 25\% (or 50\%) reduction in viscosity}}$$

B. Estimation of cellulase activity by the measurement of the reducing groups liberated from CMC

The method used was similar to that used for the estimation of PG (reducing groups) activity except that a 1 % w/v solution of CMC was used in the reaction mixtures instead of

pectin or NaPP. Cellulase (reducing groups) activity was calculated in $\mu\text{g.}$ reducing sugars liberated, and expressed in terms of the percentage hydrolysis of the 1, 4 glucosidic linkages in the CMC polymer.

$$\% \text{ hydrolysis} = \frac{\mu\text{g. glucose liberated}}{\mu\text{g. glucose polymer originally present}}$$

EXPERIMENTAL WORK AND RESULTS

1. Determination of pathogenicity

The pathogenicity of the three fungi to cabbage, lettuce and wheat seedlings was investigated as follows:- The peat/sand/fertilizer mixture (see page 32), was put into bitumen coated tins, so that each tin was about half full. About 150 g. of the mixture was infested with the sand/oats inoculum as described on page 37. The infested mixture was placed in the half full tins, and brought to a moisture content suitable for seed sowing.

Twenty five seeds were sown in each tin so that they were equidistant from each other. The cabbage and lettuce seeds were sown 5 mm. and wheat seeds 10 mm. below soil level. The experiments were done in winter in a greenhouse, with additional light for 13 hours each day from 80 watt, daylight type, fluorescent tubes spaced 5 cm. apart, and suspended 24 cm. from the soil surface. The light intensity at the surface of the soil was 800 - 850 lumens per sq. ft.

Each treatment was done in triplicate, and the seedlings were removed daily, as soon as they were seen to be diseased. During the experiment, the temperature of the greenhouse did not fall below 16°C. and exceeded this only for relatively short periods. The results given in Table 2, show the final stand of seedlings 28 days after sowing.

Table 2

Pathogenicity of the fungi towards host seedlings in the soil

Fungus	Final stand of seedlings after 28 days, as % of seed sown . Standard errors of means are given		
	Cabbage	Lettuce	Wheat
<u>R. solani</u> (S)	0	86.7 $\pm$ 8.3	89.3 $\pm$ 3.0
<u>R. solani</u> (L)	74.6 $\pm$ 8.3	10.6 $\pm$ 1.8	82.6 $\pm$ 1.8
<u>C. praticola</u>	0	0	64.0 $\pm$ 3.2
Nil	92.0 $\pm$ 3.2	85.3 $\pm$ 17.4	85.3 $\pm$ 3.2

R. solani(S) killed all the cabbage seedlings before emergence, but had no effect on lettuce or wheat seedlings. R. solani(L) had no effect on wheat and caused a little post-emergence damping-off of cabbage. It was, however, highly pathogenic to lettuce, although it killed susceptible seedlings more slowly than did the other two fungi. C. praticola killed all the lettuce and cabbage seedlings before emergence, and it also killed a few wheat seedlings before emergence, but none after emergence. C. praticola was far less pathogenic to wheat than it was to the other two hosts.

From these experiments, the pathogenicity of the three fungi to these hosts can be summarized as shown in Table 3.

Table 3

Pathogenicity of the fungi towards the host seedlings

Fungi	Cabbage	Lettuce	Wheat
<u>R.solani</u> (S)	++	-	-
<u>R.solani</u> (L)	-	++	-
<u>C.praticola</u>	++	++	+

++ = Severely pathogenic
 + = weakly pathogenic
 - = not pathogenic (no damping-off)

These results were confirmed in another experiment which was carried out aseptically. Cabbage and lettuce seeds were surface sterilized by washing for 3 minutes with HgCl_2 as described on page 34. Wheat seeds were sterilized by washing with HgCl_2 , and then, AgNO_3 as described on page 34 for 5 minutes in each sterilant. A small number (c. 30), of each type of seed was placed on a disk of sterile Whatman No. 1 filter paper, moistened with 10 ml. of sterilized nutrient solution (see page 33), in sterile Petri dishes. All ungerminated seeds, and seedlings in excess of 25 were removed after 5 days. In the centre of each dish was placed a 5 mm. diameter disk taken from the edge of a young colony of the fungus,

growing on potato-dextrose agar. The dishes were incubated in sterile plastic boxes, with the Petri dish lids removed, at 22°C. Each treatment was replicated five times and the results, after 12 days are shown in Table 4.

Table 4

Pathogenicity of the fungi in pure culture

Fungus	Final stand of seedlings as a % of the initial		
	Cabbage	Lettuce	Wheat
<u>R.solani</u> (S)	2	100	100
<u>R.solani</u> (L)	100	6	100
<u>C.praticola</u>	0	0	100
Nil	100	100	100

These results fully confirm those obtained earlier, and showed clearly that both the strains of R. solani were highly specific in their parasitism, that C.praticola parasitized both cabbage and lettuce, and that wheat seedlings were tolerant to each of the three fungi.

Before proceeding further, isolations were made from diseased seedlings from the soil experiment, and a typical isolate of each fungus was used in another soil experiment

The results were almost exactly the same as before. These new isolates were used in all subsequent experiments.

2. Behaviour of the fungi on the surfaces of the hosts

Direct observation of the surfaces of the seedlings growing in soil, contaminated with one or another of the fungi was carried out. Each fungus was observed to produce well-developed infection cushions on the surfaces of the hypocotyls of susceptible plants. On species not normally attacked, infection cushions were either not formed, or were poorly developed, in comparison to those on the surfaces of the susceptible species. The well developed infection cushions were similar to those described by Flentje (1957), and Shephard (1957).

Infection cushions were characterized by excessive clumping of the hyphae, (see Plate 3), and the profuse

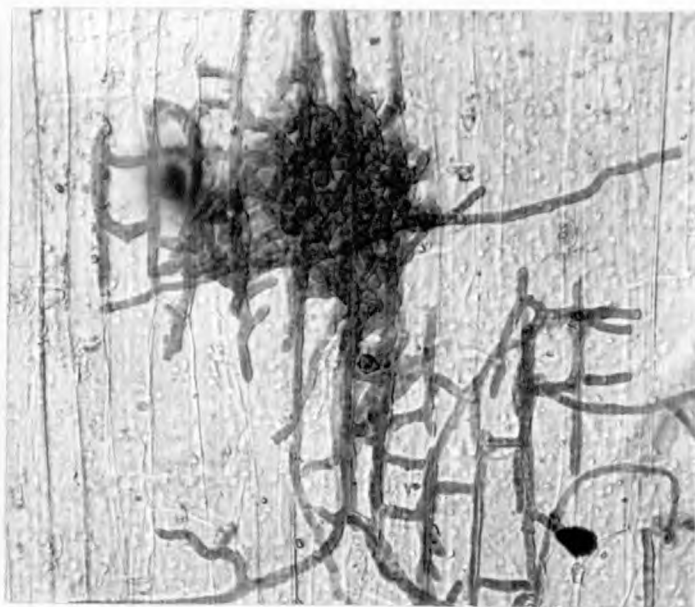


Plate 3 Infection cushions of C. praticola on the surface of a lettuce hypocotyl

(X 300)

development of short lateral branches, between which anastomoses were often formed. It is clear that the fungi R. solani(S) and R. solani(L) used in the present work, are what Flentje (1957) regards as "specialized stem-attacking strains", and that the cabbage strain R. solani(S) is similar to the radish strain used by Flentje, who stated that it attacked stems but not roots. Although not stated, this strain was presumably specific in its parasitism.

3. Effect of host exudates on the fungi

The effect of host exudates of the fungi was investigated as described below. The apparatus described on page 35 and shown in Plate 2, was used to grow the seedlings under aseptic conditions. Cabbage and lettuce seeds were surface sterilized with 0.1 % HgCl_2 for 3 minutes, and wheat seed with 0.1 % HgCl_2 for 2 minutes and then, 0.1 % AgNO_3 for 4 minutes as described on page 34. After thorough washing, the surface disinfected seeds were placed on the nylon net so as to cover most of it. The conditions of temperature and illumination are given on page 35.

In some experiments, the water level in the beakers was at the level of the nylon net, at the start of the experiment. Each day the beakers were gently swirled for a time, so that the surfaces of the seeds and the lower parts of the plants were gently washed, to dissolve any substances that might have accumulated on these surfaces. The liquid in these beakers was considered to

contain exudates from the roots, and some exudates from the seeds and the the other parts of the plants.

In other experiments the cylinders with the germinating seeds were removed 4 days after sowing, and were washed well in sterile water. They were then put into fresh lots of sterile water, the level of which was 10 mm. below the nylon net. In this way it was hoped to get exudates from the roots, uncontaminated with substances from the other parts of the plants. A few beakers became contaminated and these were discarded.

After incubation for 2 weeks, the liquid in the beakers was collected, and samples were removed to test for contaminants. The rest was split up into a number of small volumes, (about 20 ml.) stored at $-20^{\circ}\text{C}.$, and thawed as required. After thawing, the exudates were centrifuged at 10,000 g for 20 minutes to remove all the insoluble matter, and then concentrated in a rotary evaporator under reduced pressure at $30^{\circ}\text{C}.$, so that 1 g. of dried root material corresponded to 15 ml. of the final solution. The method of bio-assay is described on page 38.

Table 6 summarizes the results for a series of experiments in which the exudates used came from seedlings which had not been washed. They were placed with their roots in the final solution. The exudates are, therefore, in respect of root, seed and hypocotyl (or coleoptile) together. Each of the figures shown is the mean for 24 areas above 24 islands, and each is the percentage

increase in growth relative to the control in which the filter paper was moistened with water. In all, exudates from eight beakers were tested for each species.

Table 6

Effect of exudates from roots, seeds and hypocotyls of cabbage and lettuce, and roots seeds and coleoptiles of wheat seedlings on the growth of the fungi

Exudate	% change in growth of the fungi		
	<u>R.solani(S)</u>	<u>R.solani(L)</u>	<u>C.praticola</u>
Cabbage	+ 32.9	+ 37.9	+ 44.6
Lettuce	+ 120.0	+ 147.0	+ 147.0
Wheat	+ 2.9	+ 15.0	+ 2.9

Analysis of variance:-

Interaction effects - exudate x fungi were not significant; Exudate effects were just significant at $P = 0.01$ and very highly significant at $P = 0.05$; Fungus effects were just significant at $P = 0.05$. The significant differences between any pair of means of exudate effects were:- 6.9 at $P = 0.05$ and 9.1 at $P = 0.01$.

Exudates from wheat roots had practically no effect on the growth of R. solani(S) and C. praticola, and stimulated the growth of R. solani(L) only slightly. Exudates from cabbage roots stimulated the growth of each fungus to about the same extent (30-40 %). There was some indication that the growth of C. praticola was stimulated slightly more than the growth of the other two fungi. Exudates from the roots of lettuce were by far the most effective, stimulating the growth of each of the fungi by 120 to 150 %. There was an indication that the growth of the cabbage strain was slightly less stimulated than that of the other two fungi, but no evidence that the effect of cabbage and lettuce exudates on the growth of the two R. solani isolates was at all related to the susceptibility of these two hosts to disease. On the other hand, exudates from wheat which was resistant to each of the fungi, stimulated fungus growth only very slightly.

In a further series of experiments, the concentration in the exudates, of substances from plant parts other than roots were reduced by washing the young seedlings 4 days after sowing, and placing them so that only their roots were immersed in the water. The results of these experiments are summarized in Table 7, where the mean figures given are in respect of 24 islands. Each figure is the percentage increase in growth relative to the control.

Table 7

Effect of exudates from roots of cabbage, lettuce and wheat seedlings on the growth of the fungi

Exudates	% change in growth of the fungi		
	<u>R.solani(S)</u>	<u>R.solani(L)</u>	<u>C.praticola</u>
Cabbage	+ 38.5	+ 40.8	+ 39.7
Lettuce	+ 142.0	+ 147.5	+ 139.6
Wheat	+ 2.9	+ 6.6	+ 8.3

Analysis of variance:-

Interaction effects - exudates x fungi, and fungus effects, were insignificant. Exudate effects were very highly significant at $P = 0.01$; Significant differences between any pair of means of exudate effects were; 3.7 at $P = 0.05$; and 4.8 at $P = 0.01$.

These results were almost exactly the same as before. They differed only in the effects of the exudates of wheat on the growth of R.solani(L) and C.praticola, and these differences were not of great importance.

4. Effect of root exudates from plants of different ages on the growth of the fungi

The apparatus described on page 35 was used for these experiments. The surface sterilized seeds were sown on the nylon net, and the seedlings were allowed to remain in situ for 7 days. The liquid in which the roots had grown was then collected and concentrated as before - 7 day exudate.

In a second series, the seedlings were removed from their beakers at the end of 7 days, washed well with sterile distilled water, and placed with their roots dipping into a fresh lot of distilled water, where they were allowed to remain for a further 7 days. The solution was then collected and concentrated as before - 7 to 14 day exudate. This was repeated for a third series to give a 14 to 21 day exudate. These exudates were then bio-assayed as before. Each treatment had 6 replicates, and the results are summarized in Table 8.

Again, the exudate from wheat had little effect on any of the fungi. There was appreciable stimulation of fungus growth by the cabbage exudates, only when these had been collected during the first seven days of growth of the seedlings. Afterwards, there was little stimulation of growth. By far the biggest effect was observed with the lettuce exudates collected in the first seven days. There was far less stimulation by the

7 - 14 day exudates, but it was still greater than that given by the best of the cabbage exudates. The 14 - 21 day lettuce exudates had practically no effect.

Table 8

Effect of exudates from seedlings of different ages on the growth of the fungi

Exudate	Age of the exudate (days)	% change in growth of the fungi		
		<u>R.solani(S)</u>	<u>R.solani(L)</u>	<u>C.praticola</u>
Cabbage	0 - 7	+ 34	+ 26	+ 29
	7 - 14	+ 10	+ 11	+ 11
	14 - 21	+ 7	+ 8	+ 12
Lettuce	0 - 7	+ 133	+ 111	+ 120
	7 - 14	+ 37	+ 44	+ 39
	14 - 21	+ 6	+ 2	+ 5
Wheat	0 - 7	+ 6	+ 4	+ 11
	7 - 14	+ 5	+ 4	+ 14
	14 - 21	+ 5	+ 6	+ 4

Analysis of variance:-

The following effects were not significant; fungus; interaction fungus x exudate; interaction - fungus x time; interaction - fungus x exudate x time; The following effects were highly significant at $P = 0.01$ exudate; time; interaction - exudate x time. The significant differences

between any pair of means of exudate or time effects were;

3.2 at $P = 0.05$; and 4.2 at $P = 0.01$. Significant differences between means of exudate x time effects were; 5.5 at $P = 0.05$; and 7.3 at $P = 0.01$.

Once again, the cabbage and lettuce exudates did not affect the growth of either of the R.solani isolates, in ways corresponding with the pathogenicity of these isolates.

5. Attachment of the fungi to, and their organization on plant surfaces

The behaviour of the fungi on the surfaces of the host seedlings were examined in the following way. Cabbage, lettuce and wheat seeds was sown on filter paper, moistened with the host liquid nutrient described on page 33, in Petri dishes. The germinated seedlings were picked up 4 days later, washed in sterile water, and placed in sterile dishes, which were in turn placed in sterile plastic boxes. The sterile dishes carried a piece of filter paper as before moistened with the same nutrient solution.

The seedlings were inoculated with rings of agar placed around the hypocotyls (or coleoptiles), and examined periodically over the seven days after inoculation, after staining with 1 % aqueous cotton blue. The observations are summarized below.

1. Cabbage seedlings

a) R.solani(S)

Well developed infection cushions, as described by Shephard (1957), for this isolate, were present in abundance, and there was a characteristic orientation of the hyphae along the longitudinal axis of the hypocotyls. The majority of the hyphae followed the lines of junction of the epidermal cells, and most of the remainder grew parallel to these junctions. No infection cushions were observed on roots, cotyledons or leaves, although on young stems, the hyphae were orientated to some extent along the lines of junction of the epidermal cells.

b) R.solani (L)

There was great variation in the formation of infection cushions by R.solani(L) on cabbage plants. On some hypocotyls many infection cushions were present, on others, only a few were observed, while on still others there were none. Sometimes the infection cushions were only loosely attached to the surface of the hypocotyls. Marked orientation of the hyphae along the lines of junction of the epidermal cells was observed, even on plants which had no infection cushions on them. Orientation of hyphae was a constant feature, but the formation of infection cushions was somewhat erratic. The structure of the infection cushions was similar to that described by Flentje (1957), and Shephard (1957). No infection cushions or orientation of hyphae, were observed on roots, stems, cotyledons or leaves.

c) C.praticola

Massive infection cushions were produced on the hypocotyls in great abundance. The hyphae characteristically followed the lines of junction of the epidermal cells, and grew in the same directions on the rest of the cell surfaces as well. Roots, cotyledons, and leaves carried no infection cushions, and there was no orientation of hyphae on any of these organs, though cotyledons were parasitized and contained masses of hyphae.

2. Lettuce seedlingsa) R.solani(S)

Infection cushions were formed only rarely, and those that were produced, were not well developed, and were only loosely attached to the surfaces. The hyphae, however, grew along the lines of junction of the epidermal cells, and over the faces of the cells. No infection cushions or orientation of hyphae were observed on roots, cotyledons, leaves and stems.

b) R.solani(L)

Numerous, large, well developed infection cushions were observed on hypocotyls. Some were also present on the petioles of the cotyledons. There was much orientation of hyphae along the lines of junction of the epidermal cells, and along the cell faces as well. (see Plates 4 and 5) Roots, stems and cotyledons were free from infection cushions, and orientation of hyphae was not observed on these organs.

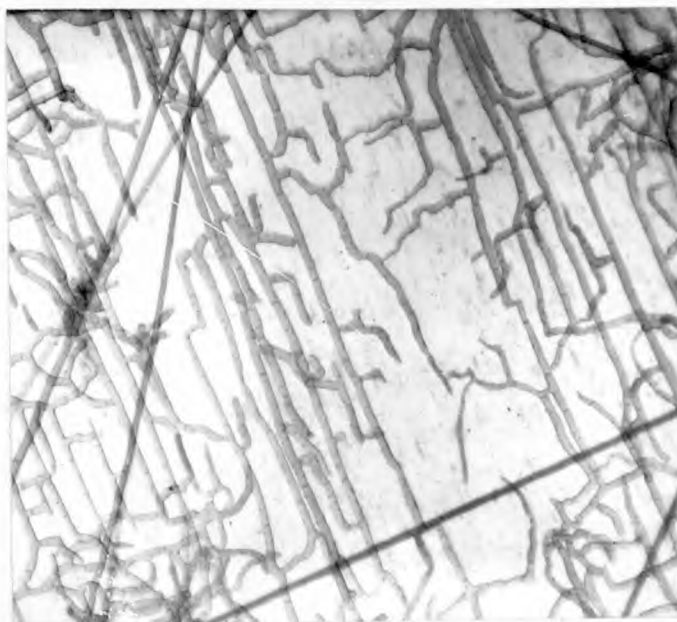


Plate 4 - Orientation of hyphae of *R. solani*(L) along the lines
of junction of the epidermal cells of a lettuce hypocotyl
(X 100)

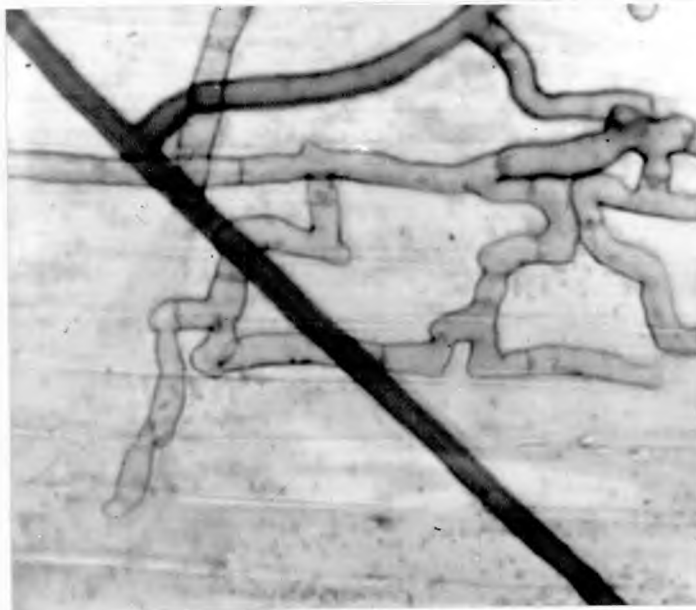


Plate 5 - Orientation of hyphae of *R. solani*(L) over the cell faces,
away from the lines of junction of the epidermal cells of
a lettuce hypocotyl (X 480)

c) C.praticola

Numerous massive infection cushions were produced on the hypocotyls, and there was profuse growth of the hyphae along the lines of junction of the epidermal cells, and elsewhere, along the surfaces of the cells. Infection cushion were not present on the roots or cotyledons, although the cotyledons were parasitized. Although the hyphae grew over the cotyledons there was no characteristic orientation.

3. Wheata) R.solani(S)

Infection cushions formed occasionally on the coleoptiles. They were sometimes firmly attached to the coleoptile but more often, they were only loosely attached. The hyphae often, though not always, grew along the lines of junction of the epidermal cells. Otherwise they grew more or less at random over the cell surface. No infection cushions or orientation of hyphae were observed on roots and leaves.

b) R.solani(L)

A few infection cushions were observed on the coleoptiles, but none on the roots or leaves. Hyphae grew along the lines of junction of the epidermal cells, but were loosely attached to the coleoptile surface.

c) C.praticola

Infection cushions were formed on the coleoptile surface. The hyphae grew along the lines of junction of the coleoptile epidermal cells. The infection cushions were comparable in size, to those formed on the hypocotyls of cabbage and lettuce. Roots and leaves were free from infection cushions, though the hyphae grew over the leaves. In many cases the hyphae were only loosely attached to the coleoptile.

The above observations are summarized in Table 9

Table 9

Attachment to, and organization of the fungi on host surfaces

Host	Organ	Fungus		
		<u>R.solani(S)</u>	<u>R.solani(L)</u>	<u>C.praticola</u>
Cabbage	Root	-	-	-
	Hypocotyl	ABC	ABC	ABC
	Stem	-	-	A
	Cotyledon	A	A	A
	Young leaf	A	-	A
Lettuce	Root	-	-	-
	Hypocotyl	ABC	ABC	ABC
	Stem	-	-	A
	Cotyledon	-	A	A
	Young leaf	A	A	A
Wheat	Root	-	-	-
	Coleoptile	ABC	ABC	ABC
	Young leaf	-	-	A

A - Fungi attached to surface, growing over it

B - Fungi growing along lines of junction of epidermal cells

C - Infection cushions formed

From Table 9 it is clear that the three fungi are not root parasites. None of the fungi formed infection cushions or grew along the lines of junction of the epidermal cells of cotyledons, young leaves or stems. There is no strict relation between the ability to parasitize a host, and the orientation of hyphae and the formation of infection cushions on it. There is, however, an indication that these fungi do form adhesive structures more frequently on hosts which they can parasitize than on hosts which they do not attack. In this respect these results do not agree with those of Kerr and Flentje (1957), who stated that strains not pathogenic to a host, fail to become attached to, or organize on the stem.

6. Nature of the stimuli leading to infection cushion formation

According to Kerr and Flentje (1957), infection cushions are formed in response to substances diffusing through the cuticle from the underlying epidermal cells, acting in conjunction with a contact stimulus. To investigate the applicability of this assertion to the host-parasite combinations used in the present work, a series of experiments were carried out with epidermal strips removed from cabbage and lettuce hypocotyls and wheat coleoptiles. They were placed cuticular surface facing upwards, on the surface of 1.5 % distilled water agar in Petri dishes. A 5 mm. diameter disk, taken from the edge of a young colony growing on potato dextrose agar, was placed in the centre of each Petri dish.

In the treatments outlined below, the epidermal strips were from 8 days old plants grown, as described on page 35, under sterile conditions, in nutrient solution (see page 33).
Treatments:-

1. The epidermal cells were well washed in sterile water before being placed on the agar surface.
2. The epidermal strips were washed with water, in 70 % ethanol, in diethyl ether, and finally in sterile distilled water.
3. The epidermal strips were washed in water, autoclaved in a Mc Cartney bottle in water for 10 minutes at 15 p.s.i. pressure and washed again in sterile distilled water.
4. Epidermal strips were placed directly on the agar surface without washing.

Five days after inoculation, the epidermal strips were examined for infection cushions and for the orientation of the hyphae. The results are summarized in Table 10.

On each type of epidermis, the three fungi grew along the lines of junction of the epidermal cells, except R. solani (S) on wheat coleoptile epidermis strips which had been treated with ethanol and ether. All three fungi formed infection cushions on each of the untreated epidermis strips, and on those washed in water. C. praticola also produced them on cabbage hypocotyls washed in ethanol and ether, but this was the only combination where infection cushions were formed on autoclaved or ethanol and ether treated epidermis strips.

Table 10

Behaviour of the fungi on epidermal strips of cabbage and lettuce hypocotyls and wheat coleoptiles, treated in various ways

Epidermis	Fungus	Treatments			
		Washed in water	Washed in ethanol & ether	Washed & autoclaved	Nil
Cabbage	<u>R.solani</u> (S)	AB	A	A	AB
	<u>R.solani</u> (L)	AB	A	A	AB
	<u>C.praticola</u>	AB	AB	A	AB
Lettuce	<u>R.solani</u> (S)	AB	A	A	AB
	<u>R.solani</u> (L)	AB	A	A	AB
	<u>C.praticola</u>	AB	A	A	AB
Wheat	<u>R.solani</u> (S)	AB	-	A	AB
	<u>R.solani</u> (L)	AB	A	A	AB
	<u>C.praticola</u>	AB	A	A	AB

A - The hyphae grew along the lines of junction of the epidermal cells

B - Infection cushions were formed

It is clear that washing in water had no effect on the orientation of the hyphae, or on the formation of infection cushions. The ethanol and ether, and the autoclaving treatments, however, apparently did not alter the surfaces of the cuticles sufficiently to prevent the characteristic orientation of the hyphae, although some significant change did occur, because infection cushions were not formed except in one case.

These results however, did not provide a complete answer, as they could also be explained on the hypothesis that substances present on the surfaces of the cells had not been washed off completely, the residues causing the hyphae to orientate.

This line of investigation was pursued further by attempting to grow the fungi on dried pieces of epidermis of cabbage and lettuce hypocotyls, and wheat coleoptiles. Here again, the object was to remove any water soluble substances, so that it could be assumed that the dried pieces of epidermis would be chemically inactive.

Epidermal strips from cabbage and lettuce hypocotyls and wheat coleoptiles were rinsed in distilled water, and placed cuticular surface upwards, on cover glasses measuring 5.6 by 2.1 cm. The two ends of each epidermal strip were attached to the cover glasses with adhesive cellophane. The strips were then allowed to dry for one hour, after which they were well washed with a fine jet of sterile distilled water from a pipette. The cover glasses were allowed to dry for a further 24 hours, after which they were placed with the epidermal strip uppermost, on the surface of 1.5 % distilled water agar in Petri dishes. The agar plates were prepared one week before the cover glasses were placed on them, and kept at 25°C., to remove any traces of moisture which might have condensed on the Petri dishes. The agar was inoculated with a 5 mm. diameter disk cut from the edge of a young colony of fungus, growing on potato dextrose agar.

After 5 days the fungus had grown over the agar, the cover glasses, and the dried epidermal strips. These were stained in 0.1 % aqueous cotton blue before examination.

In a second experiment the strips were not washed before fixing to the cover glasses and drying. Each of the three fungi formed infection cushions, and grew along the lines of junction between epidermal cells on the three types of epidermal strips, irrespective of whether they were washed or not.

7. Effect of host exudates on the formation of infection cushions on epidermal strips

Epidermal strips from 7 day old cabbage and lettuce hypocotyls and wheat coleoptiles were rinsed in distilled water, and placed cuticular surface upwards, on the surface of 7 ml. of 1.5 % distilled water agar in a Petri dish. The epidermal strips were equidistant (20 mm.) from a centrally placed inoculum of the fungus consisting of a 3 mm. diameter disk cut from the edge of a young colony growing on 1.5 % distilled water agar. On some strips was placed 0.005 ml. of a 0 - 7 day concentrated host exudate, and on the controls, the same quantity of water. After 5 days the number of infection cushions in a standard field of view under the low power of the microscope was counted for each epidermal strip. Each treatment was replicated five times and the results are summarized in Table 11.

Table 11

The effect of host exudates on the formation of infection cushions on strips of epidermis of hypocotyls of cabbage and lettuce, and coleoptiles of wheat

Fungus	Epidermis	Mean number of infection cushions in a field of view (x 100) under low power magnification			
		Exudates			
		Cabbage	Lettuce	Wheat	Water
<u>R.solani</u> (S)	Cabbage	4.4	4.0	5.4	4.0
	Lettuce	1.2	1.0	0.8	1.2
	Wheat	0.6	0.4	0.6	0.6
<u>R.solani</u> (L)	Cabbage	1.0	1.0	0.8	0.8
	Lettuce	4.8	4.4	6.2	4.6
	Wheat	0.8	0	0.6	0.4
<u>C.praticola</u>	Cabbage	7.6	7.8	6.0	8.4
	Lettuce	7.2	7.0	5.8	7.2
	Wheat	2.0	1.0	1.6	1.4

Analysis of variance:-

The following effects were not significant; exudate; interaction exudate x fungus; interaction exudate x epidermis x fungus; The following effects were highly significant at $P = 0.01$: epidermis; fungus; interaction epidermis x fungus. The significant differences for fungus and epidermis effects were:- 0.04 at $P = 0.05$ and 0.06 at $P = 0.01$ between any pair of means. The significant differences between means

for interaction epidermis x fungus effects were 0.8 at $P = 0.05$, and 1.1 at $P = 0.01$.

The effects of the exudates as compared with water was not significant at $P = 0.05$, but the formation of infection cushions differed very significantly with fungus, and with different types of epidermis. The mean values for the numbers of infection cushions formed on the different types of epidermis are given in Table 12.

Table 12

Formation of infection cushions on different types of epidermis

Fungus	Epidermis		
	Cabbage	Lettuce	Wheat
<u>R.solani(S)</u>	4.45	1.05	0.55
<u>R.solani(L)</u>	0.90	5.0	0.45
<u>C.praticola</u>	7.45	6.80	1.15

The significant differences were:- 0.4 at $P = 0.05$ and 0.6 at $P = 0.01$.

It is clear that each of the fungi formed infection cushions much more readily on pieces of epidermis from host plants to which they were normally pathogenic. Relatively few infection cushions were formed on the epidermis of resistant hosts. This is particularly well illustrated with wheat, which was less susceptible to attack by each of the fungi, and none of them formed many infection cushions on the epidermis taken from wheat coleoptiles.

8. Effect of destroying the configuration of the cuticular surface, on the formation of infection cushions on cabbage and lettuce hypocotyls and wheat coleoptiles

Pieces of cabbage and lettuce hypocotyls, and wheat coleoptiles 10 mm. long, were taken from 8 day old plants, and immersed momentarily in a concentrated solution (c. 25 % w/v) of collodion in a mixture of ethanol and diethyl ether in the proportion of 3 : 1. The solvent was allowed to evaporate immediately in air. In this way the cuticular surface was covered with a film of collodion, so that any effects on the fungi of the physical character of the surface of the cuticle would be eliminated. The tissues were then placed on clean cover glasses 10 mm. in diameter which were placed on Czapek-Dox agar in Petri dishes, inoculated with a 5 mm. diameter disk, taken from the edge of a colony of the fungus growing on potato dextrose agar. Czapek-Dox agar was used as it had a known standard composition.

In the controls the tissues were dipped in the solvent, which was allowed to dry immediately in air. After 5 days, the behaviour of the fungi on the tissues were examined. No infection cushions were formed and there was no growth of the hyphae of any of the three isolates, along the lines of junction of the epidermal cells on the tissues covered with collodion. On the controls there was the normal formation of infection cushions with the characteristic orientation of the hyphae. These results indicate the importance of the surface on the organization of the hyphae on it.

9. Behaviour of the fungi on replicas of the plant surfaces

Carbon-formvar replicas of epidermal strips of cabbage and lettuce hypocotyls and wheat coleoptiles were prepared by the method described on page 46, and the fungi were grown on these replicas as described on pages 46 - 47. The following observations were made.

On almost every replica there was some orientation of the hyphae along the lines of junction of the epidermal cells, and elsewhere, on the cell surfaces, in a longitudinal direction. The degree of orientation, however, was variable, even between replicates of the same treatments. For instance, the hyphae appeared to be more orientated on some lettuce replicas than on others, but all the replicas showed it to some extent.

Infection cushions, identical in appearance with those on the hosts, were never formed, but with C.praticola on cabbage and lettuce replicas, clumping of the hyphae and the formation of short lateral branches were observed (see Plate 6). R.solani(L) produced aggregations of hyphae on lettuce and on cabbage replicas, and R.solani(S) produced similar clumps on lettuce replicas. All these structures were somewhat loosely organized tufts of hyphae and cannot be regarded as true infection cushions. Plate 7 shows the hyphae of R.solani(S) orientated on the replica of a cabbage hypocotyl surface. These results are summarized in Table 13.



Plate 6 - Formation of tufts of hyphae similar to infection cushions
by C.praticola on the replica of a cabbage hypocotyl surface
(X 240)

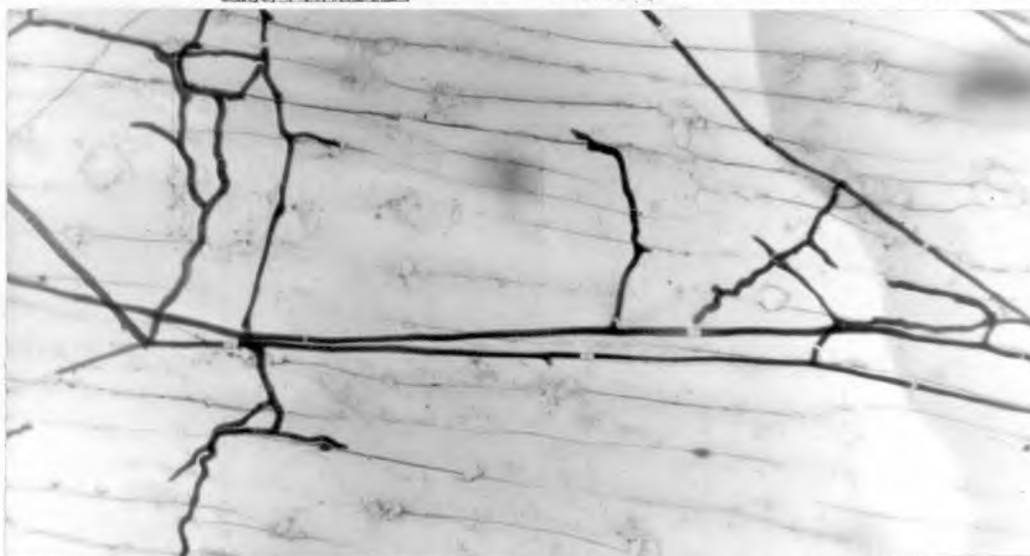


Plate 7 - Orientation of the hyphae of R.solani(S) along the lines
of junction of epidermal cells on a replica of a cabbage
hypocotyl surface (X 225)

Table 13

Behaviour of the fungi on replicas of surfaces of cabbage and lettuce hypocotyls and wheat coleoptiles

Fungus	Replicas		
	Cabbage hypocotyl	Lettuce hypocotyl	Wheat coleoptile
<u>R.solani</u> (S)	A	AB	A
<u>R.solani</u> (L)	AB	AB	AB
<u>C.praticola</u>	AB	AB	AB

A - Hyphae orientated longitudinally

B - Tufts of hyphae formed

It would be difficult to attach importance to the tufts of hyphae, which were produced haphazardly over the surface of the replicas. It is however, of significance that there was some orientation of the hyphae on each of the replicas.

10. Behaviour of the fungi on inverse replicas of surfaces of hypocotyls of cabbage and lettuce and coleoptiles of wheat

Inverse replicas of the surfaces were prepared as follows. Epidermal strips from cabbage and lettuce hypocotyls and wheat coleoptiles of 7 day old plants were placed on glass slides with the cuticular surface uppermost. They were dried in air, rinsed in distilled water and dried again, and then shadowed with a film of carbon. The slides were then flooded with a 5 % formvar solution on chloroform, and the solvent allowed to evaporate in a draught free

atmosphere. The carbon-formvar replica was stripped from the epidermis, with gummed paper. It was floated on water to remove the gummed paper and the replicas were picked up on glass slides and allowed to dry. The fungi were grown on these replicas as described on page 46.

The replicas were examined 5 days after inoculation. No infection cushions were formed on any of the replicas, but on all of them, the hyphae were orientated in a longitudinal direction, though the amount of orientation was less than on true replicas. There was more orientation of each of the fungi on the lettuce replicas than on those of cabbage and wheat. It is clear that the surface of the inverse replicas, was able to direct the growth of hyphae.

11. Examination of the cuticular surfaces under the electron microscope

The material for examination under the electron microscope was prepared as described on page 44. The electron micrographs revealed the following information. The surfaces of the two types of hypocotyl, and the coleoptile carried longitudinal ridges, which varied somewhat in width as shown below.

Cabbage - 1.2 - 3.5 μ

Lettuce - 15 - 50 μ

Wheat - 0.5 - 2 μ

(Average diameter of the mature hyphae - 5 μ)

None of the surfaces were completely smooth. It is possible, therefore, that these ridges played some part in directing the growth of the hyphae.

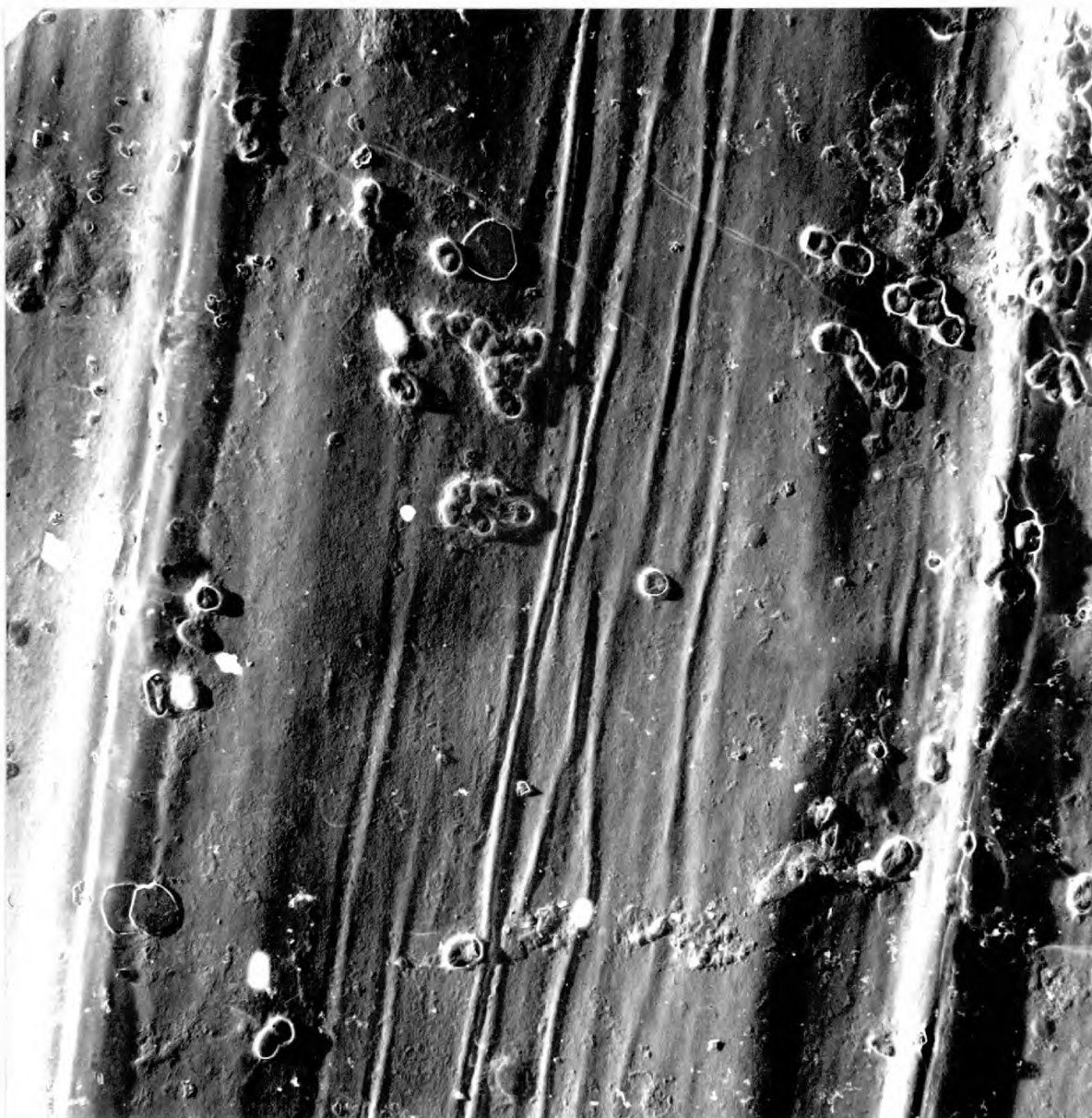


Plate 8 - Electron micrograph of the surface of the cuticle of a
cabbage hypocotyl showing longitudinal ridges
(X 4,200)

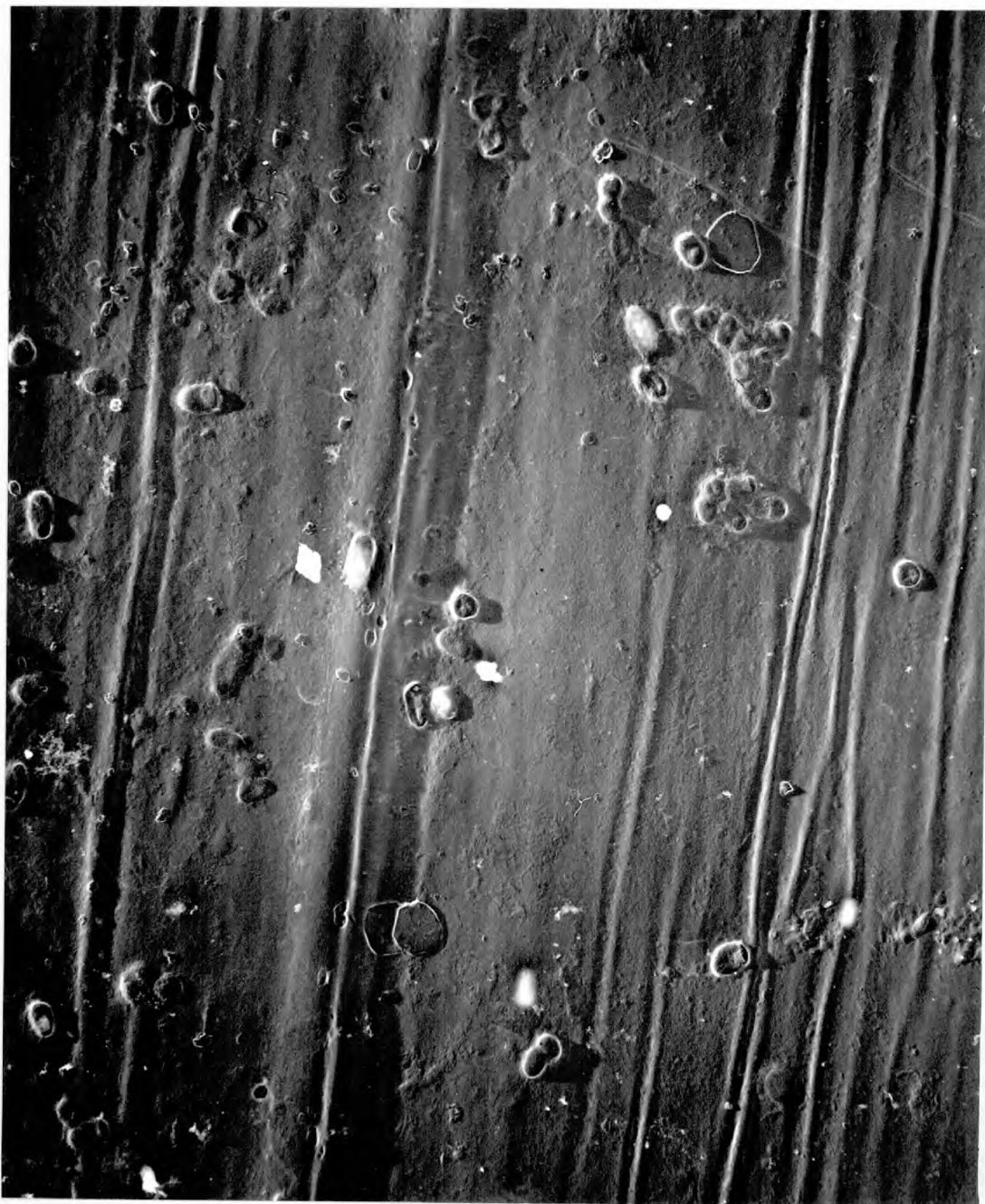


Plate 9 - Electron micrograph of the surface of a cabbage hypocotyl
showing the lines of junction of epidermal cells
(X 4,200)

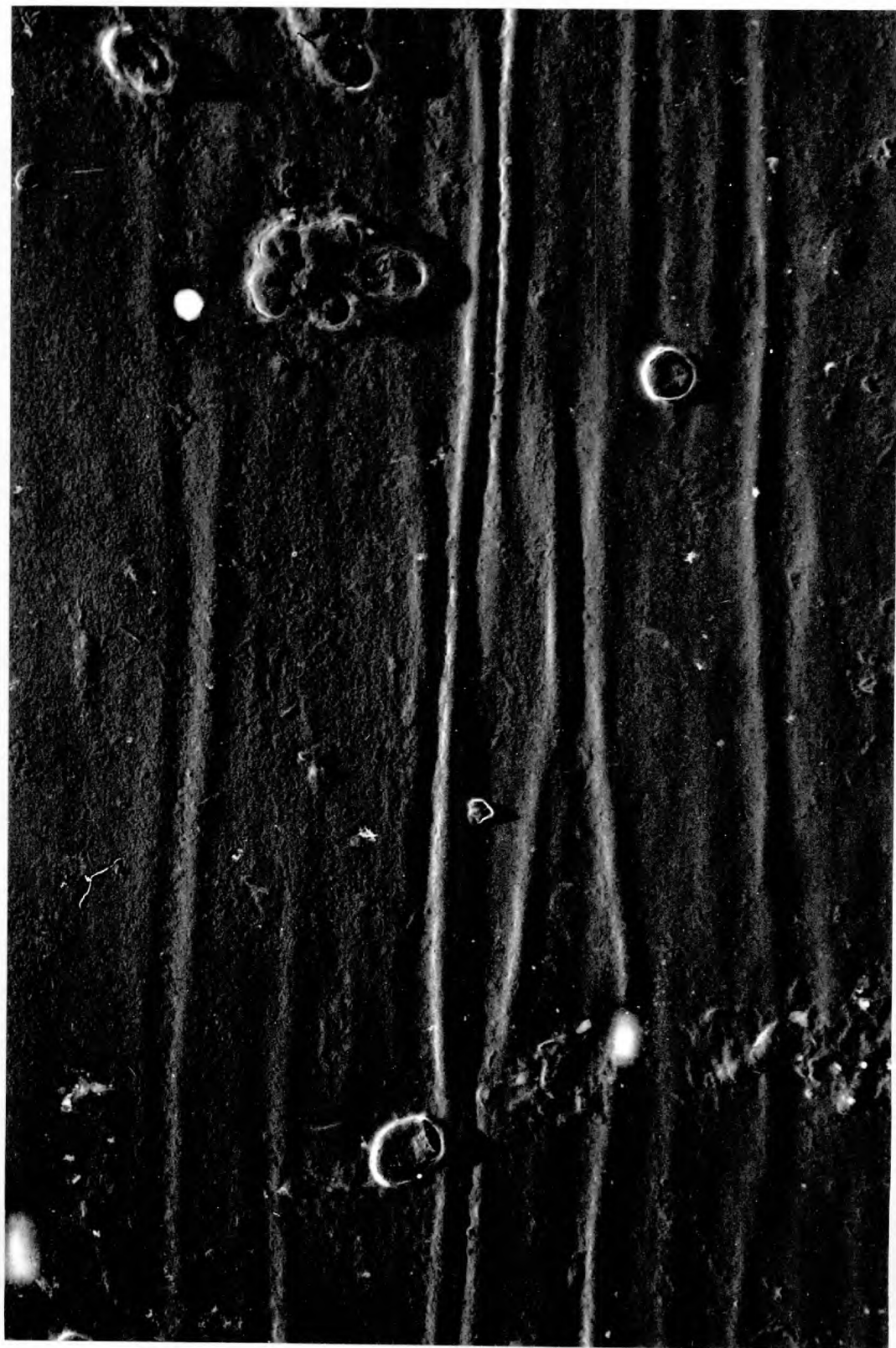


Plate 10 - Electron micrograph of the surface of the cuticle of a
cabbage hypocotyl showing longitudinal ridges
(X 9,360)



Plate 11 - Electron micrograph of the surface of the cuticle of a
lettuce hypocotyl showing longitudinal ridges
(X 4,200)



Plate 12 - Electron micrograph of the cuticular surface of a
lettuce hypocotyl showing longitudinal ridges
(X 10,200)



Plate 13 - Electron micrograph of the cuticle surface of a
lettuce hypocotyl epidermal cell
(X 25,500)

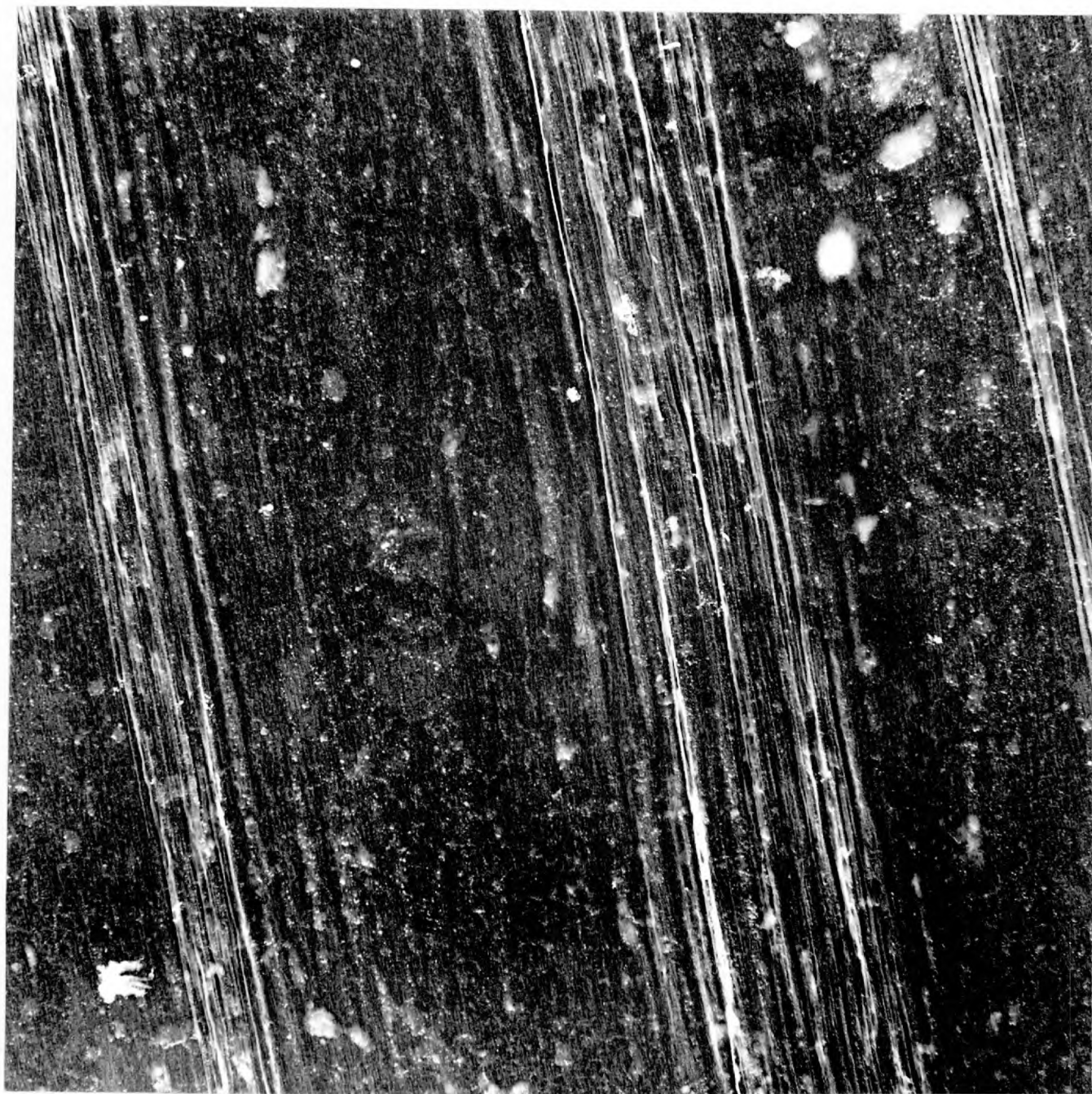


Plate 14 - Electron micrograph of the cuticle surface of a
wheat coleoptile showing longitudinal ridges
(X 4,200)



Plate 15 - Electron micrograph of the cuticle surface of a
wheat coleoptile showing longitudinal ridges
(X 4,200)



Plate 16 - Electron micrograph of the ridges on the surface
of a wheat coleoptile
(X 8,400)

The most prominent ridges appear to be on cabbage (see Plates 8,9 & 10); whereas less well defined ones are present on lettuce (Plates 11,12 & 13), and wheat (Plates 14,15 & 16). Apart from these ridges the cuticles appeared to have no characteristic configuration, although some peculiarities were observed on some preparations. These could not be correlated with the behaviour of the fungi on the surfaces of the hosts. Their haphazard distribution may indicate that they were impurities or artefacts. It is conceivable that the ridges could account for the orientation of the hyphae on the plant surfaces. Their presence also offers an explanation as to why the hyphae grow longitudinally along cell faces, as well as along the lines of junction of the cells.

12. Behaviour of the fungi on non-living surfaces.

The behaviour of the fungi on three non-living surfaces cellophane, glass and polystyrene, was next investigated. An 8 mm. diameter disk, cut from the edge of a young colony of fungus growing on potato dextrose agar, was placed on each of the surfaces after they had been cleaned of contaminating substances, and made sterile. The glass surface was that of an empty autoclaved Petri dish. The polystyrene surface was also a sterile Petri dish. The cellophane (grade PT 300) was boiled and leached in distilled water, and sterilized before use. The following observations were made.

On glass

R.solani(S) did not produce any characteristic structures, although the hyphae sometimes grew parallel to each other after they had come in contact. (Plate 17). R.solani(L) produced club



Plate 17 -- Hyphae of R.solani(S) growing parallel to each other after they had come in contact (X 120)

club-shaped, lateral branches which were comparatively short. (Plates 18 & 19) At junctions between adjacent hyphal cells there appeared to be constrictions, which gave the hyphae undulating surfaces. Later, the club-shaped side branches aggregated to form a clump of short hyphal cells. These did not develop into sclerotia as often happens in culture on agar. C.praticola produced peculiar stellate adhesive structures which consisted of central tightly packed masses of hyphae which stained deeply with cotton blue, and which were surrounded by radiating hyphae which stained lightly. (Plates 20 & 21) It seemed that only the radiating hyphae adhered closely to the glass. These composite structures were quite distinct from sclerotia, shown for comparison in Plate 22, and the term "contact cushions" is proposed for them.



Plate 18. - Growth of R.solani(L) on glass showing the club-shaped lateral branches of the hyphae (X 120)

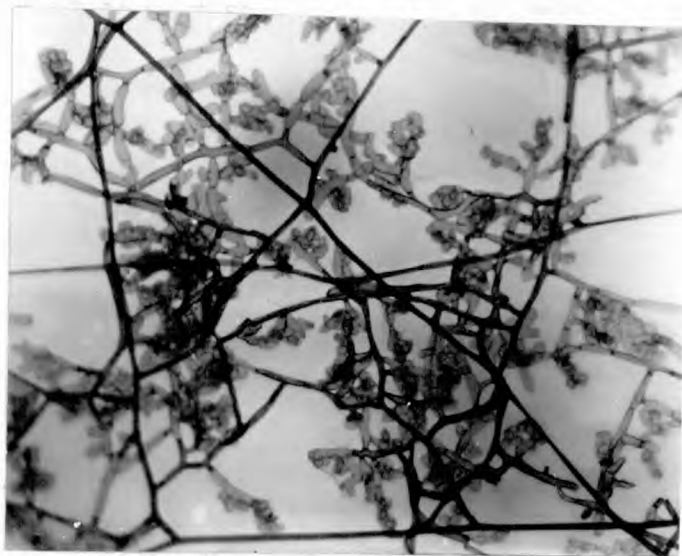


Plate 19 - Growth of R.solani(L) on glass showing "contact cushions" (X 160)



Plate 20 - Growth of C. praticola on glass showing contact cushions
(X 160)

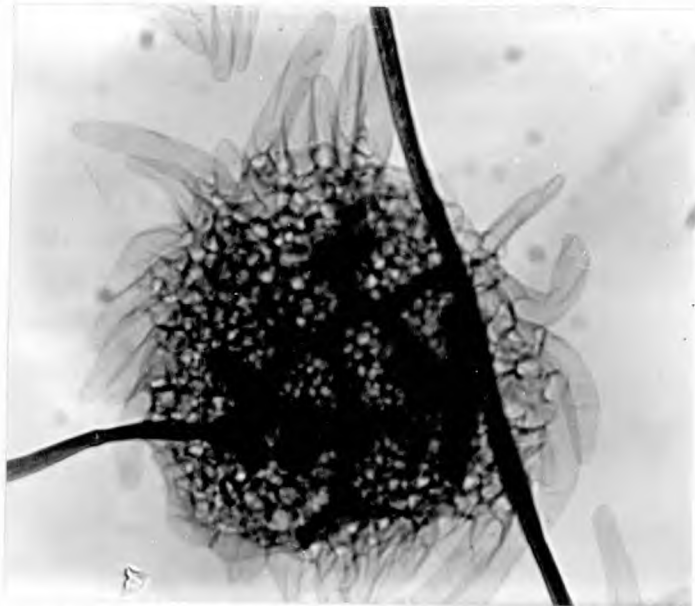


Plate 21 - A contact cushion formed by C. praticola on glass,
showing the lightly stained outer radiating hyphae and
the densely stained central mass of hyphae (X 480)

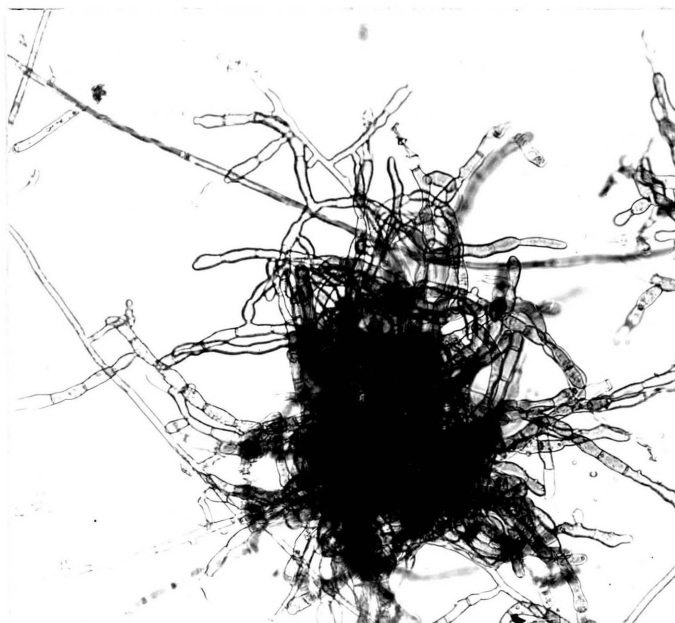


Plate 22 - Structure of a sclerotium of C.praticola (X 160)

On polystyrene

R.solani(S) developed no characteristic structures. R.solani(L) produced laterals from the main hyphae. These laterals branched repeatedly into very short branches to form an adhesive structure or contact cushion (Plate 23). The hyphae stained less lightly with increasing distance from the main hyphae. C.praticola produced contact cushions which resembled infection cushions very closely (Plate 24). The main hyphae produced lateral branches each of which branched several times more to produce the contact cushion. The distal cells were lightly stained with cotton blue, whereas the main hyphae and first laterals were stained deeply.

On cellophane

R.solani(S) produced massive adhesive structures similar to those observed by Shephard (1957), for this fungus.



Plate 23 - Contact cushions formed by R. solani(L) on polystyrene
(X 160)

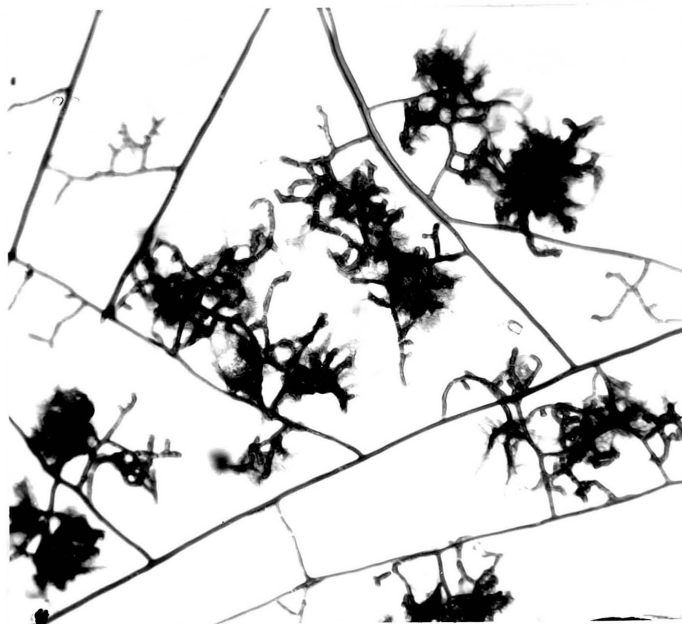


Plate 24 - Contact cushions formed by C. praticola on polystyrene
(X 120)

These contact cushions arise from a branch of a single hypha and progressively enlarge to form a large thallus-like structure, composed of fungal cells with clear cross walls, closely compacted, and regularly arranged. (Plates 25, 26 & 27) Neither R.solani(L) nor C.praticola produced any characteristic structures on cellophane.



Plate 25 - Contact cushion formed by R.solani(S) on cellophane
(X 120)



Plate 26 - Contact cushion formed by R. solani(S) on cellophane

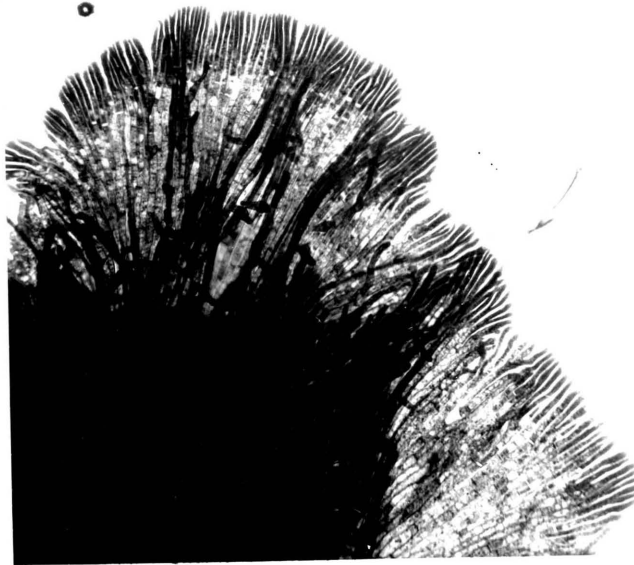


Plate 27 - Contact cushion formed by R. solani(S) on cellophane

13. Penetration and post-penetration stages of parasitism

The process of parasitism was further investigated by microscopic examination of sections of infected host plants, grown aseptically, either in beakers as described on page 35, or in Petri dishes placed in plastic boxes, as described on page 33. This reduced the possibility of contaminants affecting the experiments. The seedlings were inoculated in one of two ways. When the plants were grown in the beakers, a liquid inoculum as described on page 38, was used. When the seedlings were grown in plastic boxes, a ring of agar, or a disk, cut from the edge of a young colony growing on potato dextrose agar was used.

The infected material of cabbage and lettuce seedlings was fixed in the formalin/acetic acid/ethanol mixture and the infected wheat seedlings were fixed in the Craib fixative as described on page 40. The material was dehydrated in the tertiary butyl alcohol series, embedded in paraffin wax, sectioned and stained as described on pages 40 - 44. Transverse, radial and tangential longitudinal sections were examined and the observations are summarized below.

1. Cabbage

a) R.solani(S)

Hypocotyls were penetrated, but the penetration of roots and cotyledons was never observed. The fungus always penetrated the hypocotyls from infection cushions, directly through the cuticle and epidermis. (Plate 28) From some infection

cushions, only a few hyphae penetrated the epidermis. (Plate 29) These hyphae then branched several times inside the cortex, grow intercellularly at first, but penetrated into cells later. The hyphae then proceeded to grow extensively through the cortical tissues. From other infection cushions, masses of hyphae broke through the epidermal surfaces, and grew in between cells, as well as through them (Plate 30).

In most seedlings, whether the initial infection was by a few, or masses of hyphae, the fungus, after entering the cortex at or near soil level, grew rapidly towards the stelo. Invasion of the upper portions of the hypocotyls was slow, and often took place only after the seedling had been damped-off and had fallen over.

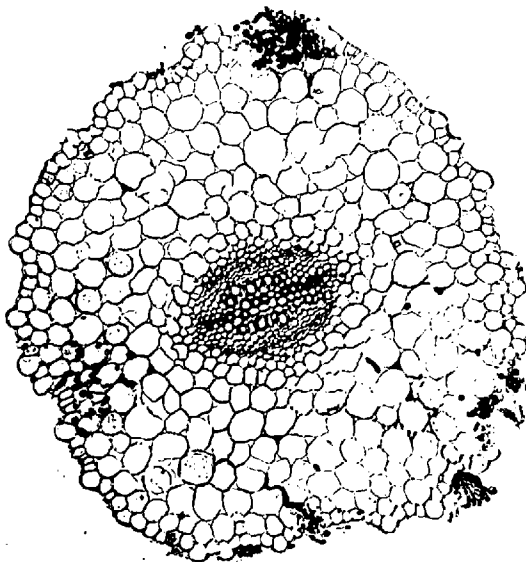


Plate 28. T.S. cabbage hypocotyl infected by R.solani(S) (X 90)

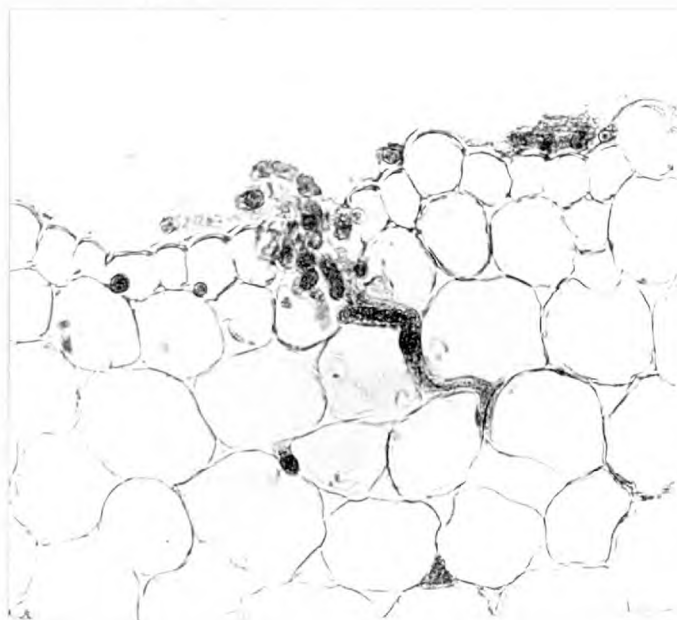


Plate 29 - T.S. cabbage hypocotyl showing the intercellular invasion of the cortex by hyphae of R.solani(S) (X 360)

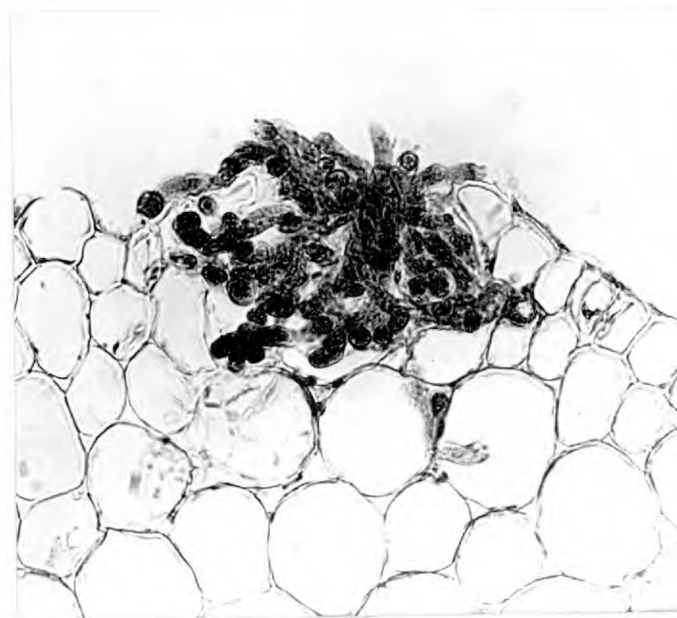


Plate 30 - T.S. cabbage hypocotyl showing the penetration of masses of hyphae into the cortex from an infection cushion of R.solani(S) (X 360)

The hyphae proceeded to grow onto the cotyledons and young leaves of the damped-off plants. In most cases, penetration was followed by damping-off one or two days later.

b) R.solani(L)

Hypocotyls were penetrated but roots and cotyledons were never penetrated. Many seedling which had infection cushions on the surface of their hypocotyls were not penetrated. Some seedlings were penetrated by hyphae, from infection cushions. Wherever penetration took place, it was directly from an infection cushion through the cuticle and epidermis of the hypocotyl.

Some seedlings were penetrated by single, or a few hyphae, which grew through the cortex intercellularly, but only very slowly. They sometimes branched, and the branches spread out from the infection cushion longitudinally, instead of towards the stole as did the hyphae of R.solani(S). Intracellular invasion was sometimes observed. In other seedlings, masses of hyphae broke through the epidermis from a single infection cushion. These hyphae either did not grow into the cortex, or did so only very slowly. (Plate 31) More often than not, the hyphae merely formed a static mass on the surface, and did not develop further.

In the later stages, sclerotia were formed on the hypocotyl surface and in the cortex. The majority of the infected seedlings were not damped-off, although there were hyphae inside their hypocotyls.

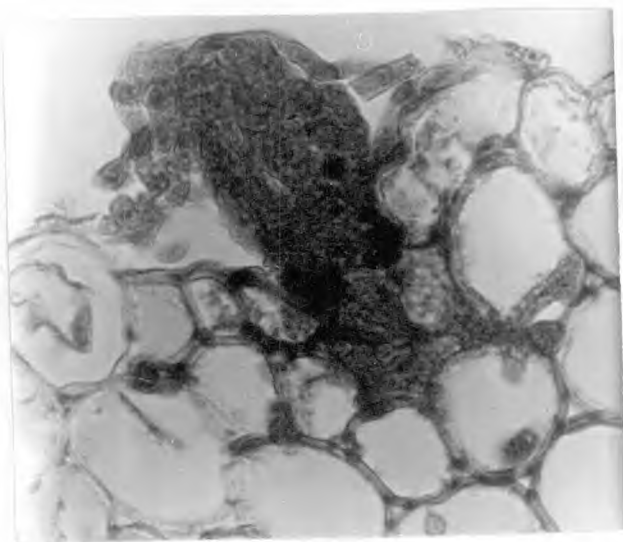


Plate 31 - T.S. cabbage hypocotyl showing invasion of the cortex
by hyphae of R.solani(L) (X 360)

c) C.praticola

Hypocotyls were penetrated by hyphae from infection cushions. Roots and cotyledons were never penetrated. No penetration was observed in the absence of infection cushions. Penetration of the hypocotyl cuticles and epidermis took place by individual, a few, or masses of hyphae. After penetration, the hyphae invaded the cortex intercellularly, very rapidly reaching the stele, often within 24 hours. (Plates 32 & 33)

When masses of hyphae invaded the hypocotyl from a single infection cushion, the colonization of the whole tissue rapidly followed, the cells and tissues being completely disorganized. Cotyledons and young leaves were also parasitized, but mostly after the plants had been damped-off. In general, invasion and damping-off

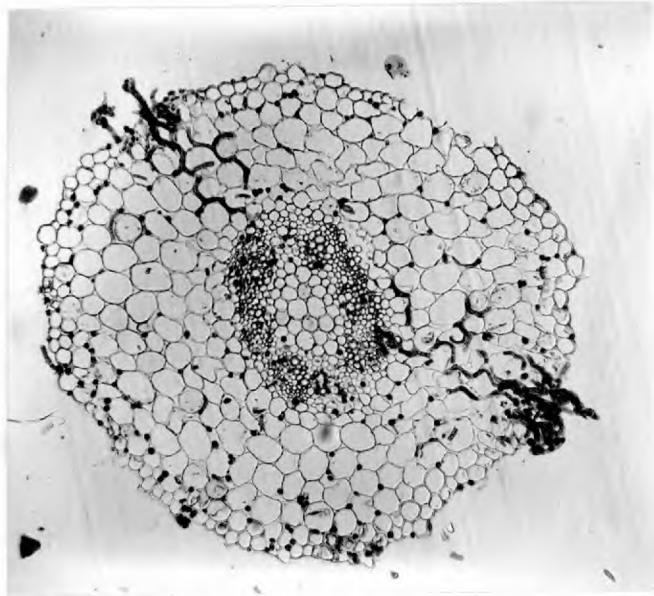


Plate 32 - T.S. cabbage hypocotyl showing invasion of the cortex
by the hyphae of C.praticola (X 90)

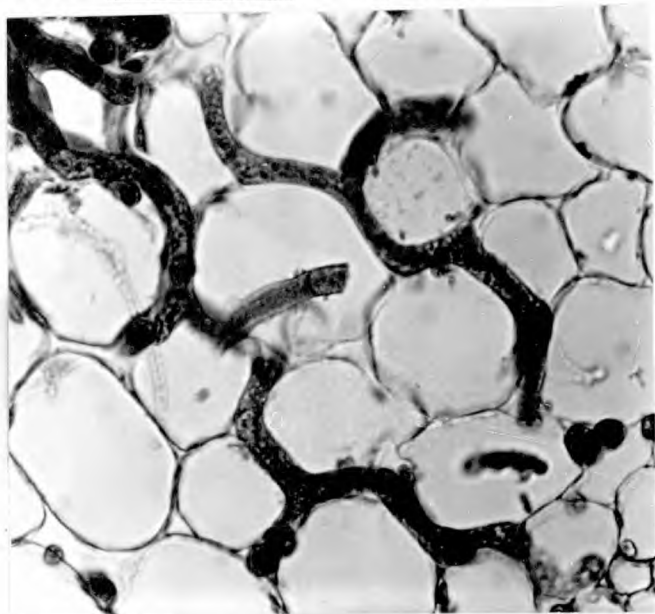


Plate 33 - T.S. cabbage hypocotyl showing the intercellular growth
of the hyphae of C.praticola (X 480)

of the seedlings was very much more rapid than with R.solani(S). No sclerotia were formed until all the host tissue had been completely filled with fungus (Plate 34).



Plate 34 - T.S. cabbage hypocotyl, which has been completely disorganized by the invading hyphae of C.praticola
There are no sclerotia (X 120)

2. Lettuce

a) R.solani(S)

Hypocotyls were penetrated but roots and cotyledons were never penetrated. Penetration of the hypocotyls was rather infrequent, and it always took place from an infection cushion. Penetration from infection cushions was never by masses of hyphae as was observed when this fungus parasitized cabbage. Instead, only a few hyphae, sometimes only one, penetrated the hypocotyl. (Plate 35)

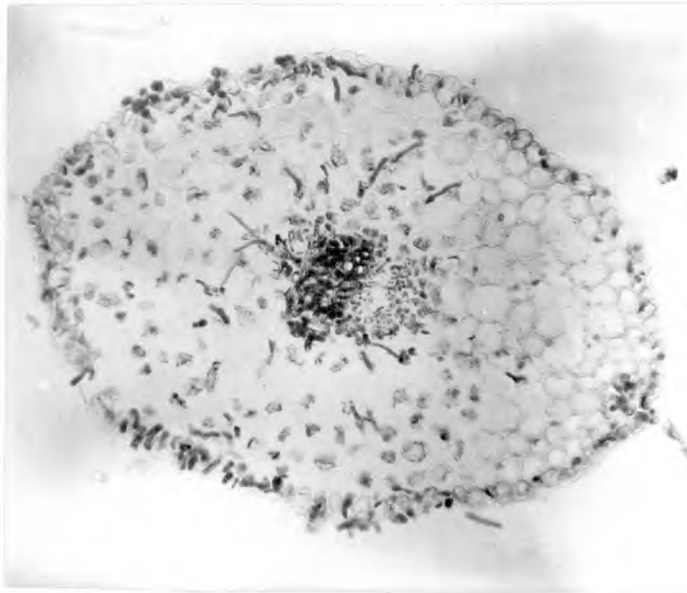


Plate 35 - T.S. lettuce hypocotyl showing sparsely distributed hyphae of R.solani(S) (X 240)

Very often penetration did not take place from some of the infection cushions. Occasionally, one or two layers of cortical cells were parasitized, just beneath the infection cushion, and the hyphae did not grow any further. In some infected seedlings, two distinct types of hyphae were observed, (Plate 36) one thick, the other slender. When infected plants containing the two types of hyphae were dissected and pieces of the two types planted on agar, only cultures containing the thick-celled mycelium were produced. It is very unlikely that the slender hyphae present in the tissues were contaminants, because the plants were grown under sterile conditions, and because the isolations produced only R.solani(S). It must therefore be assumed that the fungus can produce two distinct types of hyphae in parasitized tissues.

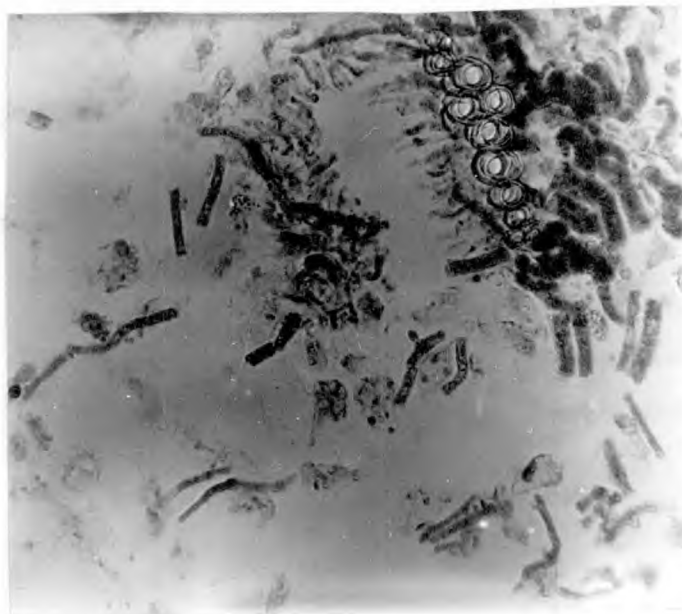


Plate 36 - T.S. lettuce hypocotyl showing two distinct types of hyphae of R.solani(S) (X 360)

Only comparatively few hyphae of either type were found in the hypocotyls, and these invaded the tissues only very slowly. Cotyledons and leaves were almost free from hyphae, and sclerotia were not observed in the tissues.

b) R.solani(L)

Hypocotyls were penetrated, but roots and cotyledons were never penetrated. Hypocotyl penetration was frequent and always took place from infection cushions. From a few of the infection cushions, single, or two to three hyphae penetrated, and branched inside the cortex, but from the large majority of the infection cushions, masses of hyphae penetrated the hypocotyl and invaded the cortex both inter- and intracellularly, rapidly spreading through the cortex and stele, causing damping-off. (Plate 37)



Plate 37 - Radial L.S. of a lettuce hypocotyl through an infection cushion of R.solani(L) showing invading hyphae and the macerated cortex (X 360)

Later, leaves and cotyledons were parasitized and after the seedling had been killed, sclerotia were formed on the surfaces of the destroyed tissues.

c) C.praticola

Hypocotyls were penetrated, but roots and cotyledons were never penetrated. Infection cushions were produced on the surface of the hypocotyls and penetration always took place from them. (Plate 38) The growth of the hyphae in the cortex was both inter- and intracellular, by large numbers of hyphae which rapidly disorganized the seedling, causing damping-off. Complete invasion of the cortex (Plate 39) was even more rapid, and damping-off occurred quicker than with R.solani(L). Sclerotia were not observed.



Plate 38 - Radial L.S. of a lettuce hypocotyl through an infection cushion of C.praticola showing the hyphae invading the cortex (X 360)



Plate 39 - T.S. lettuce hypocotyl completely invaded by hyphae of C.praticola (X 480)

3. Wheat

a) R.solani(S)

Infection cushions were produced on the surface of the coleoptiles, and penetration took place from a few, but not all of them. Roots were never penetrated. The hyphae which did penetrate the coleoptiles grew for a short distance into the coleoptile (Plate 40), in most cases. Hyphae were slow in invading the cortex, and hardly ever reached the inner epidermis of the coleoptile. In all the sections that were examined, the hyphae were rather slender, and had the appearance of those grown under poor nutrient conditions. Sclerotia were found on the surface, and sometimes, embedded inside the cortex of the coleoptile.

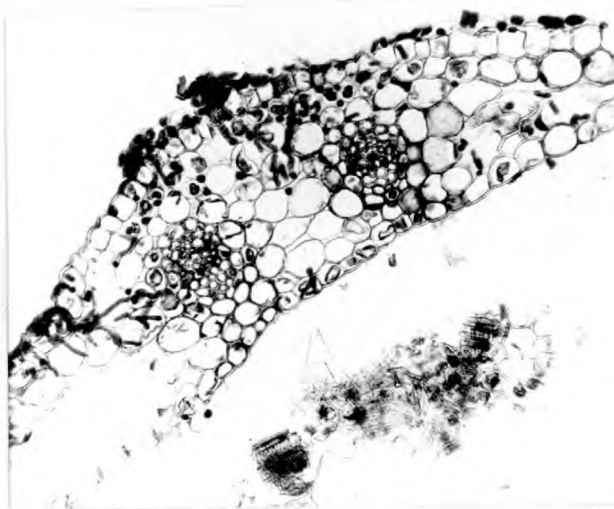


Plate 40 - T.S. wheat shoot showing the hyphae of R.solani(S)
in the coleoptile (X 40)

b) R.solani(L)

Coleoptiles were penetrated, but roots were never penetrated. However, penetration of the coleoptiles was infrequent, and when it occurred, it always took place from infection cushions. From the majority of infection cushions however, no penetration occurred. In most instances of penetration, only a very few hyphae penetrated the coleoptile. The growth of the hyphae inside the coleoptile was largely intercellular, and only a relatively few cells appeared to have been penetrated.

When it did occur, invasion was a little more rapid than it was with R.solani(S), and a few of the hyphae proceeded as far as the inner epidermis of the coleoptile.(Plate 41) Sclerotia were formed in fair abundance on the coleoptile surface.

c) C.praticola

Coleoptiles were penetrated, but roots were never penetrated. Groups of hyphae were sometimes observed to penetrate the coleoptiles from infection cushions. The hyphae penetrated the epidermis immediately below the infection cushion and branched out repeatedly into several laterals, just under the point of penetration. (Plates 42 & 43) Hyphae were both inter- and intracellular. The rate of invasion of the host tissues, was considerably more rapid than for both strains of R.solani. The inner epidermis of the coleoptile was generally reached and disorganized, (Plates 44 & 45) and hyphae were also found in the space between the first leaf and the coleoptile. The first leaf was sometimes breached.

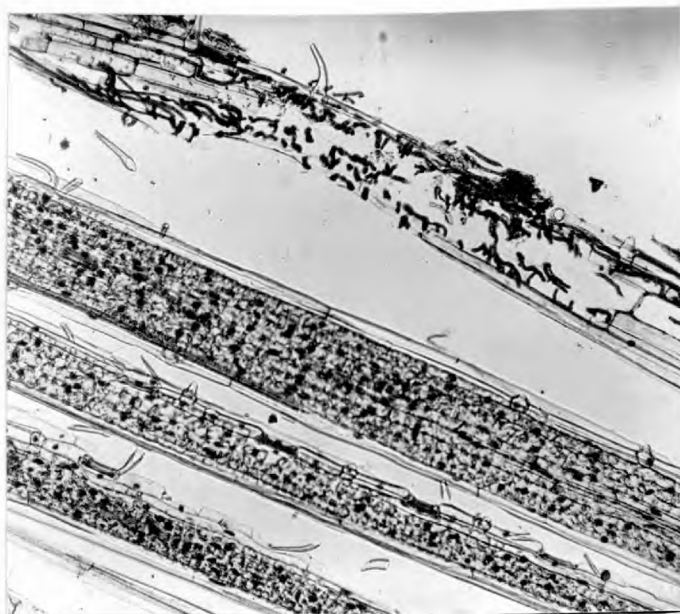


Plate 41 - Tangential L.S. wheat shoot showing the coleoptile invaded by hyphae of R.sclani(L) but the leaf sheaths are not penetrated (X 90)

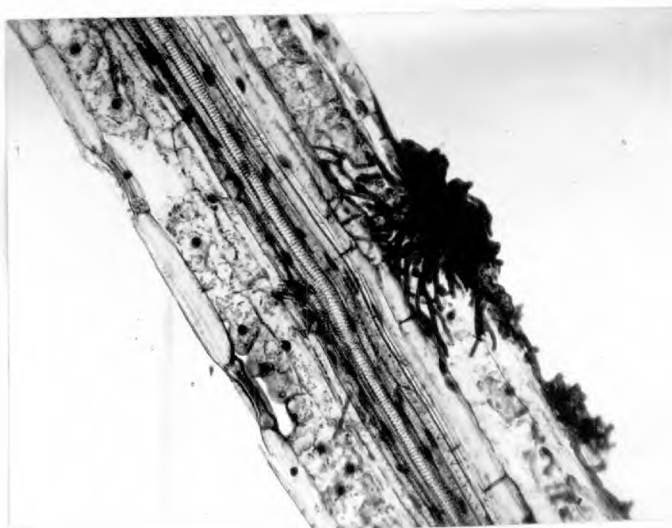


Plate 42 - Radial L.S. wheat shoot, showing longitudinal spread of the hyphae of C.praticola from an infection cushion (X 120)

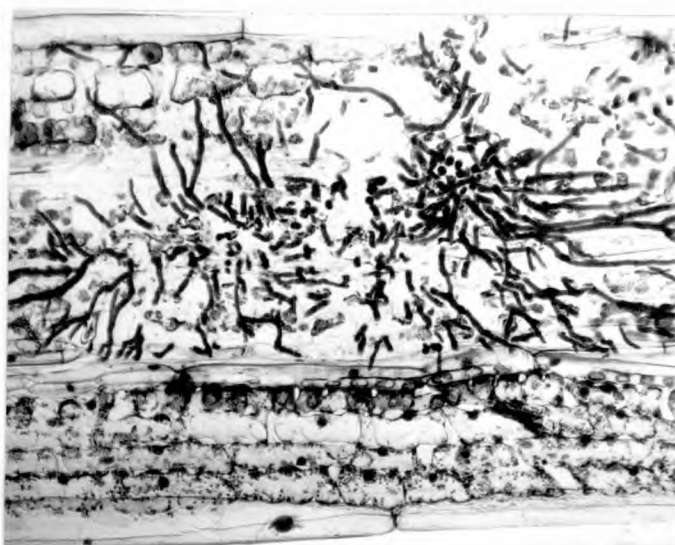


Plate 43 - Tangential L.S. wheat coleoptile, taken just under an infection cushion of C.praticola showing the spread of the invading hyphae (X 120)

sometimes breached. Although prominent lesions appeared on the coleoptiles (Plate 46), the seedlings were never killed because the fungus did not spread rapidly enough to invade the leaves as they were being developed. Occasionally, hyphae were found in the second leaf sheath, but never in the third, or in subsequent ones. Sclerotia were found on the lesions, but they were not abundant.

The observations on the penetration and post-penetration stages of parasitism are summarized in Table 14.

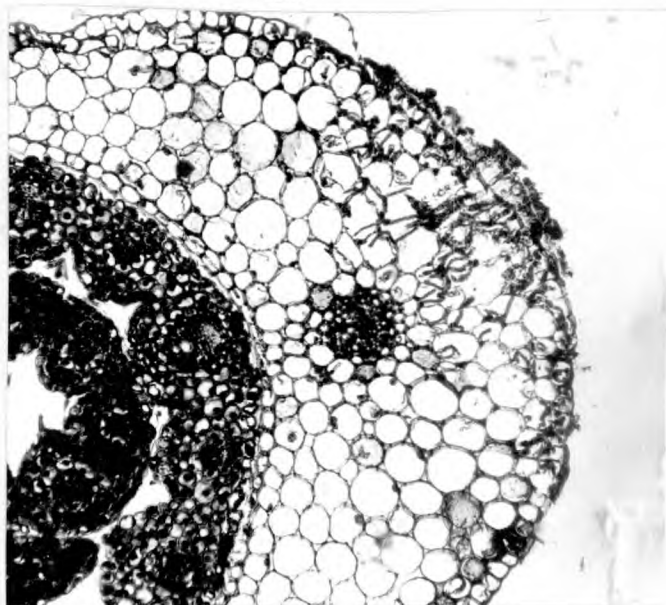


Plate 44 - sec below

(X 60)

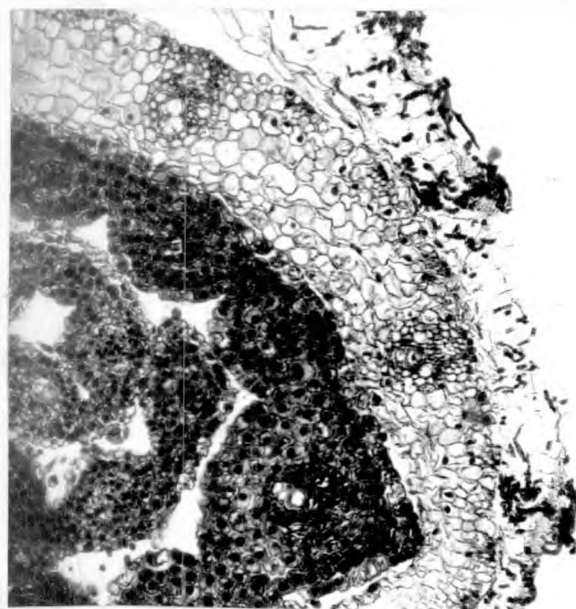


Plate 45 - sec below

(X 60)

Plates 44 and 45 show transverse sections of wheat shoots invaded by C.praticola. Plate 44 shows a young wheat shoot with only the coleoptile infected. Plate 45 shows an older wheat shoot with a disorganized coleoptile, and a new leaf free of fungus.



Plate 46 - Wheat seedlings showing lesions on the coleoptiles
caused by C.praticola (X 1)

Table 14

Observations on the invasion of the three seedlings

Fungus	Organs penetrated	Relative extent of penetration by hyphae	Speed of invasion of tissues	Main direction of advance of hyphae	Extent of invasion 4 days after infection	Sclerotia
C A B B A G E						
<u>R.solani</u> (S)	hypocotyl	abundant	+++	lateral	entire hypocotyl	absent
<u>R.solani</u> (L)	hypocotyl	erratic	+	longitudinal	cortex	abundant
<u>C.praticola</u>	hypocotyl	abundant	++++	lateral & longitudinal	entire hypocotyl	rare
L E T T U C E						
<u>R.solani</u> (S)	hypocotyl	rare	+	lateral	cortex	present
<u>R.solani</u> (L)	hypocotyl	abundant	++++	lateral & longitudinal	entire hypocotyl	present
<u>C.praticola</u>	hypocotyl	abundant	++++	lateral & longitudinal	entire hypocotyl	rare
W H E A T						
<u>R.solani</u> (S)	coleoptile	rare	+	lateral	a few cell layers of coleoptile	present
<u>R.solani</u> (L)	coleoptile	rare	+	lateral	inner epidermis of coleoptile	abundant
<u>C.praticola</u>	coleoptile & leaf sheaths	not common	++	lateral & longitudinal	inner layer of 1st leaf sheath	rare

- + - Extremely slow
 ++ - The majority of hyphae penetrate slowly but a few advance rapidly
 +++ - Rapid
 ++++ - abundant growth, at a rapid rate

From the results given above, and summarized in Table 14, it is clear that only the hypocotyls of cabbage and lettuce seedlings, and the coleoptiles and leaf sheaths of wheat were penetrated by these fungi. Penetration took place in each of the host-parasite combinations, although the success of penetration as estimated by the quantity of fungus entering the host by breaching the cuticle and epidermis, was greater when susceptible hosts were parasitized.

The speed of invasion of the cortex, and the time interval between penetration of the outer surface and the colonization of the stele by the host, seemed to be an important factor in determining whether the seedling would damp-off or not. Further, the tendency of the hyphae to spread laterally rather than longitudinally within the seedling would ensure more rapid damping-off. When C.praticola parasitized cabbage, masses of hyphae were observed at soil level, but in the upper parts of the hypocotyl, little or no hyphae were present (Plate 47).

The pathogenic fungi have a more efficient mechanism for breaching the outer host barrier, but this barrier does not exclude the less pathogenic fungi. However, it is significant that although each of the fungi penetrates each of the hosts, damping-off occurred only in some of the host-parasite combinations. This suggests that it is the rate of invasion of the host tissue that determines whether the seedling will succumb to the attack or not.

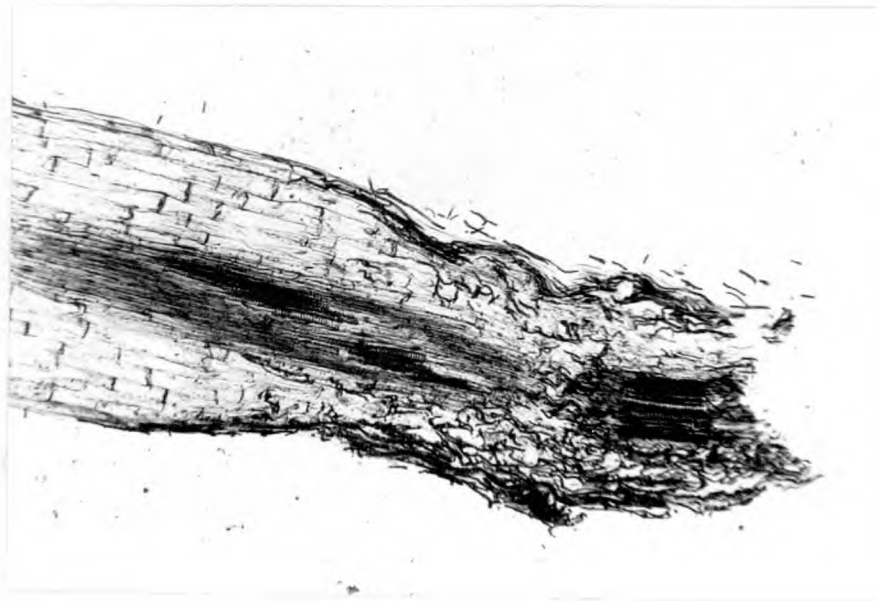


Plate 47 - Radial L.S. cabbage hypocotyl from a damped-off plant showing the hyphae of C.praticola near soil level but not on the upper parts of the hypocotyl (X 90)

From Table 14 it is clear that these rates of invasion are very different, but that the pathogenic strains invade faster than the non-pathogenic ones. Further investigations were carried out in the light of these observations. The presence or absence of sclerotia does not appear to be highly significant at this stage of parasitism.

14. Host tissues as a nutrient substrate for fungus growth

To investigate the possibility that host tissues may contain one or more substances toxic to the growth of one or more of the fungi, two series of experiments were carried out. In the first, the growth of the fungi on heat sterilized decoctions of host tissue was assessed. In the second, the growth of the fungi on homogenates of host tissue was investigated.

1. Growth of the fungi on host decoctions

Hypocotyls of seven day old cabbage and lettuce seedlings, and wheat coleoptiles from seedlings seven days old, grown in beakers in a nutrient solution as described on page 35, were used. The hypocotyls and coleoptiles were rinsed in distilled water, drained on blotting paper, and dried in air for a few minutes to remove excess moisture; 100 g. of the tissues were boiled in 1 litre of distilled water for 30 minutes, and the liquid was squeezed through muslin. The liquid was made up, to a litre again, to compensate for the water evaporated during the boiling.

Portions of the liquid, were dispensed into 10 oz. medicine flats (50 ml. in each), and autoclaved for 15 minutes at 15 p.s.i. pressure. 1 ml. of a standard liquid inoculum as described on page 38 was added. Three dilutions of each decoction were used; a) undiluted b) diluted 1:1 with distilled water c) c) diluted 1: 2 with distilled water.

The medicine flats were incubated at 25°C. for seven days, and the dry weights of the mycelium from five replicates for each treatment were recorded. The results are shown in Table 15; each figure represents the mean of five replicates. The standard error of each mean is indicated next to each mean.

Table 15

Growth of the fungi on heat sterilized decoctions

Decoctions	Dilutions with water Dec.: H ₂ O	Growth of fungus in mg. dry weight (mean of 5 replicates) The standard error of each mean is given. Figures in brackets are relative to the most dilute decoction (1:2), which is made 100		
		<u>R.solani(S)</u>	<u>R.solani(L)</u>	<u>C.praticola</u>
Cabbage	1 : 0	4.9 ± 0.4 (163)	4.3 ± 0.6 (172)	4.4 ± 0.6 (162)
	1 : 1	3.6 ± 0.7 (120)	3.0 ± 0.5 (120)	3.0 ± 1.1 (111)
	1 : 2	3.0 ± 0.4 (100)	2.5 ± 0.3 (100)	2.7 ± 0.6 (100)
Lettuce	1 : 0	6.1 ± 0.7 (203)	4.8 ± 0.7 (133)	5.1 ± 0.5 (188)
	1 : 1	4.0 ± 0.3 (133)	4.0 ± 1.1 (111)	3.5 ± 0.7 (129)
	1 : 2	3.0 ± 0.8 (100)	3.6 ± 0.2 (100)	2.7 ± 1.2 (100)
Wheat	1 : 0	7.1 ± 0.3 (208)	7.0 ± 0.9 (269)	8.2 ± 0.8 (315)
	1 : 1	5.3 ± 1.3 (156)	4.5 ± 0.4 (173)	5.2 ± 0.9 (200)
	1 : 2	3.4 ± 0.8 (100)	2.6 ± 0.7 (100)	2.6 ± 0.6 (100)

All the decoctions stimulated growth of each of the fungi. All three fungi grew better when the decoctions were more concentrated, but better growth was obtained on the wheat decoctions with all three fungi, than on lettuce and cabbage decoctions. There is an indication that either growth promoters may be present in the wheat decoctions, or inhibitors may be present in the lettuce and cabbage decoctions, as the increase in growth with increasing concentration of the decoctions, is less than might be expected. This could mean that inhibitors might have some influence, although there does not appear to be any relation between the lack of pathogenicity of a strain to a host and inability to grow on decoctions from the host.

A further explanation could be that during the process of autoclaving, some changes took place, which ~~already~~ destroyed the inhibitors that were already present, and gave new products which may have had similar effects. However, the inhibitory effects are only marginal, and should there have been a substance that was really potent, the growth of the fungi should have increased with increasing dilution of the decoction.

A further experiment was carried out using decoctions obtained in the same way as described for the previous experiment. This time, 1.5 % agar was used to set the decoctions, in Petri dishes as described on pages 28 to 29.

The agar surfaces were inoculated with a disk, cut from the edge of a young colony of each fungus growing on potato dextrose agar. The diameter of the inoculum was 3 mm. Colony diameters (two taken at right angles to each other) were measured each day. The growth of each of the fungi with respect to time was linear. The results are shown in Table 16.

Table 16

Growth of the fungi on hypocotyl and coleoptile extract agar

Decoction	Dilution with water	Fungus growth in terms of the increase in diameter of colonies per day in cm. Each figure is the mean of 4 replicates. The standard errors of means are given. Figures in brackets are relative to the most dilute decoction (1:1) which is made 100		
		<u>R.solani(S)</u>	<u>R.solani(L)</u>	<u>C.praticola</u>
Cabbage	1 : 0	1.8 $\pm$ 0.1 (112)	3.6 $\pm$ 0 (144)	3.1 $\pm$ 0.1 (119)
	1 : 1	1.6 $\pm$ 0 (100)	2.5 $\pm$ 0.1 (100)	2.6 $\pm$ 0.1 (100)
Lettuce	1 : 0	2.0 $\pm$ 0 (111)	3.7 $\pm$ 0 (115)	3.3 $\pm$ 0.1 (117)
	1 : 1	1.8 $\pm$ 0.1 (100+)	3.2 $\pm$ 0.1 (100)	3.4 $\pm$ 0 (100)
Wheat	1 : 0	2.0 $\pm$ 0.1 (117)	3.6 $\pm$ 0.2 (114)	3.4 $\pm$ 0 (130)
	1 : 1	1.7 $\pm$ 0 (100)	2.9 $\pm$ 0.1 (100)	2.6 $\pm$ 0.1 (100)

These results are somewhat similar to those obtained in the previous experiment, in that there was better growth with the higher concentration of the decoction. However, no conclusions can be drawn about the presence of inhibitors, as linear growth on agar cannot increase arithmetically, with increasing concentration of the decoction, because these fungi can grow to some extent on distilled water agar in the absence of nutrient. It is concluded, therefore, that no potent toxin, capable of depressing fungus growth significantly, is present in the decoctions.

2. Growth of the fungi on homogenates of host plants

Homogenates of the host plants were obtained by the method described on page 47. The technique used to investigate their effects on the growth of the fungi, was similar to that used for the bio-assay of root exudates as described on page 38, with 0.005 ml. of homogenate being added to the filter paper disks instead of root exudate. The homogenates were sterilized by membrane filtration as described on page 47.

In one treatment, each homogenate was diluted with an equal volume of water. A 3 mm. diameter disk, cut from the edge of a young colony of fungus growing on distilled water agar, was used to inoculate each plate. The plates were incubated at 25°C. for five days, and drawings of hyphae were made with a camera lucida and hyphal lengths measured as before.

The results are shown in Table 17. Each of the figures shown, is the mean for 8 areas above 8 islands. Each is the percentage increase of growth relative to the control, in which the filter paper disk was moistened with 0.005 ml. of distilled water.

Table 17

Effect of homogenates of cabbage and lettuce hypocotyls, and wheat coleoptiles on the growth of the fungi

Homogenates	Dilution with water	% change in growth of fungi (Each figure is the mean of 8 replicates. The standard error for each mean is given. Figures in brackets are relative to the most dilute homogenate which is made 100		
		<u>R.solani(S)</u>	<u>R.solani(L)</u>	<u>C.prat:cola</u>
Cabbage	1 : 0	+182 $\pm$ 16 (127)	+218 $\pm$ 12 (126)	+231 $\pm$ 11 (127)
	1 : 1	+143 $\pm$ 8 (100)	+172 $\pm$ 10 (100)	+181 $\pm$ 14 (100)
Lettuce	1 : 0	+170 $\pm$ 6 (116)	+202 $\pm$ 9 (124)	+212 $\pm$ 11 (121)
	1 : 1	+146 $\pm$ 12 (100)	+163 $\pm$ 7 (100)	+174 $\pm$ 16 (100)
Wheat	1 : 0	+189 $\pm$ 8 (125)	+242 $\pm$ 11 (131)	+260 $\pm$ 7 (131)
	1 : 1	+151 $\pm$ 12 (100)	+184 $\pm$ 16 (100)	+198 $\pm$ 20 (100)

All the homogenates stimulated the growth of each fungus. Dilution of the homogenates brought about smaller increases in growth relative to the control. This means that any

inhibitors which might have been present, were not able to bring about a reduction in growth, to less than that, when the fungus was grown on distilled water agar, which was the only nutrient source. In other words, the nutrients present in the homogenates affected growth to a greater extent than the inhibitors. That inhibitors were not present cannot, however, be overruled, because the decreases in growth obtained by dilution of the homogenates were not proportional to the extent of dilution, as indicated by the figures shown in brackets in Table 17.

In fact, it appears likely that some inhibitors are present. Their presence, however, does not correspond to specificity of parasitism, because all three fungi behaved similarly under the influence of the homogenates of all three hosts. R.solani(S) appeared to be slightly less stimulated than the other two fungi by the homogenates from all three hosts.

15. Hypersensitive reactions

Flentje(1957), found that hosts resistant to attack by strains of R.solani produced a hypersensitive reaction to the invading hyphae. He reported that invaded cells and their immediate neighbours were killed, and showed "a definite yellow-brown reaction", and the invading hyphae were presumed to be killed as they did not grow when dissected out and placed on agar. Flentje further suggested that hypersensitive reactions did not occur in leaf petioles, but were confined to the stem.

The possibility of a hypersensitive reaction taking place in the three host plants was investigated. The following host-parasite combinations were studied;

R.solani(S) on lettuce and wheat

R.solani(L) on cabbage and wheat

C.praticola on wheat

The seedlings were grown under aseptic conditions as described on page 33 in Petri dishes placed in plastic boxes. When the plants were 7 days old they were inoculated with a ring of agar cut from the edge of a young colony growing on potato dextrose agar. Six days after inoculation, the plants showing lesions were removed, rinsed in sterile distilled water, and the epidermis of the hypocotyls or coleoptiles near the lesions were removed by peeling them off. This was done so that any fragments of mycelium that might have been present on the host surfaces could no longer interfere with the experiments.

A small portion of the lesion was then dissected with a sterile needle and transferred to potato dextrose agar in Petri dishes, and incubated at 25°C. for one week. In all the combinations tested, the fungi grew out on the agar surface to form colonies of mycelium, of the isolate used to inoculate the plants.

It was concluded that in the host plants which were fairly resistant to parasitism by the fungi, some fungistatic action was present, because the fungi did not progress through the tissues. However, the fungi were not killed.

Alternatively, as was observed with C.praticola on wheat, the fungus did progress into the tissues from lesions, but the rate of progress was slow, in comparison to the growth of this fungus on plants which it normally parasitized. In both cases there was evidence of fungistasis which stopped growth completely in many lesions of R.solani(S) on lettuce and wheat, and R.solani(L) on cabbage and wheat, or which retarded growth considerably in the above host-parasite combinations, as well as when C.praticola parasitized wheat.

There was no evidence of a fungicidal substance being produced by any of the three hosts, as all three fungi grew out from portions of dissected lesions when placed on potato dextrose agar. These observations are in conflict with those of Flentje (1957), who states that the hypersensitive reaction resulted in the fungus being definitely killed.

16. Death of the host cells before penetration by hyphae

Sections of hypocotyls of infected cabbage and lettuce seedlings, and coleoptiles of infected wheat seedlings, 15 μ thick were cut using a freezing microtome. These sections, as well as whole plants, were examined microscopically, to investigate whether there was discolouration or death of cells before penetration by the hyphae. The results obtained were rather inconsistent. In some plants, the cells showed a brown discolouration, while at the same time, these stained with neutral red. In others the brown discolouration was absent, but the cells did not take up the stain

satisfactorily. It was difficult to come to any definite conclusions regarding the nature of the reaction.

17. Action of cell wall degrading enzymes

It has been suggested that the production of cell wall degrading enzymes by R.solani bears relation to pathogenicity (Barker & Walker, 1962). In the present work, the pectolytic and cellulose degrading enzymes produced by the three fungi have been investigated, in order to ascertain the following;

- 1) The capacity of these fungi to produce pectolytic and cellulolytic enzymes, when grown on synthetic substrates.
- 2) Whether the relative amounts of the enzymes produced, bears any relationship to pathogenicity.
- 3) Whether cell wall degrading enzymes are produced when these fungi parasitize seedlings.
- 4) Whether these enzymes are produced in different amounts on different seedling species.
- 5) Whether the enzymes produced by growing the fungi in culture on synthetic substrates bear any relation to the enzymes produced when these fungi parasitize host seedlings.
- 6) Whether the enzymes produced when the fungi parasitize the host seedlings bear any relation to pathogenicity of the fungi towards the hosts on which these enzymes are produced.
- 7) Whether this relationship, if present, could account for the specificity of parasitism.

18. Production of extra-cellular enzymes on synthetic media

The media used for the growth of the fungi on synthetic substrates, and the procedure adopted, are described on page 50. The carbon sources used for the various experiments were 1 % pectin, pectate, CMC, WdC, and sucrose. The basal macro- and micro-nutrients were the same in all the media. All three fungi grew satisfactorily at pH 5.7, and therefore, the experiments were carried out at this pH.

19. Growth of the fungi on media used as a substrate for enzyme production

In the first set of experiments, the growth rates of the three fungi were compared on different media. These were both solid, being set with gelatine, as well as liquid.

a) Growth of the fungi on solid media

The media described above were brought to a pH of 5.7. They were autoclaved for 15 minutes with 1.5 % gelatine at 15 p.s.i. pressure. Each Petri dish (9 cm. in diameter) had 15 ml. of the medium, the surface of which was inoculated with a 3 mm. diameter disk, cut from the edge of a young colony growing on distilled water agar. The cultures were incubated at 25°C. The growth of the colonies was measured by observing two diameters at right angles to each other, each day. The results are given in Table 18 in terms of the increase in diameter of the colonies per day.

Table 18

Growth of the fungi on solid media

Fungus	Substrate (Basal medium + C source)	Fungus growth in increase in diameter in mm. of the colonies per day. Each figure is the mean of 5 replicates. Standard errors of the means are given	
<u>R.solani</u> (S)	1% pectin	16	± 2
	1% NaPP	15	± 0
	1% CMC	14	± 1
	1% WdC	15	± 2
	1% Sucrose	14	± 2
<u>R.solani</u> (L)	1% pectin	43	± 1
	1% NaPP	41	± 1
	1% CMC	42	± 3
	1% WdC	41	± 3
	1% Sucrose	50	± 2
<u>C.praticola</u>	1% pectin	31	± 0
	1% NaPP	30	± 1
	1% CMC	33	± 1
	1% WdC	33	± 2
	1% Sucrose	34	± 1

There were some differences in the growth of the three fungi, but the response of each to different media was not very marked. R.solani(S) was the slowest growing fungus. However, it grew almost equally well on all the media. R.solani(L) was the fastest growing fungus. It grew slightly better on 1 % sucrose than on the other media, between which there were only very small differences. C.praticola had a growth rate intermediate

between R.solani(S) and R.solani(L). C.praticola responded similarly to all the media.

b) Growth of the fungi in liquid media

The media used, and the procedure adopted for these experiments are described on page 50. The fungi were grown in still cultures, in 10 oz. medicine flats, each containing 50 ml. of the nutrient medium, and incubated at 25°C. for seven days. At the end of this period, the mycelium was washed, and dry weights were measured. The results are shown in Table 19.

Table 19

Growth of the fungi in liquid culture

Fungus Dry weight of mycelium in mg. after 7 days growth.
Each figure is the mean of 10 replicates. Standard
errors are given for each mean in brackets.

	Growth substrates				
	1% pectin	1% NaPP	1% CMC	1% WdC	1% sucrose
<u>R.solani</u> (S)	27.5 (6.1)	18.2 (4.3)	44.3 (4.1)	8.4 (3.9)	40.3 (6.1)
<u>R.solani</u> (L)	228.6 (12.6)	119.4 (19.1)	298.2 (11.0)	214.3 (10.4)	282.3 (14.1)
<u>C.praticola</u>	215.4 (16.8)	210.9 (21.4)	207.2 (16.1)	216.1 (18.3)	472.0 (14.1)

Again, R.solani(S) was the slowest growing fungus. It grew better on CMC than on pectin or pectate. Its growth on wood cellulose was very poor, whereas that on sucrose was satisfactory. Both R.solani(L) and C.praticola had rapid rates of growth

as compared with R.solani(S). On sucrose, as well as on CMC, R.solani(L) produced luxuriant growth. On pectin and wood cellulose this fungus grew satisfactorily, but on pectate, the growth was about half that on pectin. C.praticola produced luxuriant growth on sucrose, and slightly less growth on all the other media.

Enzyme preparations used in the experiments

The enzyme preparations were culture filtrates of the fungi, grown on synthetic media. In each case, the concentration of the enzyme preparation was adjusted by evaporation under reduced pressure (and/or by dilution), to make 100 ml. of the final enzyme preparation equivalent to 500 mg. dry weight of mycelium, obtained by the growth of the fungi for 7 days. The enzyme preparations are, therefore, comparable in respect of fungus growth.

20. Pectin methylesterase(PME)

Pectin methylesterase catalyses the hydrolysis of methyl ester groups of pectinic acids to give pectic acids, with the liberation of free acid groups which can be estimated.

A. Detection of PME

In preliminary experiments, PME activity was detected by the change in colour of the indicator methyl red which had been added to the reaction mixture as described on page 53. The mixtures were examined at different time intervals of up to 60 hours, and those which had changed the colour of the indicator are mentioned below.

Culture filtrates of R.solani(S) and R.solani(L) grown on pectin, changed the colour of the indicator within 12 hours. The culture filtrate of C.praticola grown on pectin did so within 48 hours, indicating that the filtrate of this fungus was less active than those of the two R.solani isolates, when grown on pectin. Culture filtrates of all three fungi grown on pectate failed to change the colour of the indicator even within 60 hours.

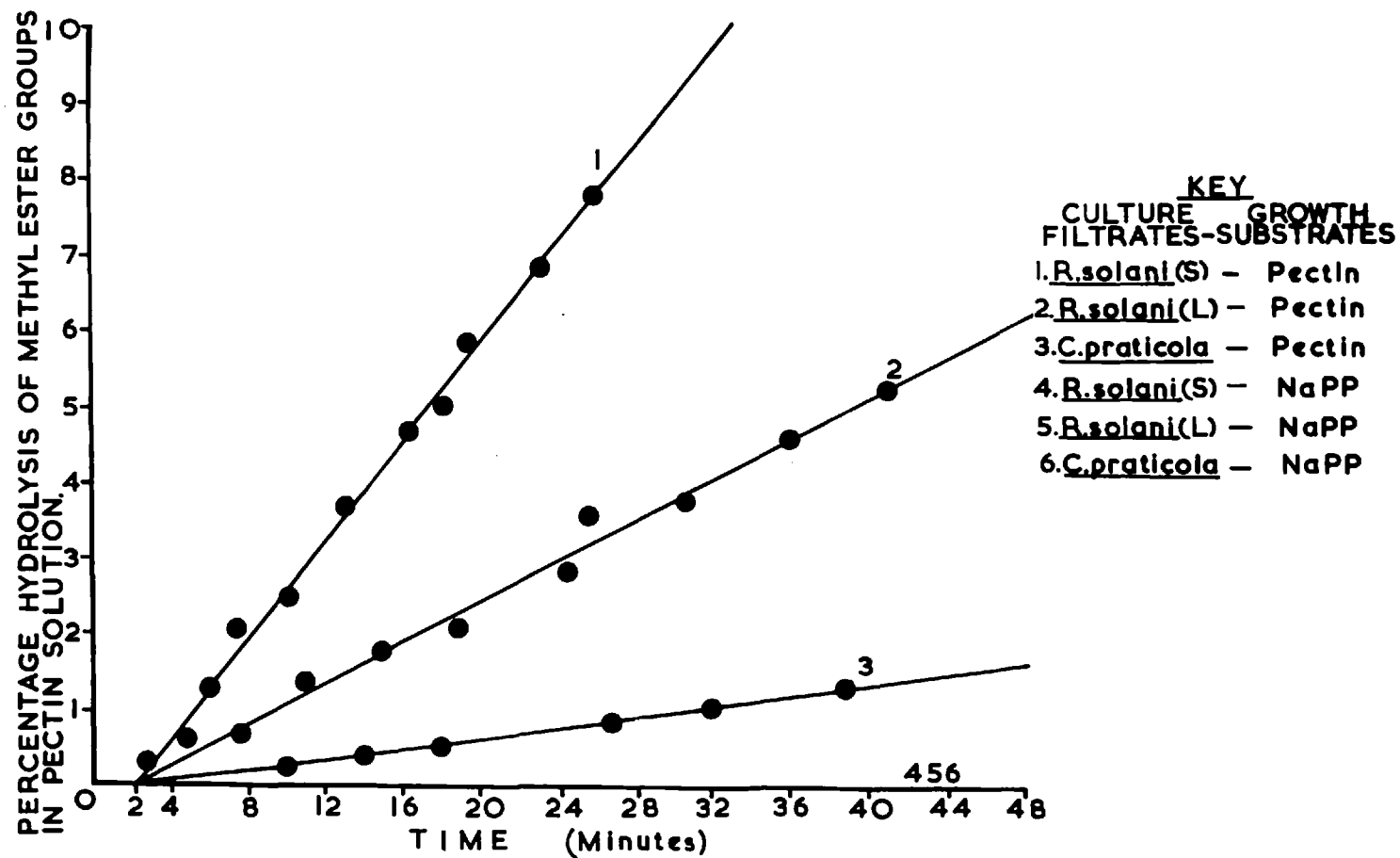
B. Estimation of PME activity by titration

Standardized culture filtrates of the fungi grown on 1% pectin and 1% pectate substrates were tested for PME activity titration (with 0.01 N NaOH) of the acid groups liberated, according to the procedure described on page 53. Figure 1 shows the relation between percentage hydrolysis of methyl ester groups in pectin solution with time, after the addition of the enzyme preparation.

Table 20 shows the PME activity of the culture filtrates in terms of the rate of hydrolysis of methyl ester groups in pectin solution, and also the growth of the fungi in mg. dry weight of mycelium produced after 7 days under the conditions described earlier. The growth figures are taken from Table 19, and are included here to compare growth and PME activity.

From Figure 1 it is clear that all the fungi produced no PME when grown on pectate. Of the culture filtrates of the fungi grown on pectin, that of R.solani(S) was the most active, and that of C.praticola the least, although C.praticola

Fig. 1. PECTIN METHYLESTERASE ACTIVITY OF CULTURE FILTRATES OF THE FUNGI GROWN ON SYNTHETIC SUBSTRATES.



was the faster growing fungus. R.solani(S) was the most slow growing fungus of the three, yet its filtrates had the highest activity. It appears therefore, that PME activity is unrelated to the growth of these fungi on the substrates used, and that PME activity is adaptive or inducible, rather than constitutive for these fungi.

Table 20

PME activity of culture filtrates of the fungi grown on pectin and pectate substrates

Fungus	Growth substrate	Fungus growth in mg. dry wt. after 7 days	Rate of hydrolysis of methylester groups in pectin at pH 5.7 (Hydrolysis after 20 min.)
<u>R.solani</u> (S)	Pectin	27.5	5.9
	NaPP	18.2	0
<u>R.solani</u> (L)	Pectin	288.6	2.4
	NaPP	119.4	0
<u>C.praticola</u>	Pectin	215.4	0.6
	NaPP	210.9	0

C.praticola was the most virulent pathogen of these three fungi, yet, its PME activity was the lowest. It is therefore concluded that the mere ability of a fungus to secrete PME on suitable synthetic media is not directly related with the pathogenicity of these fungi to cabbage and lettuce plants.

21. Polygalacturonase(PG)

PG breaks down pectic or pectinic acids by the hydrolysis of the glycosidic linkages, with the production of reducing groups. Standardized enzyme preparations obtained by growing the three fungi on pectin and pectate, were used for all the experiments in the estimation of PG activity.

A. PG(Viscosity)activity

The technique used for estimating PG(Viscosity)activity is described on page 55. Table 21 shows PG(Viscosity)activity calculated as explained on page 56 for a 50 % reduction in viscosity of the test substrates, which were pectin and pectate.

From Table 21 it is evident that none of the fungi grown on pectate produced active culture filtrates. When the fungi were grown on pectin, R.solani(L) produced the most active filtrates. These reduced the viscosity of pectate nearly twice as fast as they did the viscosity of pectin. Culture filtrates of C.praticola were inactive. R.solani(S) grew more slowly than either of the other fungi, yet its filtrates had some activity. There is, therefore no relationship between fungus growth and PG(Viscosity)activity.

Further, C.praticola which was the most virulent pathogen, parasitizing both cabbage and lettuce plants, produced inactive filtrates, whereas the fungi which parasitized

only one of these two hosts, produced filtrates with some activity. There was therefore no direct correlation between pathogenicity and PG(Viscosity)activity of culture filtrates of these fungi.

Table 21

PG(Viscosity)activity of culture filtrates of the fungi grown on 1 % pectin and 1 % polypectate, estimated by the ability of the culture filtrates to reduce the viscosity of pectin and polypectate

Fungus	Growth substrate	Fungus growth in mg. dry wt. after 7 days	Substrate in reduction of viscosity was tested	PG(Viscosity) activity (100/time taken for 50% reduction in viscosity)
<u>R.solani</u> (S)	Pectin	27.5	Pectin	1.6
	Pectin		NaPP	2.2
	NaPP	18.2	Pectin	0
	NaPP		NaPP	0
<u>R.solani</u> (L)	Pectin	228.6	Pectin	12.5
	Pectin		NaPP	25.0
	NaPP	119.4	Pectin	0
	NaPP		NaPP	0
<u>C.praticola</u>	Pectin	215.4	Pectin	0
	Pectin		NaPP	0
	NaPP	210.9	Pectin	0
	NaPP		NaPP	0

B. PG(Reducing groups) activity

The reducing groups produced by the breakdown of pectin and pectate by standardized enzyme preparations of the fungi grown on pectin and pectate were estimated by the method described on page 56. Figures 2 and 3 show the relation between the percentage hydrolysis of glycosidic linkages in pectin and pectate respectively with time. Table 22 shows the percentage hydrolysis of glycosidic linkages in pectin and pectate at the end of 24 hours. It also shows the growth of the fungi on pectin and pectate substrates, in order to relate growth to activity.

The main conclusions drawn from Figures 2 and 3, and Table 22 are given below.

a) Enzyme activity of the filtrates of the fungi grown on pectin

Filtrates of R.solani(S) and C.praticola were about $3/4$ and $1/2$ as active respectively, as those of R.solani(L) in degrading pectin, and about $1/2$ and $1/3$ as active respectively as those of R.solani(L) in degrading pectate. The culture filtrates of all three fungi produced many more reducing groups in 24 hours from pectin than from pectate.

b) Enzyme activity of the filtrates of the fungi grown on pectate

Filtrates of R.solani(S) and C.praticola were about $1/2$ and $1/4$ as active respectively, as those of R.solani(L) in degrading pectin, and about $1/2$ and $1/3$ as active respectively, as those of R.solani(L) in degrading pectate. Here again, the culture

Fig. 2. PECTIN POLYGALACTURONASE (REDUCING GROUPS) ACTIVITY AT pH 5.7 OF CULTURE FILTRATES OF THE FUNGI GROWN ON SYNTHETIC SUBSTRATES.

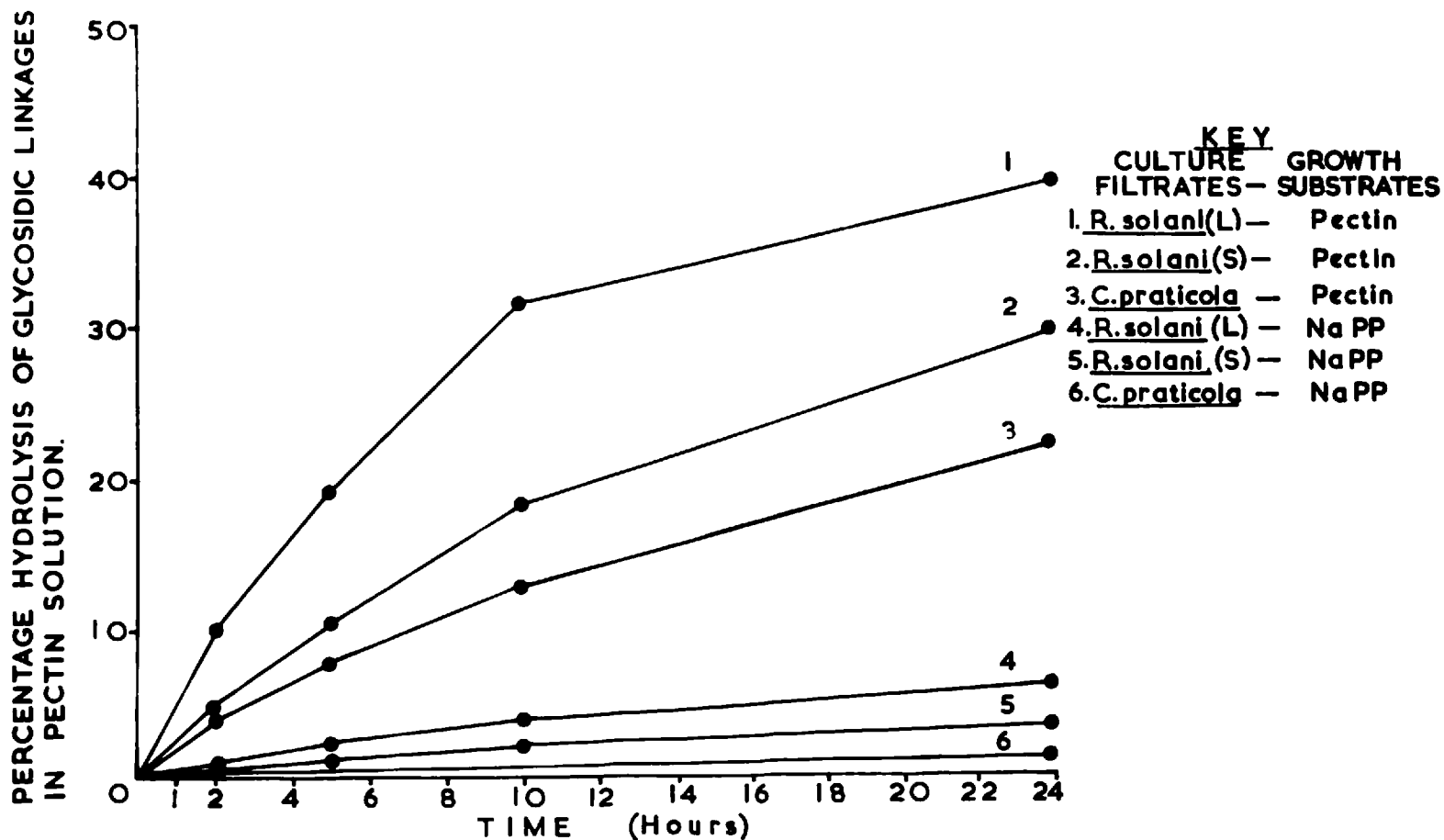
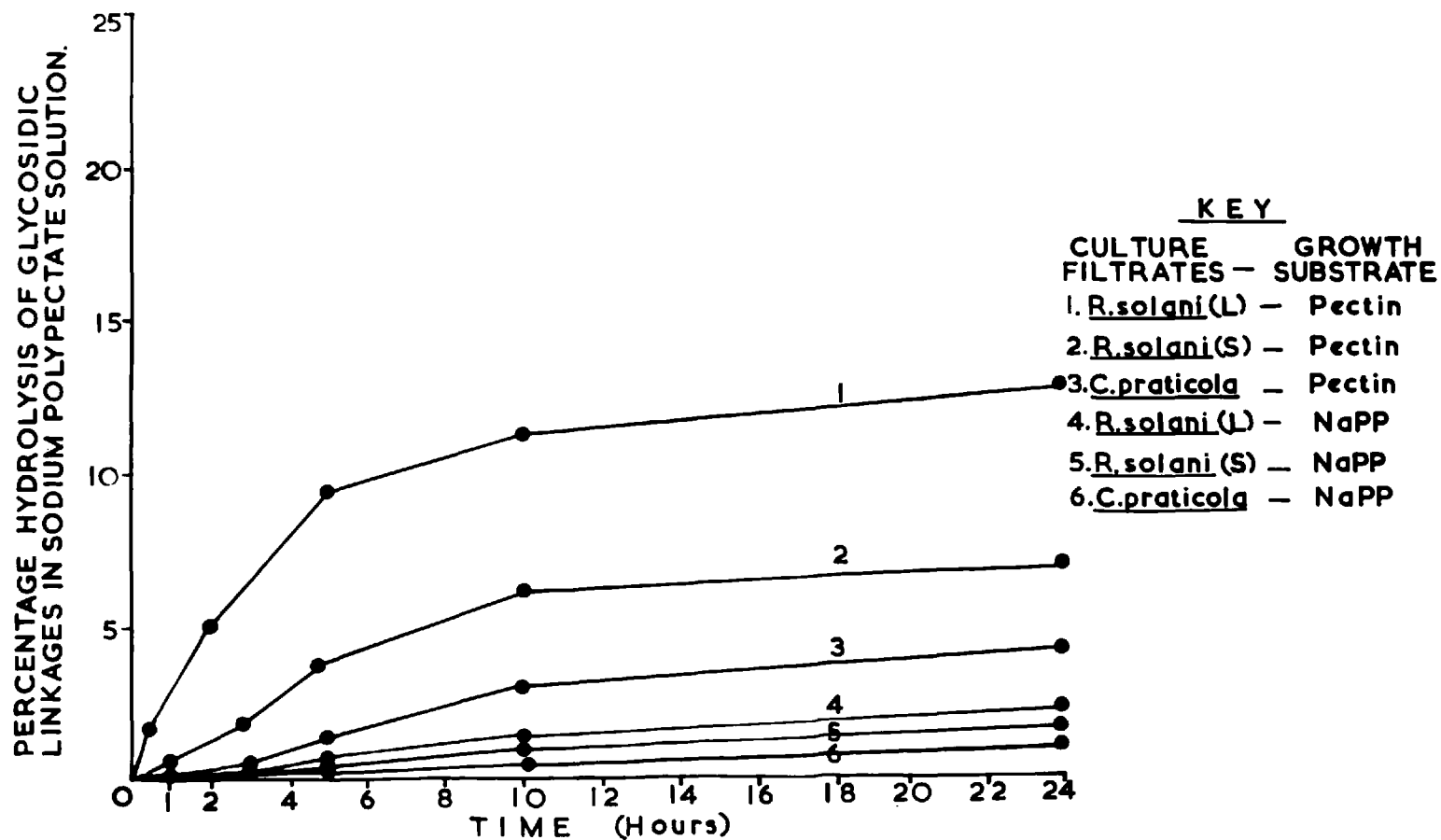


Fig. 3. PECTIN POLYGALACTURONASE (REDUCING GROUPS) ACTIVITY AT pH 5.7
OF CULTURE FILTRATES OF THE FUNGI GROWN ON SYNTHETIC SUBSTRATES.



filtrates of all three fungi had produced more reducing groups in 24 hours from pectin than from pectate.

Table 22

PG(Reducing groups)activity of culture filtrates of the fungi grown on 1 % pectin and 1 % pectate substrates, estimated in terms of the % hydrolysis of glycosidic linkages in pectin and pectate after 24 hours

Fungus	Growth Substrate	Fungus growth in mg. dry wt. after 7 days	Substrate in which activity was tested	PG(Reducing groups) activity. (% hydrolysis of glycosidic linkages after 24 hours)
<u>R.solani</u> (S)	Pectin	27.5	Pectin	29.7
			NaPP	7.0
	NaPP	18.2	Pectin	3.5
			NaPP	1.5
<u>R.solani</u> (L)	Pectin	228.6	Pectin	40.0
			NaPP	12.8
	NaPP	119.4	Pectin	6.0
			NaPP	2.8
<u>C.praticola</u>	Pectin	215.5	Pectin	21.6
			NaPP	4.1
	NaPP	210.9	Pectin	1.2
			NaPP	0.9

c) Relation of fungus growth to PG(Reducing groups) activity

Since the culture filtrates had been standardized to compensate for differences in growth between fungi, if there was a correlation between fungus growth and PG(reducing groups)activity the figures for the percentage hydrolysis of glycosidic linkages should be equal. However, this was not so, and it is concluded that there was no such relationship. For instance, R.solani(L) and C.praticola had comparable growth rates, yet, PG(reducing groups)activity varied considerably. Again, R.solani(S) grew much more slowly than C.praticola, yet, the culture filtrates of R.solani(S) were more active than those of C.praticola.

C. Comparison of PG(Viscosity) and PG(Reducing groups) activities

Table 23 summarizes the results obtained in the experiments on PG activity of culture filtrates of the fungi grown on synthetic media, for both viscosity reduction and reducing groups estimations, on the two substrates pectin and pectate. To facilitate comparison, the culture filtrates with the highest activities are assigned the figure 100, and the others are given figures relative to this. The viscosity figures indicate activity in terms of the time taken for a 50 % reduction in viscosity of the test substrate. The reducing groups figures are a measure of the reducing groups produced in the first hour of the hydrolysis of glycosidic linkages in the test substrates.

Table 23

PG activity of the culture filtrates of the fungi, relative to the most active culture filtrate which is made 100

Fungus	Growth substrate	Test substrate on which activity was estimated	PG activity(relative)	
			Viscosity	Reducing groups
<u>R.solani</u> (S)	Pectin	Pectin	10	50
		NaPP	13	24
	NaPP	Pectin	0	4
		NaPP	0	0
<u>R.solani</u> (L)	Pectin	Pectin	64	100
		NaPP	100	60
	NaPP	Pectin	0	10
		NaPP	0	2
<u>C.praticola</u>	Pectin	Pectin	0	40
		NaPP	0	4
	NaPP	Pectin	0	2
		NaPP	0	0

The following conclusions are drawn regarding PG activity;

a) Pectin was a better substrate for pectic enzyme production by each of the fungi than pectate. When the fungi were grown on Pectate, activity of the culture filtrates was low.

b) When the fungi were grown on pectin, R.solani(L) produced the most active filtrates, whereas those of C.praticola were the least active.

- c) There was no correlation between the growth of the fungi on pectin or pectate, and PG activity
- d) The culture filtrates of C.praticola were less active than those of the two R.solani isolates, yet each R.solani isolate parasitized either cabbage or lettuce, whereas C.praticola parasitized both these hosts. It must therefore be concluded that there was no ~~relat~~ relationship evident between PG activity and the pathogenicity of these fungi towards cabbage and lettuce.

22. Cellulase

Cellulase activity was estimated in terms of the ability of the culture filtrates of the fungi to break down cellulose chains, thereby reducing the viscosity of solutions of CMC, and also by the estimation of the reducing groups produced by the hydrolysis of the glucosidic linkages present in CMC chains. The enzyme preparations used for these experiments were culture filtrates of the fungi grown on the basal medium together with CMC or wood cellulose as the carbon sources, as described on page 50.

The culture filtrates were standardized as described for the experiments on PG activity, to compensate for differences in growth between different fungi. However, this standardization was subject to a source of error where the fungi were grown on wood cellulose. The mycelium grew over the surface of the liquid medium in the form of a mat. This was removed with

a bent needle and floated on water to remove adhering particles of wood cellulose. The mycelium was then dried and weighed. The dry weight measurements however, were not strictly accurate, as some particles of wood cellulose might have been present on the mat, and possibly, some fragments of mycelium may have been lost in the removal process. However, it does give some measure of growth. Since the standardizations of the enzyme preparations were dependent on determination of dry weights, the enzyme preparations too, are subject to this source of error.

A. Cellulase(Viscosity)activity

Cellulase viscosity activity was estimated by the ability of the culture filtrates to reduce the viscosity of CMC solutions as described on page 57. Table 24 shows cellulase (viscosity) activity calculated as explained on page 57 for a 50 % reduction in viscosity of CMC solution. The growth rates of the fungi on the two substrates CMC and WdC are given, to facilitate comparison of activity of the filtrates and fungus growth.

From Table 24 it is clear that when the fungi were grown on CMC, R.solani(S) and C.praticola produced filtrates of nearly equal activity. These, however, were only about a third as active as the filtrates of R.solani(L). R.solani(S) produced very poor growth on WdC, and the filtrates were inactive. R.solani(L) filtrates were much more active than those of C.praticola.

Table 24

Cellulase(Viscosity)activity of culture filtrates of the fungi grown on 1 % CMC and 1 % WdC, estimated by the ability of the culture filtrates to reduce the viscosity of CMC solution

Fungus	Growth substrate	Fungus growth in mg. dry wt. after 7 days	Cellulase(viscosity)activity (100/time for a 50% reduction in viscosity of CMC solution)
<u>R.solani</u> (S)	CMC	44.3	12.5
	WdC	8.4 (?)	0
<u>R.solani</u> (L)	CMC	298.2	40.0
	WdC	214.3 (?)	15.3
<u>C.praticola</u>	CMC	207.2	11.7
	WdC	260.1 (?)	2.8

(?) see source of error on page 152

Although the culture filtrates had been standardized to compensate for differences in growth, there were significant differences between their activities. It is therefore concluded that there is no direct relationship between cellulase (viscosity) activity of the culture filtrates of the fungi, and their growth on CMC and WdC.

B. Cellulase(Reducing groups)activity

The production of reducing groups from solutions of CMC by standardized culture filtrates of the fungi, grown on CMC and WdC substrates were estimated by the method described

on page 57. Figure 4 shows the percentage hydrolysis of glucosidic linkages in CMC plotted against time. Table 25 shows the percentage hydrolysis of glucosidic linkages in CMC at the end of 24 hours after the addition of the enzyme preparation. The growth rates of the fungi on CMC and WdC are included, to relate growth to activity.

Table 25

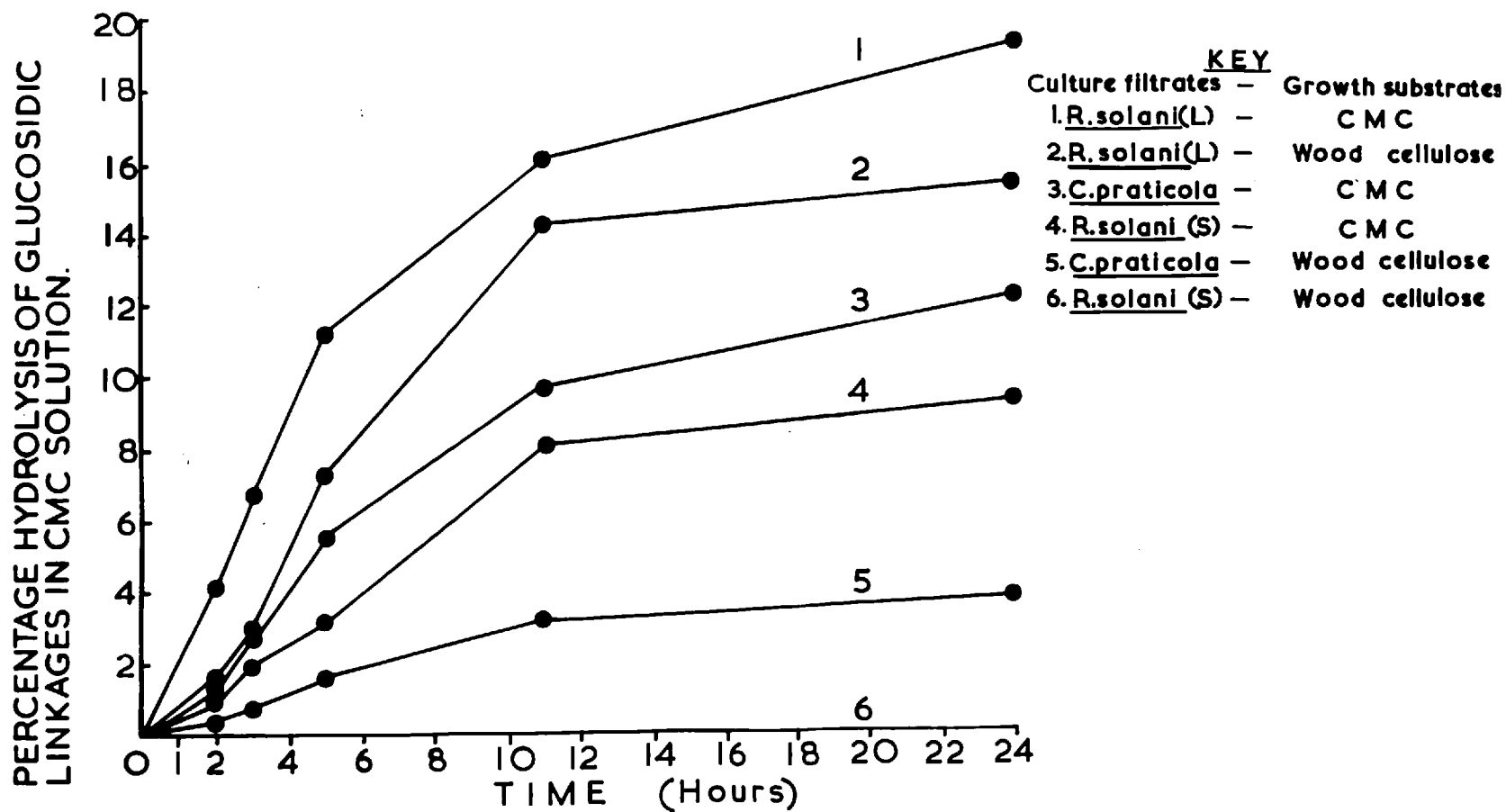
Cellulase (Reducing groups) activity of culture filtrates of the fungi grown on 1 % CMC and 1 % WdC in terms of the % hydrolysis of glucosidic linkages in CMC

Fungus	Growth substrate	Fungus growth in mg. dry wt. after 7 days	Cellulase(reducing groups) activity. % hydrolysis of glucosidic linkages in CMC after 24 hours
<u>R.solani</u> (S)	CMC	44.3	9.4
	WdC	8.4 (?)	0
<u>R.solani</u> (L)	CMC	298.2	19.9
	WdC	214.3 (?)	15.4
<u>C.praticola</u>	CMC	207.2	12.2
	WdC	260.1 (?)	3.8

(?) see source of error on page 152

From Table 25 and Figure 4 it is clear that culture filtrates of each fungus grown on CMC had some activity. R.solani(L) filtrates were twice as active as those of R.solani(S) which had the lowest activity of the three fungi.

Fig. 4. CELLULASE (REDUCING GROUPS) ACTIVITY AT pH 5.7 OF CULTURE FILTRATES OF THE FUNGI GROWN ON SYNTHETIC SUBSTRATES.



Culture filtrates of R.solani(S) grown on WdC had no activity. Filtrates of R.solani(L) were more active than those of C.praticola. Again, there was apparently no direct relation between cellulase (reducing groups) activity and the growth of the fungi on either of the two substrates used.

Table 26 summarizes the results obtained in the experiments on cellulase activity of culture filtrates of the fungi grown on synthetic media, for both viscosity reduction and reducing groups estimations. To facilitate comparison, the filtrates with the highest activity are assigned the figure 100, and the others are given figures relative to this. The viscosity figures indicate activity in terms of the time taken for a 50 % reduction in viscosity of the test substrate, CMC. The reducing groups figures are a measure of the amount of reducing groups produced in the first hour of the hydrolysis of glucosidic linkages in CMC.

The following conclusions are drawn regarding cellulase activity.

- a) CMC was a satisfactory substrate for the growth of the three fungi, but WdC was not suitable as a substrate for either growth or enzyme production by R.solani(S). Both R.solani(L) and C.praticola grew on WdC, and produced culture filtrates with some activity.
- b) The filtrates of R.solani(L) had a higher cellulase activity than those of the other two fungi.
- c) There was apparently no relationship between fungus growth and cellulase activity of the culture filtrates.

Table 26

Cellulase activity of the culture filtrates of the fungi, relative to the most active filtrate which is made 100

Fungus	Growth substrate	Cellulase activity (relative)	
		Viscosity	Reducing groups
<u>R.solani</u> (S)	CMC	75	45
	WdC	0	0
<u>R.solani</u> (L)	CMC	100	100
	WdC	60	80
<u>C.praticola</u>	CMC	50	65
	WdC	13	20

d) C.praticola, the most virulent pathogen, parasitizing both cabbage and lettuce, had a smaller capacity to produce cellulase than R.solani(L) which much less pathogenic to cabbage than C.praticola. It can therefore be concluded that cellulase activity of culture filtrates of the fungi growing on synthetic substrates bears no relationship to their pathogenicity towards cabbage and lettuce seedlings.

23. Production of extracellular cell wall degrading enzymes by the fungi when they parasitize host plants

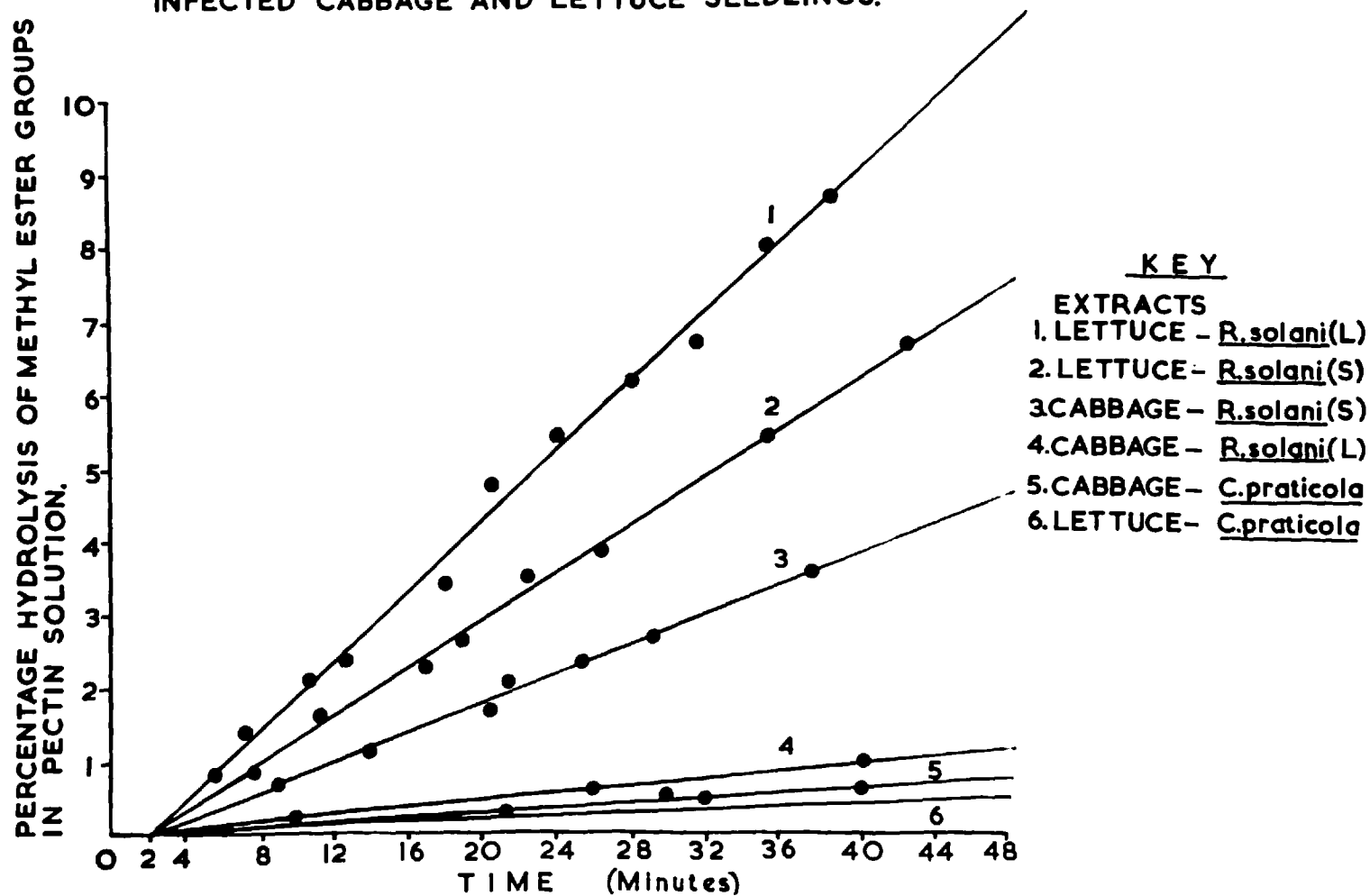
The pectolytic and cellulolytic enzyme activity of 0.2 M NaCl extracts of cabbage and lettuce seedlings infected with the fungi was next investigated. The enzyme preparations were obtained according to the procedure described on page 51. The enzyme preparations were dialysed and standardized as described on page 52. The standardized preparations were used in all subsequent experiments.

24. Pectin methylesterase (PME)

The enzyme preparations were tested for PME activity by titration (with 0.01 N NaOH solution) of the acid groups liberated according to the procedure described on page 53. Figure 5 shows the relation between the percentage hydrolysis of methyl ester groups in pectin with time, after the addition of the enzyme preparation. Table 27 shows the activity of the enzyme preparations, as a measure of the rate of hydrolysis of methyl ester groups in pectin, in terms of the hydrolysis for the first 20 minutes, after the addition of the enzyme preparation.

From Figure 5 and Table 27 it is clear that extracts of both the R.solani strains from lettuce seedlings were more active than those from cabbage. However, the cabbage extracts of C.praticola were more active than the lettuce extracts. This however, is not very significant, as there was more native enzyme

Fig.5. PECTIN METHYLESTERASE ACTIVITY OF 0.2 M NaCl EXTRACTS OF INFECTED CABBAGE AND LETTUCE SEEDLINGS.



present in cabbage than in lettuce. If this is taken into consideration both the cabbage and lettuce extracts of C.praticola were almost equally active, and their activities were much less than those of the two R.solani isolates.

Table 27

PME activity of 0.2 M NaCl extracts of diseased cabbage and lettuce seedlings

Fungus	Extract		Rate of hydrolysis of methyl ester groups in pectin (Hydrolysis for the first 20 minutes)
		Host	
<u>R.solani</u> (S)		Cabbage	1.75
		Lettuce	2.90
<u>R.solani</u> (L)		Cabbage	0.45
		Lettuce	4.20
<u>C.praticola</u>		Cabbage	0.25
		Lettuce	0.15
Nil		Cabbage	0.16
		Lettuce	0.10

The lettuce extract of R.solani(L) was more active than its cabbage extract, and the cabbage extract of R.solani(S) was more active than the cabbage extract of R.solani(L). There might appear to be a suggestion here, of a relationship with pathogenicity. This, however, is not quite so clear, because the lettuce extract of R.solani(S) was more active than its cabbage extract. Further, it may mean that R.solani(L) produced more enzyme

on lettuce because it grew better on lettuce than on cabbage.

If there was a correlation between PME activity of extracts if the diseased plants and pathogenicity, it would have been expected extracts of both cabbage and lettuce infected with C.praticola would be very active. This however, was not so; and it is therefore concluded that there is no strict correlation between pathogenicity, and PME activity of these extracts.

25. Pectin polygalacturonase (PG)

A - PG(Viscosity) activity

PG (viscosity) activity of dialysed 0.2 M NaCl extracts of cabbage and lettuce seedlings infected with the fungi, was estimated by their ability to reduce the viscosity of solutions of pectin and pectate, according to the method described on page 55. The enzyme preparations were obtained according to the method described on pages 51 - 52. Table 28 shows PG(viscosity) activity of the extracts in terms of the time taken for a 25 % reduction in viscosity of the test substrates, pectin and pectate. The results shown in Table 28 are considered below.

a) Extracts of infected cabbage seedlings

Extracts of cabbage seedlings infected with each of the three fungi reduced the viscosity of pectate more rapidly than they did that of pectin. R.solani(L) extracts were a little more active in reducing the viscosity of both test substrates than those of the other two fungi, the activities of which were nearly equal to each other.

Table 28

PG (Viscosity) activity of 0.2 M NaCl extracts of cabbage and lettuce seedlings infected with the fungi

Extract		PG(Viscosity) activity	
Fungus	Host	100/time for a 25% reduction in viscosity of pectin solution	100/time for a 25% reduction in viscosity of pectate solution
<u>R.solani</u> (S)	Cabbage	1.3	10.0
	Lettuce	1.5	8.3
<u>R.solani</u> (L)	Cabbage	1.8	12.5
	Lettuce	0.5	25.0
<u>C.praticola</u>	Cabbage	1.6	10.0
	Lettuce	2.0	5.8

b) Extracts of infected lettuce seedlings

R.solani(L) extracts reduced the viscosity of pectate solutions nearly three times faster than those of R.solani(S), and nearly five times faster than those of C.praticola, but these R.solani(L) extracts reduced the viscosity of pectin a little more slowly than the extracts of the other two fungi.

c) Comparison of fungus extracts from the two hosts

The activities of cabbage and lettuce extracts of R.solani(S) were about the same, on both test substrates, pectin and pectate. However, the lettuce extracts of R.solani(L) reduced the viscosity of pectate twice as fast as the cabbage extracts,

whereas the cabbage extracts were more active in reducing the viscosity of pectin than the lettuce extracts. Cabbage extracts of C.praticola reduced the viscosity of pectate nearly twice as fast as the lettuce extracts of this fungus, but both extracts reduced the viscosity of pectin at about the same rate.

B - PG(Reducing groups) activity

The reducing groups produced by the enzymic breakdown of pectin or pectate were estimated by the method described on page 56. The enzyme preparations were similar to those used for the estimation of PG(viscosity)activity, and were dialysed 0.2 M NaCl extracts of cabbage and lettuce seedlings infected with each fungus. Figures 6 and 7 show the percentage hydrolysis of glycosidic linkages in pectin and pectate respectively, with time, after the addition of the enzyme preparation to the substrate.

From Figures 6 and 7 the following conclusions were drawn.

a) Reducing groups produced from pectin

None of the extracts had high activities on pectin, the percentage hydrolysis of glycosidic linkages for each of them being less than 1.5 after 24 hours.

b) Reducing groups produced from pectate

The production of reducing groups from pectate were higher than that from pectin, for each extract. The lettuce extracts were more active than cabbage ones for each fungus.

**Fig. 6. PECTIN POLYGALACTURONASE (REDUCING GROUPS)ACTIVITY OF
0.2 M NaCl EXTRACTS OF INFECTED CABBAGE AND LETTUCE SEEDLINGS
AT pH 5.7.**

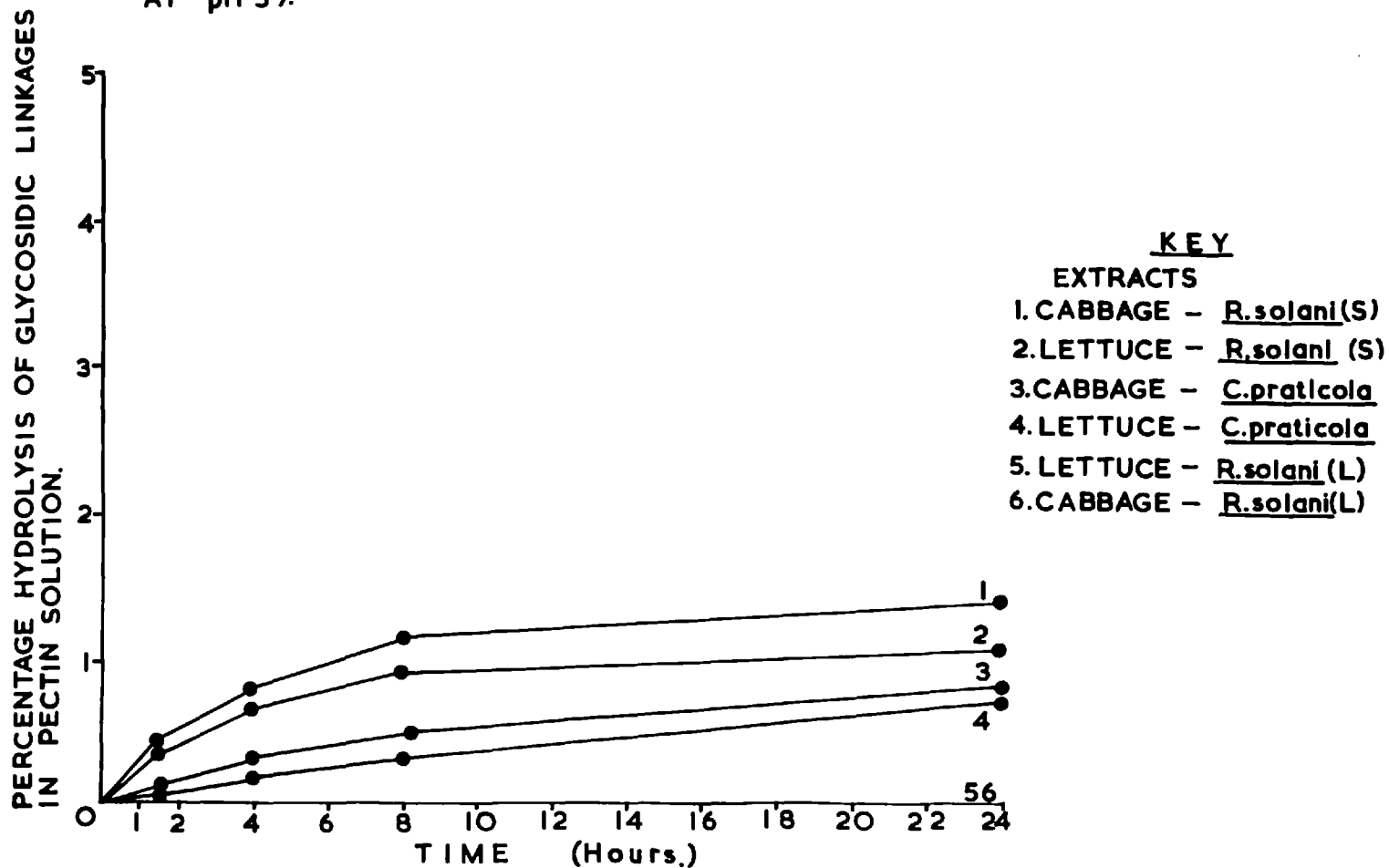
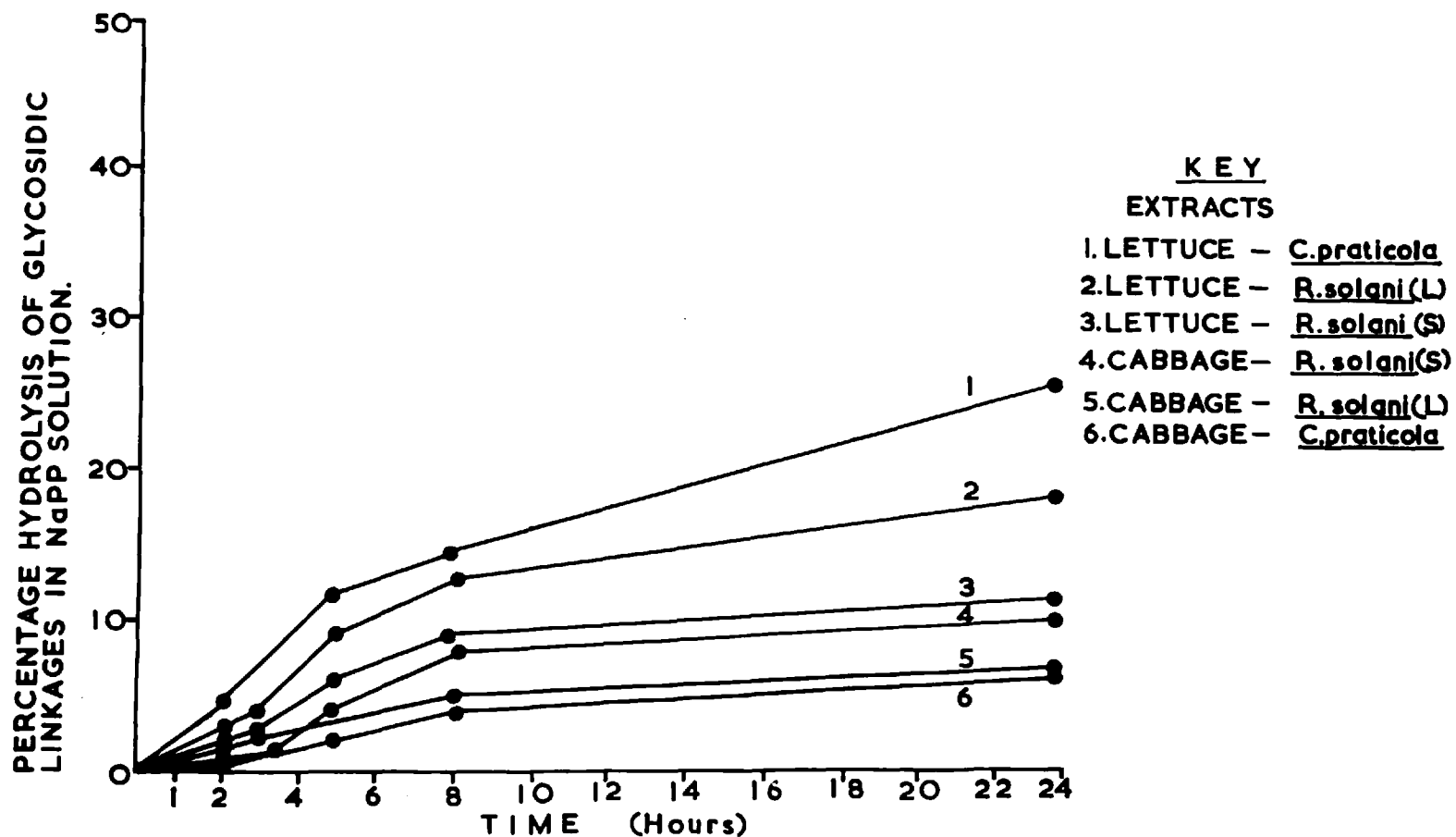


Fig.7. PECTIN POLYGALACTURONASE (REDUCING GROUPS)ACTIVITY OF 0.2 M NaCl EXTRACTS OF INFECTED CABBAGE AND LETTUCE SEEDLINGS AT pH 5.7.



The lettuce extract of C.praticola was more active than any of the other extracts, but the cabbage extract of this fungus was less active than those of the other fungi. There is no indication, therefore, of a relation between activity and pathogenicity.

The cabbage extracts of R.solani(S) were more active than those of the other two fungi, but its lettuce extracts were less active than those of the other two fungi. Here, there might be considered to be some indication of a relationship with pathogenicity, but in actual fact this is somewhat unrealistic, as the lettuce extract of this fungus was more active than its cabbage extract.

Lettuce extracts of R.solani(L) were more active than cabbage extracts of this fungus, indicating a relation with pathogenicity. Here again, however, the cabbage extracts of R.solani(L) were as active as the cabbage extracts of C.praticola, whereas the pathogenicity of C.praticola to cabbage was very much more severe than that of R.solani(L) to this host.

It was considered that there was no direct relationship between the ability of extracts of diseased host plants to hydrolyse glycosidic linkages in pectate, and pathogenicity.

Table 29 summarizes the results obtained in the experiments on PG activity of the extracts of infected cabbage and lettuce plants, for both viscosity reduction and reducing groups estimations, on the substrates pectin and pectate. To facilitate comparison, the extracts with the highest activity are assigned the

figure 100, and the others are given figures relative to this. The viscosity figures are calculated from the activity in terms of the time taken for a 25 % reduction in viscosity of the test substrate. The reducing groups figures are a measure of the reducing groups produced in the first hour of the hydrolysis of glycosidic linkages in the test substrates.

Table 29

PG activity of extracts of diseased cabbage and lettuce seedlings relative to the most active filtrate, which is made 100

Extract		Test substrate	PG Activity	
Fungus	Host		Viscosity	Reducing groups
<u>R.solani</u> (S)	Cabbage	Pectin	5.2	24
		NaPP	40.0	20
	Lettuce	Pectin	6.0	16
		NaPP	33.2	80
<u>R.solani</u> (L)	Cabbage	Pectin	7.2	0
		NaPP	50.0	60
	Lettuce	Pectin	2.0	0
		NaPP	100.0	100
<u>C.praticola</u>	Cabbage	Pectin	6.4	6
		NaPP	40.0	40
	Lettuce	Pectin	8.0	4
		NaPP	23.2	20

From the results shown in Table 29, it is clear that the extracts were more active in degrading pectate than pectin. Further, although there was some correlation between PG activity as assessed by viscometry, and that assessed by the estimations of reducing groups, this did not apply to all the enzyme preparations. For example, the R.solani(S)/cabbage extracts and the C.praticola/cabbage extracts had comparable power to reduce the viscosity of pectate, whereas the C.praticola filtrate produced more reducing groups from pectate than the R.solani(S) filtrate. There is no correlation between pathogenicity and PG activity of extracts of diseased host seedlings as estimated by either viscometric or reducing groups methods.

26. Cellulase

Cellulase activity of dialysed 0.2 M NaCl extracts of cabbage and lettuce plants infected with the fungi was estimated in terms of the breakdown of CMC, as assessed by the reduction in viscosity of solutions, as well as by the estimation of the reducing groups produced by the hydrolysis of glucosidic linkages in CMC.

A - Cellulase(Viscosity)activity

The experimental procedure was the same as that used for the culture filtrates of the fungi grown on synthetic media, and is described on page 57. Table 30 shows the results obtained.

Table 30

Cellulase (viscosity) activity of 0.2 M NaCl extracts of infected cabbage and lettuce seedlings

Extract		Cellulase(Viscosity)activity (100/time for a 25% reduction in viscosity of CMC)
Fungus	Host	
<u>R.solani</u> (S)	Cabbage	12.5
	Lettuce	18.1
<u>R.solani</u> (L)	Cabbage	5.0
	Lettuce	20.0
<u>C.praticola</u>	Cabbage	3.3
	Lettuce	28.5

From Table 30 it is evident that the only extracts of infected cabbage plants having a fairly good activity were those from plants infected with R.solani(S). Extracts of lettuce plants infected with C.praticola had the highest activity, while those of plants infected with R.solani(S) and R.solani(L) were both about half as active.

Only one of the two cabbage infecting strains had a high activity when extracts of cabbage plants were tested, and whereas all three fungi growing on lettuce produced active enzyme preparations, only two of them killed lettuce plants. There is, therefore, no correlation between cellulase(viscosity) activity and pathogenicity.

b - Cellulase(Reducing groups) activity

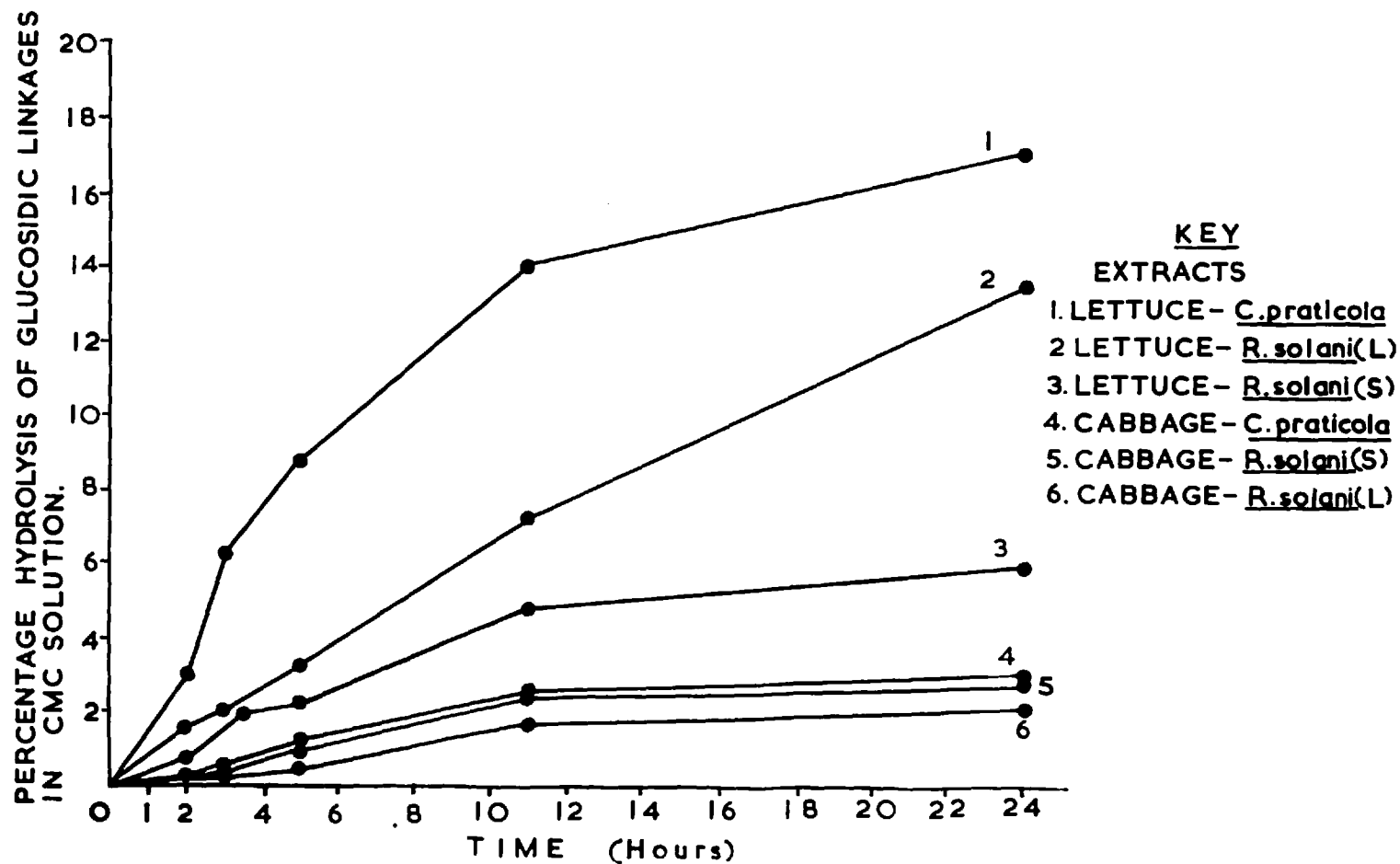
The method of estimating reducing groups produced in the enzymic hydrolysis of glucosidic linkages in CMC is described on page 57. Figure 8 shows the percentage hydrolysis with time.

From Figure 8 it is clear that there are substantial differences in the capacities of different extracts to liberate reducing groups from CMC. The extracts of cabbage plants infected with R.solani(S) had a lower activity than extracts of lettuce plants infected with the same fungus, in spite of the fact that this fungus parasitized cabbage more than it did lettuce.

On the other hand, extracts of lettuce seedlings infected with R.solani(L) had a much higher activity than cabbage plants infected with this fungus. In spite of the fact that C.praticola parasitizes both cabbage and lettuce plants to almost equal extents, the extracts of lettuce plants infected with this fungus had a very much higher activity than the cabbage extracts. There is, therefore, apparently no relationship between pathogenicity and cellulase (reducing groups) activity.

Table 31 summarizes the results obtained in the experiments on cellulase activity of extracts of infected cabbage and lettuce plants, for both viscosity reduction and reducing groups estimations. To facilitate comparison, the extracts with the highest activity are assigned the figure 100, and the others are given figures relative to these. The viscosity figures are calculated from

Fig. 8. CELLULASE (REDUCING GROUPS) ACTIVITY AT pH 5.7 OF 0.2 M NaCl EXTRACTS OF INFECTED CABBAGE AND LETTUCE SEEDLINGS.



the activity in terms of the time taken for a 25 % reduction in the viscosity of the test substrate CMC. The reducing groups figures are a measure of the reducing groups produced in the first hour of the hydrolysis of glucosidic linkages in CMC.

Table 31

Cellulase activity of extracts of diseased cabbage and lettuce plants relative to the most active extracts, which are made 100

Extract		Cellulase activity	
Fungus	Host	Viscosity	Reducing groups
<u>R.solani</u> (S)	Cabbage	43.8	13.3
	Lettuce	63.5	26.6
<u>R.solani</u> (L)	Cabbage	17.5	6.6
	Lettuce	71.9	60.0
<u>C.praticola</u>	Cabbage	11.6	13.3
	Lettuce	100	100

From Table 31 it is clear that there is some correlation between cellulase (viscosity) activity and cellulase (reducing groups) activity of extracts of infected host seedlings. Cellulase activity was higher with extracts of lettuce plants for each fungus, than with extracts of cabbage plants. There is no strict relationship between cellulase activity of the extracts and pathogenicity.

27. Comparison of the onzyme activities of culture filtrates of the fungi grown on synthetic substrates, and extracts of diseased host seedlings

The culture filtrates of the fungi grown on synthetic substrates were standardized by concentration, in respect of the growth of the fungi on the different media. This was not possible with the diseased plant extracts, because the actual amount of fungus could not be measured directly. Accordingly, the activities of the enzyme preparations were made relative to the most active culture filtrate which was assigned the figure 100. This was dono separately for the culture filtrates of the fungi grown on synthetic substrates, and for the extracts of diseased plants. All the comparisons made below are on this basis.

A - PME activity

Table 32 compares PME activity for the two sets of enzyme preparations. The figures shown are a measure of the percentage hydrolysis of methyl ester groups in pectin, 20 minutes after the addition of the culture filtrates.

From Table 32 it is clear that there were significant differences between the PME activity of the culture filtrates of the fungi grown on synthetic substrates, and the extracts of diseased plants. When the fungi were grown on poectin, and when they parasitized cabbage, the enzyme preparations of R.solani(S)

were the most active, and those of C.praticola, the least. When the fungi parasitized lettuce, however, the extracts of R.solani(L) were more active than those of the other two fungi. This might mean, that R.solani(L) grew better on lettuce than on cabbage, and that the reverse was true of R.solani(S), and this result would then indicate a growth effect, and not one connected directly with pathogenicity.

Table 32

Comparison of PME activity of culture filtrates of the fungi grown on synthetic substrates, with that of extracts of diseased hosts

Fungus	PME Activity			
	Fungus growth substrates			
	Pectin	NaPP	Cabbage	Lettuce
<u>R.solani</u> (S)	100	0	41.6	69.0
<u>R.solani</u> (L)	40.6	0	10.7	100
<u>C.praticola</u>	10.1	0	5.9	3.5
Nil	-	-	3.8	2.3

The two sets of culture filtrates were similar to the extent that the C.praticola filtrates were the least active, when enzyme preparations of this fungus, growing on each type of substrate were tested. This cannot be correlated with pathogenicity because C.praticola was the most virulent pathogen of the three fungi.

B - PG Activity

Table 33 compares PG(viscosity) activity of culture filtrates of the fungi grown on pectin and pectate, with

that of the extracts of seedlings infected with the fungi. The test substrates were pectin and pectate. The figures shown are a measure of the activity in terms of a 25% reduction in viscosity of the test substrate.

Table 33

Comparison of PG(viscosity) activity of culture filtrates of the fungi grown on synthetic substrates, with that of extracts of diseased hosts

Fungus	Test substrate	PG (Viscosity) activity			
		Growth substrates			
		Pectin	NaPP	Cabbage	Lettuce
<u>R.solani</u> (S)	Pectin	10	0	5	6
	NaPP	14	0	40	33
<u>R.solani</u> (L)	Pectin	64	0	7	2
	NaPP	100	0	50	100
<u>C.praticola</u>	Pectin	0	0	6	8
	NaPP	0	0	40	23

Each enzyme preparation was able to break down pectate more rapidly than they did pectin. None of the fungi produced active filtrates when grown on pectate. All the R.solani(L) filtrates were more active than those of the other two fungi. However, the pectin filtrate was able to degrade pectin, whereas the cabbage and lettuce ones were only feebly active. The pectin, cabbage and lettuce filtrates of R.solani(S) did not differ widely in their abilities to reduce the viscosity of pectin solutions, but the cabbage and lettuce filtrates reduced the viscosity of pectate

more rapidly than did the filtrates of this fungus grown on pectin. C.praticola filtrates from the two synthetic substrates were inactive, while those from cabbage and lettuce had some activity.

It can be concluded that PG(viscosity) activity varies for these fungi, depending on the medium used for their growth, activity of filtrates from synthetic media being different from those from diseased host plants. Neither of these sets of enzyme preparations showed a strict relationship with pathogenicity.

C - PG(Reducing groups) activity

Table 34 compares PG(reducing groups)activity of culture filtrates of the fungi grown on pectin and pectate, with that of extracts of diseased seedlings. The figures shown are a measure of the reducing groups produced in the first hour of the hydrolysis of glycosidic linkages in the test substrates, pectin and pectate.

From Table 34 it was clear that there was great variation in the ability of the enzyme preparations to produce reducing groups from both test substrates. With the exception of the R.solani(L) filtrate, which was more active than the extracts from diseased plants for this fungus, all the pectate filtrates were less active than any of the other enzyme preparations, tested on either of the substrates. Further, the extracts of diseased plants produced more reducing groups from pectate than filtrates of the fungi grown on pectin, whereas the extracts from diseased plants produced less reducing groups from pectin than the filtrates of the fungi grown on pectin.

Table 34

Comparison of PG(Reducing groups)activity of culture filtrates of the fungi grown on synthetic substrates, with that of extracts of diseased host plants

Fungus	Test substrate	PG(Reducing groups) activity			
		Growth substrates			
		Pectin	NaPP	Cabbage	Lettuce
<u>R.solani</u> (S)	Pectin	50	4	24	16
	NaPP	24	0	20	80
<u>R.solani</u> (L)	Pectin	100	10	0	0
	NaPP	60	2	60	100
<u>C.praticola</u>	Pectin	40	2	6	4
	NaPP	4	0	40	20

It was concluded that the substrates on which the fungi grow, can profoundly affect PG activity of the filtrates. Further, there is no strict correlation between the activity of filtrates from synthetic media and that of extracts of diseased host plants, and neither of these bear a strict relation to pathogenicity of these fungi to lettuce and cabbage seedlings.

D = Cellulase activity.

The basis of comparison of the activities of the culture filtrates of the fungi grown on synthetic media, with that of extracts of diseased hosts was the same as that described for PG activity. Table 35 compares cellulase(viscosity)activity for the two sets of enzyme preparations.

Table 35

Comparison of cellulase(viscosity)activity of the culture filtrates of the fungi grown on synthetic substrates, with that of extracts of diseased host plants

Fungus	Cellulase (Viscosity) activity			
	Growth substrates			
	CMC	WdC	Cabbage	Lettuce
<u>R.solani</u> (S)	75	0	44	63
<u>R.solani</u> (L)	100	60	17	70
<u>C.praticola</u>	50	13	12	100

C.praticola grown on CMC and lettuce plants produced active filtrates, but when grown on WdC and cabbage, they were very much less active. However, C.praticola parasitized both cabbage and lettuce, so that there cannot be a strict correlation between cellulase (viscosity) activity and pathogenicity.

Further, there was great variation in activity between the enzyme preparations obtained by growing the fungi on different substrates. This was particularly well illustrated by the filtrates of R.solani(L) and C.praticola.

Table 36 compares cellulase(reducing groups) activity of the culture filtrates of the fungi grown on CMC and WdC with that of extracts of infected seedlings. The figures shown are a measure of the reducing groups produced in the first hour of the hydrolysis of glucosidic linkages in CMC.

Table 36

Comparison of cellulase(reducing groups)activity of culture filtrates of the fungi grown on synthetic substrates, with that of extracts of diseased host plants

Fungus	Cellulase (Reducing groups) activity			
	Growth substrates			
	CMC	WdC	Cabbage	Lettuce
<u>R.solani</u> (S)	50	0	17	37
<u>R.solani</u> (L)	100	83	14	84
<u>C.praticola</u>	70	20	19	100

There was great variation in cellulase(reducing groups) activity with the different extracts of each fungus. Cabbage appeared to be a poorer substrate for enzyme production by each fungus than lettuce or CMC. On cabbage, there were no great differences in the amount of enzyme produced, as there were in the pathogenicity of the three fungi to this host. It is concluded that the substrates on which these fungi grow, have a marked effect on cellulase(reducing groups) activity, which in turn, cannot be directly correlated with pathogenicity.

28. Presence of pectic substances in host tissues

The presence or absence of pectic substances in hypocotyls of cabbage and lettuce seedlings was investigated by the histochemical methods described on page 43. These methods are not highly specific, in that a positive reaction does not necessarily mean that all the stained portions of the tissues consist of pectic

substances. A negative reaction, however, does indicate their absence. When sections of cabbage and lettuce hypocotyls were cut with a freezing microtome at thickness of 15 μ , and tested, positive reactions were obtained as expected.

The lettuce hypocotyls appeared to have a greater proportion of their cortical cell walls stained, than did the cabbage hypocotyls. The intensity of staining too was deeper in lettuce than in cabbage, and this suggested that the lettuce hypocotyls were composed of cells with a greater content of pectic substances in their walls than cabbage.

In the hypocotyls of both hosts, the outer cells of the cortex stained deeper than the inner cells, towards which there was a diminishing gradation in staining intensity. The fact that lettuce hypocotyl cell walls probably contained more pectic materials than cabbage might explain why generally higher pectic enzyme activities were observed in lettuce than in cabbage, but the validity of this conclusion must be subject to confirmation after further investigation.

29. Effect of enzyme preparations on host tissue

A - Culture filtrates of the fungi grown on synthetic substrates

The macerating activity of culture filtrates of the fungi grown on synthetic media were investigated as follows. Hypocotyls from 8 days old cabbage and lettuce seedlings grown under aseptic conditions, were detached and placed in sterile Petri dishes in the cell free culture filtrates of the fungi grown on sucrose,

pectin and pectato substrates, obtained as described earlier. The dishes were kept at room temperature and examined after various intervals. The results obtained, are shown in Table 37

Table 37

Maceration of cabbage and lettuce hypocotyls by culture filtrates of the fungi grown on synthetic substrates

Fungus	Growth substrate	Time taken for maceration (hours)	
		Cabbage hypocotyl	Lettuce hypocotyl
<u>R.solani</u> (S)	Pectin	12	12
	NaPP	48	48
	Sucrose	-	-
<u>R.solani</u> (L)	Pectin	6	6
	NaPP	48	48
	Sucrose	-	-
<u>C.praticola</u>	Pectin	12	12
	NaPP	-	48
	Sucrose	-	-

The sucrose culture filtrates of all three fungi did not macerate the hypocotyls even in 48 hours. The pectin culture filtrates of all three fungi macerated both cabbage and lettuce hypocotyls. The latter became very flaccid and gelatinous. Culture filtrates of R.solani(L) from pectin macerated both cabbage and lettuce hypocotyls within 6 hours. These observations in general, do not indicate any correlation between the macerating activity of the culture filtrates of the fungi grown on synthetic substrates and pathogenicity.

B - Extracts of infected host plants

Water extracts of cabbage and lettuce seedlings infected with the fungi were obtained as described on page 51. The extracts were sterilized by membrane filtration. The concentration of the extracts were adjusted by evaporation under reduced pressure, so that 100 ml. of the filtrate were equivalent to the extract from 1000 infected seedlings. These concentrated extracts were diluted in some treatments to varying degrees as indicated below.

Surface sterilized seeds were germinated on nylon net in test tubes under aseptic conditions as described on page 37. The extracts of infected plants were placed in the test tubes up to the level of the nylon net. After 14 days from the date of sowing, the seedlings were removed, and the lengths of their tap roots were measured. Dry weights of the roots and shoots were also determined. The conditions of temperature and illumination for the growth of the seedlings are given on page 37. In each treatment there were three replicates, each replicate consisting of one test tube containing 20 seedlings. The results of the experiments are shown in Tables 38 - 42.

From Table 38, it is clear that all the extracts had a marked effect on the lengths of the tap roots of cabbage plants. The tap roots became longer as the concentration of the extracts were reduced. Extracts of lettuce plants infected with the R.solani strains depressed the tap root lengths to a slightly lesser extent than did the corresponding extracts from infected cabbage plants. However, extracts from both types of plants infected with

C.praticola depressed tap root length to about the same extent.

Table 38

Effects of extracts of infected cabbage and lettuce seedlings on the tap root lengths of cabbage seedlings

Tap root length of cabbage seedlings in mm. The figures are the means for 10 seedlings, with the standard error for each mean. Figures in brackets are values relative to those for seedlings in water, which are made 100

Extract

Fungus	Host	Dilution - Extract : Water			
		1 : 0	3 : 1	1 : 1	1 : 3
<u>R.solani</u> (S)	Cabbage	0.6 ± 0.08 (0.8)	5.2 ± 1.1 (7.5)	9.0 ± 1.8 (13.0)	21.8 ± 2 (31.5)
	Lettuce	12.0 ± 1.4 (17.3)	22.2 ± 1.8 (32.0)	27.0 ± 2.1 (39.0)	59.0 ± 2.9 (85.2)
<u>R.solani</u> (L)	Cabbage	5.6 ± 1.4 (8.1)	8.0 ± 0.6 (11.5)	15.8 ± 1.1 (22.8)	22.4 ± 1.6 (32.3)
	Lettuce	15.8 ± 1.1 (22.8)	20.6 ± 1.7 (29.7)	24.2 ± 1.8 (34.9)	26.2 ± 6.3 (37.8)
<u>C.praticola</u>	Cabbage	0.6 ± 0.1 (0.8)	6.6 ± 2.0 (9.5)	17.2 ± 1.6 (24.8)	21.2 ± 0.9 (36.6)
	Lettuce	0.4 ± 0.08 (0.5)	9.0 ± 1.2 (13.0)	18.0 ± 1.2 (26.0)	29.6 ± 1.3 (42.7)
Water		69.2 ± 2.5 (100)	-	-	-

It is clear from these results that depression of tap root length by the extracts cannot be related to pathogenicity. The results for lettuce are shown in Table 39.

Table 39

Effect of extracts of infected cabbage and lettuce seedlings on the tap root lengths of lettuce seedlings

Extract		Tap root length of lettuce seedling in mm. The figures are the means for 10 seedlings, with the standard error for each mean. The figures in brackets are values relative to those for seedlings in water, which are made 100			
Fungus	Host	Dulution - Extract : water			
		1 : 0	3 : 1	1 : 1	1 : 3
<u>R.solani</u> (S)	Cabbage	1.8 ± 2.3 (3.5)	8.6 ± 1.4 (16.8)	12.6 ± 1.5 (24.6)	19.6 ± 1.0 (38.4)
	Lettuce	16.0 ± 1.9 (31.3)	19.6 ± 1.4 (38.4)	26.0 ± 2.0 (50.9)	33.0 ± 2.6 (64.7)
<u>R.solani</u> (L)	Cabbage	10.6 ± 1.9 (20.7)	12.6 ± 1.3 (24.6)	21.8 ± 2.4 (42.7)	26.0 ± 2.7 (50.9)
	Lettuce	11.4 ± 1.2 (22.6)	13.4 ± 1.0 (26.2)	19.6 ± 3.0 (38.4)	32.0 ± 2.0 (62.7)
<u>C.praticola</u>	Cabbage	5.6 ± 1.2 (10.9)	15.0 ± 1.7 (29.4)	21.2 ± 1.9 (41.5)	27.6 ± 2.0 (51.1)
	Lettuce	10.0 ± 1.0 (19.6)	14.6 ± 1.6 (28.6)	26.0 ± 2.3 (50.9)	33.2 ± 1.5 (65.0)
Water		51.0 ± 2.3 (100)	-	-	-

From Table 39 it is seen that each of the extracts had some effect on the length of the tap roots of lettuce seedlings, which were depressed to varying extents with increasing concentration of the extracts. Those of R.solani(S) grown on lettuce depressed tap root length to a slightly lesser extent than corresponding extracts of R.solani(L). On the other hand, extracts of

R.solani(S) grown on cabbage, depressed tap root length to a greater extent than did corresponding extracts of R.solani(L). These results are not related to pathogenicity.

Extracts of C.praticola grown on cabbage and lettuce, depressed tap root length to about the same extent. It is concluded that extracts of infected cabbage and lettuce plants are toxic to both cabbage and lettuce seedlings, but that the degree of toxicity bears no close relationship with pathogenicity.

The validity of these conclusions were tested further by the observations of the dry weights of the roots and shoots of cabbage and lettuce seedlings, growing aseptically for 14 days in extracts of diseased plants. The results are summarized in Tables 40 and 41. From Table 40 the following observations are made.

Cabbage plants

a) Effect of extracts on root growth

The extracts of both cabbage and lettuce plants not infected with fungus had no effect on the dry weight of cabbage roots. Of the R.solani(S) extracts, the one from cabbage depressed root growth more than did the one from lettuce. The extracts of lettuce plants infected by R.solani(L) however, depressed the growth of cabbage roots, whereas no inhibition was observed with the extracts of lettuce plants infected with this fungus. These results could be explained on the basis of the greater production of the toxic principle of each fungus on its normal, as compared with its resistant host. Extracts of both cabbage and lettuce plants infected

Table 40

Effect of extracts of cabbage and lettuce plants infected with the fungi on the growth of cabbage seedlings

Growth of cabbage seedlings in mg. dry weight of sets of 20 seedlings. Each figure is the mean of 5 sets, the standard errors of the means are given. The figures in brackets indicate values relative to those obtained by growing the seedlings in water, which are made 100

Extract		Root				Shoot			
Fungus	Host	Dilution-Extract : water				Dilution-extract : water			
		1:0	3:1	1:1	1:3	1:0	3:1	1:1	1:3
<u>R.solani</u> (S)	Cabbage	8.2	10.3	10.4	11.4	52.2	53.6	55.2	55.6
		±0.4	±1.1	±0.7	±0.1	±2.4	±3.1	±2.1	±0.4
		(74)	(93)	(94)	(103)	(92)	(95)	(97)	(98)
	Lettuce	9.2	10.1	10.6	11.2	53.4	55.2	54.8	56.7
		±0.3	±0.3	±0.1	±0.6	±2.2	±3.7	±1.4	±5.2
		(83)	(91)	(96)	(101)	(94)	(97)	(97)	(100)
<u>R.solani</u> (L)	Cabbage	11.0	11.6	11.7	11.6	58.8	60.0	59.8	58.8
		±0.1	±0.4	±0.7	±0.4	±1.3	±1.8	±2.9	±1.9
		(100)	(105)	(106)	(105)	(104)	(106)	(106)	(104)
	Lettuce	9.1	10.1	10.1	11.0	59.2	60.4	56.1	63.4
		±0.4	±0.2	±0.6	±0.4	±3.1	±7.2	±4.1	±4.6
		(82)	(91)	(91)	(100)	(104)	(107)	(99)	(112)
<u>C.praticola</u>	Cabbage	7.2	9.2	10.1	11.2	57.1	49.3	55.7	59.1
		±0.2	±0.4	±0.4	±0.3	±4.7	±8.6	±7.1	±6.1
		(65)	(83)	(91)	(104)	(101)	(87)	(98)	(114)
	Lettuce	7.6	10.0	10.2	11.0	49.4	50.6	50.3	53.6
		±0.2	±0.4	±0.5	±0.4	±2.2	±7.1	±8.7	±6.1
		(69)	(91)	(92)	(100)	(87)	(89)	(89)	(95)
Nil	Cabbage	11.2	11.2	11.1	11.1	57.6	57.4	59.1	58.2
		±0.4	±0.6	±0.7	±0.6	±3.4	±4.6	±5.1	±5.0
		(101)	(101)	(101)	(101)	(102)	(101)	(104)	(103)
	Lettuce	11.0	11.2	11.2	11.1	59.1	58.3	59.2	58.2
		±0.5	±0.7	±0.7	±0.5	±4.9	±3.1	±1.2	±2.9
		(100)	(101)	(101)	(101)	(104)	(103)	(104)	(103)
Water		11.0 ± 0.7 (100)				56.4 ± 2.4 (100)			

with C.praticola were more toxic to cabbage roots than any of the extracts of plants infected with either of the R.solani strains. This shows that C.praticola produces more toxin than the other two fungi.

b) Effect of extracts on shoot growth

The results with stems were not so striking as those obtained with roots. Uninfected cabbage and lettuce extracts had no inhibitory effect on growth, and in fact slightly stimulated growth. Extracts of both plants infected with R.solani(S) had a small depressing effect on shoot growth, whereas those of R.solani(L) had no such effect. Extracts of lettuce plants, but not those of cabbage, infected with C.praticola had an inhibitory effect on the growth of cabbage shoots.

From these results it can be concluded that each of the three fungi can produce toxins inhibiting the growth of cabbage shoots. The filtrates of C.praticola were the most toxic of the three fungi. In some cases the seedlings appeared to have been macerated, and were jelly-like at the bases of the hypocotyls. This indicates that the enzyme system may yet be responsible for this particular effect. Filtrates of R.solani(S) were more toxic to the roots than those of R.solani(L). Further, the least pathogenic strain, R.solani(L), had less effect on the growth of cabbage roots than either of the pathogenic strains. There is an indication here, of a connexion with pathogenicity.

The most concentrated extracts of C.praticola and R.solani(S) macerated the hypocotyls of cabbage seedlings. This

occurred with the extracts of both cabbage and lettuce plants infected with C.praticola, and with the extracts of only cabbage plants infected with R.solani(S). The hypocotyls were soft and gelatinous at the base, near the roots, and closely resembles plants which are damped-off in the normal way. Plate 48 shows some seedlings macerated by the extracts of lettuce plants infected with C.praticola.

Some of these plants were fixed in the formalin/acetic acid/ethanol mixture, embedded in wax, sectioned and stained as described on pages 39 to 43. The sections were examined for the presence of fungus as a contaminant, but were all fungus free. Plate 49 shows a portion of a transverse section of



Plate 48 - Cabbage seedlings macerated by the extracts of lettuce seedlings infected with C.praticola

a cabbage hypocotyl which had undergone maceration by the extracts of cabbage plants infected with R.solani(S). Some cells had been completely separated from the tissues, while others were only very loosely held together.

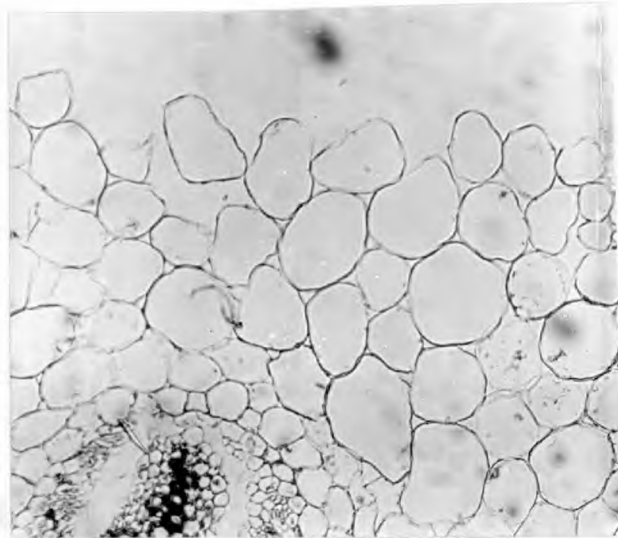


Plate 49 - T.S. cabbage hypocotyl macerated by the extracts of cabbage plants infected with R.solani(S) showing the separation of the cells after the degradation of the cell walls (X 160)

Lettuce plants

Table 41 shows the effect of extracts of infected cabbage and lettuce plants, on the dry weights of roots and shoots of lettuce seedlings.

Table 41

Effect of extracts of cabbage and lettuce plants infected with the fungi, on the growth of lettuce seedlings

Growth of lettuce seedlings in mg. dry weight of sets of 20 seedlings. Each figure is the mean of 5 sets. The standard errors for the means are given. The figures in brackets indicate values relative to those obtained by growing the seedlings in water, which are made 100

Extract		Root				Shoot			
Fungus	Host	Dilution-Extract : water				Dilution-Extract : water			
		1:0	3:1	1:1	1:3	1:0	3:1	1:1	1:3
<u>R.solani</u> (S)	Cabbage	2.7 ±0.2 (81)	3.2 ±0.4 (96)	3.3 ±0.4 (100)	2.8 ±0.6 (84)	14.1 ±1.4 (96)	16.0 ±1.0 (109)	15.6 ±1.3 (106)	14.6 ±0.9 (100)
	Lettuce	2.5 ±0.3 (75)	2.9 ±0.4 (87)	2.7 ±0.5 (81)	2.6 ±0.2 (78)	14.0 ±1.7 (95)	14.8 ±2.0 (101)	15.2 ±1.6 (104)	14.7 ±1.1 (101)
<u>R.solani</u> (L)	Cabbage	2.7 ±0.2 (81)	3.0 ±0.4 (90+)	3.2 ±0.2 (96)	3.4 ±0.2 (103)	14.0 ±1.8 (95)	13.9 ±2.2 (95)	14.2 ±1.4 (97)	15.4 ±0.9 (105)
	Lettuce	2.2 ±0.2 (66)	2.8 ±0.2 (84)	3.1 ±0.3 (93)	3.2 ±0.4 (96)	14.2 ±0.9 (97)	14.0 ±0.4 (95)	13.8 ±1.6 (94)	15.2 ±1.6 (104)
<u>C.praticola</u>	Cabbage	2.4 ±0.1 (72)	2.7 ±0.1 (81)	2.9 ±0.4 (87)	3.2 ±0.3 (96)	13.5 ±1.4 (92)	14.1 ±1.6 (96)	14.0 ±1.3 (95)	14.6 ±1.4 (100)
	Lettuce	2.2 ±0.3 (66)	2.5 ±0.4 (75)	2.8 ±0.4 (84)	3.2 ±0.2 (96)	14.2 ±1.2 (97)	14.3 ±1.6 (98)	15.8 ±1.1 (108)	14.7 ±0.9 (101)
Nil	Cabbage	3.4 ±0.2 (103)	3.3 ±0.1 (100)	3.4 ±0.2 (103)	3.3 ±0.3 (100)	14.6 ±1.4 (100)	14.7 ±1.2 (101)	14.3 ±1.2 (98)	14.6 ±2.0 (100)
	Lettuce	3.3 ±0.2 (100)	3.4 ±0.3 (103)	3.3 ±0.4 (100)	3.4 ±0.1 (103)	15.7 ±2.1 (107)	15.6 ±1.4 (106)	15.2 ±1.6 (104)	15.6 ±2.2 (106)
Water		3.3 ± 0.1 (100)				14.6 ± 1.7 (100)			

a) Effect of extracts on root growth

The extracts of both cabbage and lettuce plants uncontaminated with fungus, had no effect of the growth of the roots. Extracts of lettuce plants infected with R.solani(L), as well as those infected with C.praticola, were more toxic to the growth of lettuce roots than any of the other extracts. Here apparently, there is a connexion with pathogenicity. Both R.solani(L) and C.praticola parasitized lettuce seedlings, and produced extracts which were toxic to lettuce.

The extracts of cabbage plants infected with C.praticola were also toxic to the growth of lettuce roots. It can be assumed that this fungus, which parasitized cabbage as well as lettuce, produced the toxic principle on cabbage, and this was toxic to lettuce roots. The extracts of cabbage plants infected with R.solani(S), inhibited the growth of lettuce roots only to a very small extent. The extracts of lettuce plants infected with this fungus however, were more more toxic than the corresponding extracts from cabbage.

b) Effect of extracts on shoot growth

The effects of the extracts on lettuce shoots were much less than their effects on roots. The more concentrated extracts of both cabbage and lettuce plants, infected with each fungus had a slight inhibitory effect on shoot growth, whereas extracts from both uninfected hosts were not inhibitory. The inhibitory effects of the extracts of infected plants was a general effect, there being

no great differences between the inhibitions caused by the different extracts.

Again, as was observed in the case of cabbage shoots, some lettuce shoots showed clear evidence of maceration by the fungus extracts. Both R.solani(L) and C.praticola extracts from lettuce plants produced filtrates which caused maceration. Sections of these plants were examined for the presence of contaminants, but were found to be free of them. Plate 50 shows some lettuce seedlings macerated by the lettuce extract of R.solani(L).



Plate 50 - Lettuce seedlings showing hypocotyls macerated by the extracts of lettuce plants infected by R.solani(L)

The following general conclusions can be drawn from the experiments on the effect of extracts of infected host plants on young seedlings.

a) The extracts of cabbage and lettuce seedlings infected with the fungi were toxic to cabbage and lettuce seedlings, to varying extents,

whereas the extracts of cabbage and lettuce seedlings uncontaminated with fungus were not toxic.

b) There are indications that a fungus parasitizing its normal susceptible host, produces the toxic principles in greater quantity than when they parasitize less susceptible hosts.

c) At the concentrations used in these experiments, it appears that there is no highly potent specific toxin from any fungus, having a selective effect on either cabbage or lettuce. In other words, there are no indications to suggest that each fungus produces a toxic principle which is very different in its effects on either host, from toxic principles produced by the other fungi.

d) In most cases, root growth showed responses to a toxic principle, whereas the response on the part of the shoot was only just discernible. This may be due to the fact that the shoot consisted largely of hypocotyl and cotyledon, produced by the utilization of materials stored in the seed, and responses to external factors can largely be overcome by the presence of sufficient reserves for the growth of the shoot.

e) Although tap root lengths reduced with increase in concentration of the fungus extracts, it is unwise to attach too much importance to these results, because the observations of the tap root length do not accommodate the presence of laterals, which were, however, slow in being formed.

f) The toxic principle cannot entirely be separated from enzymes, (particularly the pectolytic and cellulolytic enzymes) and the

observations made above, may be a measure of enzyme activity, (which in turn could be connected with the degree of inactivation of enzymes) rather than a completely separate toxin.

g) Although, as stated earlier, a fungus tends to produce a more efficient toxic principle when growing its susceptible host, the effects of the various extracts on cabbage and lettuce seedlings cannot be strictly correlated with pathogenicity.

C - Effects of autoclaved extracts of infected cabbage and lettuce plants on the growth of cabbage and lettuce seedlings

The procedure adopted in this series of experiments was the same as that described for the preceding ones, except that the extracts were autoclaved for 20 minutes at 15 p.s.i. pressure. It was hoped that the pectic and cellulolytic enzymes would be completely inactivated by the autoclaving, and that the possible effects of a heat stable toxin could be observed.

Table 42 shows the effects of the autoclaved extracts on the dry weights of roots and shoots of cabbage and lettuce seedlings. From these results it is clear that the autoclaving had a profound effect on the toxicity of the extracts, none of which were as toxic to either cabbage or lettuce, as the unautoclaved extracts. It can be concluded therefore, that either the toxicity of the culture filtrates was due to the presence of thermolabile enzymes, which were themselves toxic, or that there were toxins present in the extracts, which were quite distinct from the enzymes, and that these toxins were themselves inactivated or destroyed during the autoclaving.

Table 42

Effects of autoclaved extracts of infected cabbage and lettuce seedlings on the growth of cabbage and lettuce seedlings

Growth of seedlings in mg. dry weight of sets of 20 seedlings. Each is the mean of 5 sets; standard errors for each mean are given; The figures in brackets relative to those obtained by growing the seedlings in water, which are made 100

		Cabbage				Lettuce			
Extract									
Fungus	Host	Root		Shoot		Root		Shoot	
		conc	dil	conc	dil	conc	dil	conc	dil
<u>R. solani</u> (S)	Cabbage	11.2	11.1	55.7	53.6	3.2	3.2	15.2	14.6
		± 0.4	± 0.6	± 3.6	± 4.1	± 0.4	± 0.6	± 1.8	± 1.0
		(101)	(101)	(98)	(95)	(96)	(96)	(104)	(100)
	Lettuce	10.6	11.0	55.2	55.6	3.3	3.2	14.8	14.6
		± 0.6	± 0.4	± 2.0	± 3.0	± 0.4	± 0.4	± 2.0	± 2.1
		(96)	(100)	(97)	(98)	(100)	(96)	(101)	(100)
<u>R. solani</u> (L)	Cabbage	11.0	11.2	59.2	56.1	3.4	3.2	14.3	14.7
		± 0.6	± 0.4	± 1.8	± 4.0	± 0.6	± 0.2	± 1.8	± 2.0
		(100)	(101)	(104)	(99)	(103)	(96)	(98)	(101)
	Lettuce	11.4	11.2	57.1	58.8	3.1	3.3	15.6	14.8
		± 0.4	± 0.6	± 2.2	± 3.6	± 0.8	± 0.4	± 3.0	± 2.2
		(103)	(101)	(101)	(104)	(94)	(100)	(106)	(101)
<u>C. praticola</u>	Cabbage	10.9	11.1	58.2	55.7	3.4	3.3	14.7	15.2
		± 0.6	± 0.4	± 4.0	± 2.6	± 3.3	± 0.4	± 1.7	± 1.6
		(99)	(101)	(103)	(98)	(103)	(100)	(101)	(104)
	Lettuce	11.4	11.2	57.4	58.2	3.1	3.2	14.6	14.3
		± 0.4	± 0.6	± 3.0	± 2.0	± 0.8	± 0.4	± 1.2	± 1.8
		(103)	(101)	(101)	(103)	(94)	(96)	(100)	(98)
Nil	Cabbage	10.9	11.6	57.6	56.3	3.3	3.2	14.7	14.2
		± 0.4	± 0.6	± 3.0	± 2.0	± 0.6	± 0.4	± 1.4	± 1.9
		(99)	(105)	(102)	(100)	(100)	(96)	(101)	(97)
	Lettuce	11.2	11.0	59.0	56.4	3.4	3.2	15.2	14.6
		± 0.2	± 0.4	± 2.6	± 2.2	± 0.4	± 0.4	± 2.0	± 1.4
		(101)	(100)	(104)	(100)	(103)	(96)	(104)	(100)
Water		11.0 $\pm$ 0.7		56.4 $\pm$ 2.2		3.3 $\pm$ 0.2		14.6 $\pm$ 1.7	
		(100)		(100)		(100)		(100)	

conc - Extracts were used undiluted

dil - Extracts were diluted with an equal volume of water

DISCUSSION

The present work sought to inquire into the nature of the process of parasitism of various plants , by different strains of R.solani and a related species, and was directed in particular to the causes underlying the specificity of parasitism shown towards different hosts by these fungi. The ability of the plants to resist parasitic attacks, depends on their having a satisfactory defence mechanism, or on their ability to cause the breakdown of the parasite's attacking mechanism. The latter could conceivably take place either early, or late in the parasitic process. in different host-parasite combinations.

The specificity of parasitism for the host-parasite combinations used in the present work, was very clearly defined both in experiments carried out in vitro, and in soil. Shephard (1957), found that environmental conditions had only a limited effect on this specificity, and the results obtained in the present work confirm these findings, because although the environmental conditions were the same for each host-parasite combination in the different experiments, they were not identical in the soil experiment and the in vitro experiments. Nevertheless, the pattern of specificity of parasitism, was the same in all.

No evidence was obtained that suggested a relationship between pathogenicity and the cultural features of the isolates. Compared with the other two fungi, R.solani(S) grew more

slowly on each of the media used, yet it never failed to damp-off cabbage seedlings in a number of conditions, whereas R.solani(L) failed to do so although it grew nearly twice as fast as did R.solani(S) on a variety of media. Again, although R.solani(L) and C.praticola had very similar growth rates under a variety of conditions, and both these fungi parasitized lettuce to about the same extent, R.solani(L) did not kill cabbage whereas C.praticola did. None of these facts can be explained in terms of the behaviour of the three fungi in culture.

The possibility that the host influences the parasite before contact was made between the two was suggested by Kerr, (1966), as a result of his studies on the effect of root exudates from radish, lettuce and tomato plants on the growth of R.solani. Fungus growth was stimulated only by radish and lettuce root exudates but not by exudates from tomato roots. Radish and lettuce were susceptible to attack by R.solani, but tomato was resistant. It appeared that the growth of the fungus was stimulated only by exudates from plants that it could parasitize. He suggested that host exudates "may affect the ability of a fungus to "find" a suitable host" in the sense that growth will be stimulated in the region of tissues that the fungus could later parasitize.

If exudates from resistant plants were also stimulatory, this growth would be wasted. A further point is that these effects occur only because the root is essentially a leaky structure compared with the other parts of the plant. Flentje, (1957), claimed that substances exuded by the roots of certain plants play a part in determining whether or not strains of R.solani will parasitize the plants, and that a chemical stimulus alone, or in conjunction with a contact stimulus is important in the specificity of parasitism. Kerr and Flentje, (1957), believed that there was a close correlation between the susceptibility of the seedlings to R.solani, and stimulation of the pathogens by the seedlings. An investigation of these claims was a major object in the present work.

The fungi which were used attacked their hosts through the hypocotyl, and were presumably comparable to those used by Kerr and Flentje (1957), although they did not say explicitly that their plants were parasitized in this way. Exudates from cabbage roots grown in water culture under sterile conditions did stimulate the growth of R.solani(S) which parasitizes cabbage, but they also stimulated the growth of R.solani(L) which, at the most, was only a very weak parasite of this host.

Similarly, lettuce root exudates stimulated the growth of R.solani(S) which did not parasitize lettuce, as much as they stimulated the growth of R.solani(L) which actively parasitized this host; so that these two strains, highly specific in their

parasitism of cabbage and lettuce, there was no indication of a specific relationship between susceptibility of a host to infection and colonization, and stimulation of growth from exudates from the roots. These results clearly conflict with those of Kerr and Flentje (1957), so far as stimulation of growth is concerned.

There are, of course, differences in the two sets of experiments. There is the obvious one that different fungi were used, and there is the subsidiary one, that in the present work, the plants were grown with their roots in water, whereas Kerr and Flentje grew them in sand. Although they did not state specifically that only water was used, it seems safe to assume that this was so.

It might be argued that these results support the hypothesis of Kerr and Flentje to the extent that exudates from the one plant resistant to the three fungi, did not stimulate the growth of any of them. On the other hand, the results shown in Table 2 indicate that C.praticola did kill an appreciable number of wheat seedlings before emergence, whereas the other two fungi did not. Here too, ability to parasitize was not related to the stimulation of growth of the parasite by root exudates.

An obvious deficiency in the present work was that only water was used to grow the seedlings. This however, makes these results comparable with those of Kerr and Flentje, who themselves used water and not nutrient. The use of water instead of nutrient probably explains why only exudates from very young seedlings of cabbage and lettuce were stimulatory. After the initial

period of growth, in which reserves within the seed were being used, the supply of nutrients in one form or another probably became limiting. If nutrients had been used, the stimulatory effects might have persisted longer. On the other hand, the use of very young seedlings is not too unrealistic because this is the type of plant most readily attacked by each of the three fungi.

A further point of criticism of the present work would be implied by the question, what is the point of studying the effect of root exudates on the growth of fungi that attack through the hypocotyl? Some arguments to justify the use of root exudates are that if the roots exude substances with specific effects, this might suggest that the hypocotyls from the same plant would do so also; and that if the growth of the fungus is stimulated by exudates at the root surface, the effect might persist, and influence the behaviour of the fungi at the hypocotyl.

Contrary arguments are that even if the roots did exude substances with specific activity, it would always be necessary to show that these substances were exuded by the hypocotyl, or that their effects on fungi growing at root surfaces persisted, and influenced the behaviour of the fungi at the surface of the hypocotyl. The fact is that the place to look for substances having formative effects on the hyphae of fungi invading through the hypocotyl, is the hypocotyl itself. This is, of course, technically very difficult.

In the present work, an attempt was made to obtain some effect of hypocotyl exudates, but the method was not ideal, if, for no other reason, that any hypocotyl effects would most

probably have been swamped by those of the roots. The results obtained with the experiments with, and without hypocotyl exudates did not differ significantly.

In fact the effect of root exudates on the fungi used in the present work, appears to be no more than a "rhizosphere effect", in which stimulation of growth was not general but specific.

Flentje, (1957), stated that some strains of R.solani generally attached themselves to, and grew along the lines of junction of the epidermal cells. He found that a stem-attacking strain exclusively followed these lines of junction. The three strains used in the present work behaved in this characteristic manner on the surface of each host. It was found, however, that the characteristic orientation was not confined to the lines of junction between the epidermal cells, and the hyphae were often observed to grow longitudinally along the cell surfaces, as well as along the lines of junction of cells.

Further, Flentje, (1957), stated that strains not pathogenic to a host, fail to become attached to, or organize on, the stems. In the present work however, it was found that all strains attached to, became organized on, and even produced infection cushions on the surface of each host. There was, however, some correlation between the amount of organization, both in terms of orientation of the hyphae as well as infection cushion formation and, pathogenicity. It appears then, that there are wide differences

in the capacity of different strains to organize on host surfaces.

Kerr and Flentje, (1957), suggested the hypothesis that "attachment to, organization on, and penetration of a host by the radish strain of P.filamentosa (R.solani), is controlled by the nature of the cuticle surface, and the presence of a diffusible material which is excreted onto the cuticle surface from the underlying cells". They found that structures resembling infection cushions were formed on cellophane in response to radish root exudate by a radish strain of R.solani, but not by other strains of this fungus.

They also found that hyphae of the radish strain, became attached to, organized on, and penetrated radish epidermis strips (presumably of the hypocotyl) moistened with radish root exudate, whereas on control strips with no exudate, attachment and growth along the lines of junction of cells occurred, but no infection cushions were formed, and no penetration took place.

In the present work, it was found that apart from the failure to stimulate fungus growth in a specific way, root exudates had no effect on the formation of infection cushions, which were produced on each type of epidermis by each fungus with the same frequency for each fungus-epidermis combination, with or without root exudate, from any of the three hosts. These results are therefore in conflict with those of Kerr and Flentje (1957), with respect to specificity of the effects of root exudates on

stimulation of fungus growth, as well as the effects of root exudates on infection cushion formation on strips of hypocotyl (or coleoptile) epidermis.

The present results, however, confirm the findings of Kerr and Flontje (1957), that the nature of the cuticle surface was important in the behaviour of R.solani on plant surfaces. These workers found that on damaged stems of radish, no infection cushions were formed on punctured areas, and they always formed on intact cuticle surfaces. This fact, together with the evidence that cellophane was not penetrated by their radish strain, indicated the importance of the contact stimulus in organization on, and penetration of a surface by R.solani. The results obtained in the present work, produced much evidence in support of this hypothesis.

Each of the three fungi used here, formed characteristic structures on non-living surfaces, and although they were not identical with infection cushions in appearance, they were evidently adhesive in function. The term "contact cushions" is proposed for these structures. The contact cushions formed on different surfaces were not identical in appearance, varying in structural details. This could possibly be correlated with differences in the configuration of the different surfaces. There can, of course, be no question that contact cushions only in response to particular substances, because the surfaces had been thoroughly cleaned, and were almost certainly chemically inert.

Flentje (1957), did not observe his strains of R.solani to form contact cushions on either glass, collodion, cellophane, cotton or other non-living surfaces. He cited this as evidence of the inadequacy of the contact stimulus theory to explain the behaviour of a strain of R.solani, and, with the other evidence on root exudates discussed above, brought in the chemical aspect to explain it. These criticisms are inapplicable to the strains used in the present work.

Returning to the host plants, there is no doubt that the different fungi reacted differently to different hosts, and it has been shown that those differences are not related in specific ways to exudates from the roots of the hosts. It could, of course, be argued that selective action on the fungi might have been due to some substances not extracted from the hypocotyls, by the techniques used, but this selectivity could instead depend on differences in the configuration of the surfaces of the different hosts.

Because it was found that there was some correlation between pathogenicity, the degree of organization of the fungi on the host surfaces, and the frequency of infection cushion formation, it might appear that these observations discredit the contact stimulus hypothesis. On the contrary, they provide some evidence for it, and in view of this, the work on the behaviour of the fungi on replicas of surfaces of hypocotyls and coleoptiles was undertaken.

Under the electron microscope, the preparations of the host surfaces show, that while there are differences in the detailed structure of the cuticles, there are also common features. The latter include the absence of pores (apart from stomata), and the presence of longitudinally arranged ridges on the surfaces of cabbage and lettuce hypocotyls and wheat coleoptiles. The dimensions of these ridges varied from host to host. It was known from other observations, that not only do the hyphae follow the lines of junction between epidermal cells, but that they also grow longitudinally along the faces of the cells, at some distance from the lines of junction. This behaviour could be conditioned by the longitudinal ridges seen under the electron microscope. Comparisons of the dimensions of the ridges with those of the hyphae indicated that this was well within the realms of possibility.

In the light of the above, the following is suggested to explain the behaviour of the hyphae after they have come in contact with the epidermis of the host. The growing points of the hyphae are directed both along the lines of junction of the epidermal cells, and longitudinally, along the faces of the cells, adjacent to the ridges on the surface of the cuticle. Because this orientation took place on replicas as well as on the epidermis itself, it is unnecessary to postulate that it depends in any way on substances exuding from the host and accumulating on the surface.

It can, of course, be argued that the replicas may not have been perfect, and this argument derives some

weight from the fact that although the hyphae did become orientated on the replicas, infection cushions similar in appearance to those found on the intact host surfaces were not formed.

There is another point of interest regarding the effects of host exudates on the fungi. This is implied by the question, what significance can host exudates have on a well-nourished fungus, deriving its nutrient from a substantial external inoculum? In some experiments, the fungi were very well nourished from an agar disk inoculum before they came into contact with the host, but the behaviour of different fungi on host surfaces still differed greatly. It is difficult to imagine how exudates from the host could have caused any further stimulation of fungus growth because this was already luxuriant.

There is however, the possibility that the effect of plant exudates was on the type of branching of the hyphae, rather than on growth in general. This would seem to be the only way in which exudates from hosts could act specifically, i.e. by having formative effects on the parasite; so that the type, but not the amount of growth was altered.

In this connexion, the results of two sets of experiments were interesting. Firstly, host exudates were found to have no effect on the numbers of infection cushions formed on epidermal strips from the host, and secondly, the hyphae branched repeatedly when contact cushions were formed on non-living surfaces, in the absence of any plant product.

These findings stand in contrast to those of Kerr and Flentjo (1957), who state that host exudates did have an effect on infection cushion formation, and whose work is supported to some extent by the findings of Cannon and Corbett, (1962), and Dodman and Atkinson, (1962). There is also the further point that neither Flentje, (1957), nor Kerr and Flentje, (1957), report the formation of contact cushions on non-living surfaces in the absence of substances of host origin. The present work is in conflict with these observations as well.

Further evidence as to the significance of the contact stimulus was obtained by eliminating the surface configuration of the hosts, by covering the cuticle with an inert substance, such as collodion. Infection cushions were never formed under these conditions. It could be argued here that the film prevented host exudates from reaching the fungus, so in further experiments, the hypocotyls and coleoptiles were washed with organic solvents. It was assumed that these treatments would remove most of these substances which may have diffused through the cuticle onto the surface from the underlying epidermal cells. After the treatments, the surfaces were still found to be suitable for attachment and organization of the hyphae.

This again, would seem to reduce the significance of host exudates in the formation of infection cushions. It could be argued that the washings did not completely

remove all traces of diffusible substances, and that the remaining traces could have brought about specific effects, but this would seem unlikely.

Finally, the experiments of Kerr and Flentje (1957), with damaged cuticles, and pieces of the cuticle removed, resulted in the formation of infection cushions only on the intact cuticle, and not where it had been damaged or removed. All the evidence presented above indicated the paramount significance of the contact stimulus, and it appears that the chemical stimulus may be of lesser significance than Kerr and Flentje (1957), believe it to be.

Penetration and infection cushion formation appeared to be closely linked. Roots and cotyledons of each of the three hosts were not penetrated, and infection cushions were not formed on their surfaces by any of the fungi. The only leaves that were penetrated were those of wheat by C.praticola. Here again, infection cushions were produced. Each fungus produced infection cushions on wheat coleoptile surfaces and penetrated coleoptiles from them.

Although the formation of infection cushions always preceded penetration, it often happened that structures which were not penetrated from the outside by the fungi, were parasitized. Thus hyphae grew into the cotyledons of cabbage and lettuce from the hypocotyls, which were penetrated directly. The surface configuration of the cotyledons might be important in this connexion because if the texture of the cotyledon surface is very different from that of the

hypocotyl, attachment might not take place, and the formation of infection cushions may be prevented. The presence of wax or other substances on the cotyledon surface could account for this.

Although cotyledons were not penetrated because the requirements for the formation of infection cushions may have been lacking, a similar explanation does not account for the fact that penetration occurred only from some, and not all infection cushions, produced on the surfaces of non-susceptible hosts. No definite conclusions could be drawn about these, because some of the infection cushions from a which there was no penetration, were better developed than some from which there was penetration. Further investigations along these lines may prove to be profitable.

The surface of a host was not the only critical barrier which determined the specificity of parasitism. This was clear from the fact that although each fungus did penetrate each of the three hosts, they did not invade each type of host tissue equally rapidly. That there were differences between fungi in the extent, speed, and direction of invasion, was immediately evident after penetration.

Another variable factor was the amount of mycelium entering the cortex from infection cushions, after the surface of the hosts had been breached. Although penetration may have an effect on all these factors, it appears to be indirect. It seems therefore, that what occurs after penetration is the

significant factor in determining whether or not a seedling will be damped-off.

The studies on the invasion of the host tissues provided some information regarding this aspect. The pathogenic strains invaded the tissues of susceptible hosts, and the hyphae reached the stele in a short time, relative to the speed of invasion of less pathogenic strains. The time taken for the hyphae to reach the stele, therefore, appears to be an important factor. If the conducting tissues are not damaged, then the plant would live, because the roots can obtain their source of energy, and the shoots, their moisture and mineral requirements. The plant would continue to grow, even though the cortex is infected, and would eventually become resistant by virtue of their age (Shephard 1957).

Investigations on the post-penetration stages of parasitism revealed that this was exactly what did happen. The hyphae of pathogenic strains reached the stele of susceptible plants very rapidly. There were indications that the hyphae grew more laterally than longitudinally. In other words, the invading hyphae tended to grow towards the stele rather than towards the cotyledons or roots. This factor, together with differences in the speed of invasion, could explain why some plants are not damped-off.

The question arises as to what causes these differences in the rates and direction of invasion of different hosts by different parasites. One possibility that must be considered is the occurrence of a hypersensitive reaction, which Flentje (1957), reported for a strain of R.solani, where the presence of the fungus caused the adjacent cells of the cortex to produce a hypersensitive reaction, resulting in the death of the hyphae, presumably by the production of one or more substances toxic to the fungi. In the present work there was evidence that hypersensitive reactions sometimes occurred because cells around some lesions were discoloured, and the hyphae had apparently stopped growing. However, it could not be demonstrated that the substances produced in the hypersensitive reaction were fungicidal, because the fungus grew when dissected out from the lesions and planted on agar.

It was concluded that the hypersensitive reaction resulted in fungistasis. It is clear therefore, that the nature of the hypersensitive reaction of different hosts to different strains of R.solani is variable. Sometimes, as was observed in the present work, it resulted in fungistasis, whereas, for the host-parasite combinations such as used by Flentje (1957), the effect was fungicidal.

Host resistance in the present work could not be explained entirely on the basis of hypersensitivity, because the hyphae of weakly pathogenic strains were often not affected by the hosts' hypersensitive reaction, and continued to advance into the

tissues. Extracts of the host plants were tested for the presence of toxic substances, which might have retarded the growth of the less pathogenic strains. Heat sterilized decoctions and membrane filtered homogenates of host tissues were used, to test their influence on the growth of the fungi.

There was no clear evidence of a specific toxin being produced in the tissues of more resistant hosts. All the decoctions and homogenates stimulated the growth of each fungus but the increase in each case was not proportional to the increase in concentration of the extracts. This could mean that inhibitors were present, but that their effects were swamped by the growth promoting effects of the extracts.

If toxins were present in sufficient concentration, and if they were highly potent, then it would have been expected that fungus growth would increase with increasing dilution of the plant extract. This, however, was not observed. Further investigations along these lines could be rewarding.

Some interesting observations were made on the parasitism of wheat by the R.solani strains, and more particularly by C.praticola. The latter fungus invaded wheat tissues more rapidly than did the two R.solani strains. There appeared to be a delicate balance between the rate of invasion by C.praticola and the rate of production of new leaves (and leaf sheaths) by the wheat plant. It was found that when C.praticola had reached the inner epidermis of the

coleoptile, the wheat plant had produced a leaf sheath which had to be parasitized before the plant could be killed. When C.praticola had parasitized the first leaf sheath, the plant had meanwhile produced a second, and possibly a third leaf sheath.

The ability of a plant to continue growing must depend on its conducting tissues remaining intact. It is not too difficult to imagine how this happens, because the vascular elements in wheat are diffused throughout the plant, and are not concentrated centrally as in cabbage and lettuce. If the fungus could not destroy all the vascular elements, then both roots and shoots would continue to grow and provide more barriers to the fungus until it is old enough to acquire resistance. These considerations, however, are not the only ones involved, because they do not explain why the growth of each of the fungi inside the tissues of the wheat plant was slow in the first place.

The parasitism of wheat, although somewhat different from that of the other hosts, does not provide much information as to what factors tend to influence the rate of invasion of a host by a pathogen. One of these factors must undoubtedly be associated with the ability of the fungi to push their way through the host tissues. This could be accomplished either by breaking down the cells that the fungus comes in contact with, or, by passing in between the cells, or by a combination of both these methods.

Accordingly, the ability of the fungi to produce cell wall degrading enzymes was investigated. Barker and

Walker (1962), grew several isolates of R.solani on synthetic media and tested the culture filtrates for PME, PG and cellulase activity. They stated that foliage strains showed higher PME and PG activities than did root or stem isolates. They also claimed that there was a correlation between pathogenecity and PG activity of extracts of the fungi grown on synthetic media.

It is known that R.solani and related species can grow in a wide range on hydrogen ion concentration. In the present work, all the experiments on the action of cell wall degrading enzymes were carried out at a pH of 5.7. Each fungus grow satisfactorily at this pH. Because the effects of a wide range of hydrogen ion concentration were not investigated, these results are subject to the criticism certain possible subsidiary effects of pH have been ignored.

When the fungi were grown on pectin, the filtrates of each fungus showed some PME activity. This did not happen when the fungi were grown on pectate. This shows that PME production by these fungi is adaptivo. PME activity was least in filtrates of C.praticola, which, however, was the most pathogenic fungus of the three. If it could be argued that the pH requirements were not suitable for PME production by C.praticola, then the supplementary question arises as to why the pH was suitable for PME production by the other two fungi. It appears more likely that there is no direct relation between PME activity of the culture filtrates of the fungi grown on synthetic substrates and pathogenicity. Clearly, if there was such a correlation, then the filtrates of C.praticola would be

more active than those of the other two fungi.

Similar results were obtained in the experiments on PG activity. Again, pectin was a better substrate for PG production than pectate, by each fungus. C.praticola again produced filtrates which were less active than those of the other two fungi. These results cannot be correlated with pathogenicity. It appears that the substrates on which the fungi were grown have a profound effect on the production of pectolytic enzymes, although actual amount of growth is not directly related to enzyme activity. Barker and Walker (1962), stated that they did find a correlation between PG activity and pathogenicity, but their conclusions in this respect can be questioned because they depend on assessments of pathogenicity, in terms of an index, which is itself quite arbitrary.

Even if there were a correlation between the PG activity of the filtrates and the pathogenicity index, this is still somewhat unrealistic, because it is necessary to demonstrate the presence of these enzymes in host tissues, particularly because PG activity varies when fungi are grown on different substrates. The fact that PG is produced in culture, does not necessarily mean that it is produced in the tissues of a host by a fungus. The significance of a correlation as found by Barker and Walker (1962), therefore, is rather obscure in relation to the process of parasitism.

There was some variation in cellulase activity of the culture filtrates of the fungi, depending on the substrates on which they were grown. Again, as was observed for pectolytic enzymes,

there was no good correlation between cellulase activity of the filtrates, and pathogenicity of the fungi to cabbage and lettuce. These results are in agreement with those of Barker and Walker (1962), who did not find any correlation between cellulase activity and a pathogenicity index

The fact that no quantitative relation has been observed between pathogenicity and the enzyme activity of the filtrates from cultures on synthetic media could mean no more than that the host tissues are very different from synthetic substrates, both for fungus growth and the production of cell wall degrading enzymes. Accordingly, the investigation of the enzymes present in diseased host tissues was the next logical step.

Bateman (1963), has pointed out that few attempts have been made to demonstrate whether the cell wall degrading enzymes produced by a pathogen in vitro and those found in vivo are similar or different. He attempted to study this, and found that the pectolytic enzymes produced by R.solani in culture are not the same, and do not act on their substrates in the same way as those associated with Rhizoctonia infected bean tissues.

In the present work it was found that for each of the enzymes investigated, (PME, PG and cellulase), there was no good correlation between the enzyme activities of the culture filtrates of the fungi grown on synthetic substrates, and the activities of extracts of host plants infected with the fungi. It is clear, therefore, that enzyme activities vary considerably with the substrates on which the fungi grow. These results are in agreement with those of Bateman(1963).

There is, however, a good qualitative similarity between the two sets of enzyme preparations, because the same enzymes were produced in culture on synthetic substrates, and when these fungi parasitized host plants. It was found that some extracts of host plants infected with pathogenic fungi were more active than the extracts of the same plant infected with a non-pathogenic fungus. There might seem to be a correlation here, with pathogenicity; but this may be merely because the pathogenic strains grew more vigorously. In other words, the effect may be the result of, and not the cause underlying pathogenicity.

If pathogenicity were directly related to the enzyme activities of the extracts of diseased plants, then it would have been expected that the extracts of plants infected with C.praticola would have been more active than those of plants infected with the other two fungi. This, however, was not observed except in one or two isolated instances. It was concluded that there was no quantitative relationship between the activity of cell wall degrading enzymes present in extracts of diseased plants, and pathogenicity.

In spite of the above facts, it still seems likely that cell wall degrading enzymes play some part in the parasitism of the plants by these fungi if only because each fungus did produce pectolytic and cellulolytic enzymes when they parasitized the seedlings. Further, each fungus progressed in between cortical cells, and also penetrated them, so there can be no doubt that the cell wall degrading enzyme systems are important. Their precise mode of action

needs further investigation. It could be that these systems are inactivated in some ways by host tissues, or that their effects are swamped by other factors.

The toxicity of extracts of cabbage and lettuce seedlings infected with the fungi, to the roots of young seedlings has been clearly demonstrated. Shoots were not markedly sensitive, probably because, up to 14 days from germination, they might still have been dependent to some extent on the reserves in the cotyledons or the products of photosynthesis of the cotyledons. Another factor which might possibly be even more important is that the toxic principle might not have been translocated sufficiently quickly to the upper portions of the shoots. This seems quite likely because in treatments where highly concentrated extracts were used, the base of the hypocotyl was often macerated, whereas the upper parts of it were still healthy.

Further, some seedlings which were in contact with the extracts up to the level of the cotyledons, were completely macerated. It is significant that the concentrated extracts macerated the plants at the base of the hypocotyls. The appearance of hypocotyls damaged in this way was very similar to plants damped-off by the fungi in the normal manner. It would appear that the factor responsible for damping-off is extractable, and further studies with these extracts, and attempts to characterise the substances involved may provide useful clues about their role in parasitism.

It is of course recognized that the cause of maceration, and eventual damping-off might consist only partially of enzymes. Although there was no good correlation between pathogenicity and the effect of the extracts on the young seedlings, there were indications that the toxic principle was produced in greater abundance when the fungi parasitized their normal susceptible hosts. It is possible that the differential effects might have been due to this fact.

Although the possibility is not entirely overruled, it appears to be very unlikely that a highly specific toxin is involved, because there is no indication of its selectivity, or indeed, that the toxins are highly potent. The thermolability of the toxic principle has been demonstrated. It might, of course, be that the toxic principle was entirely enzymic in nature, but at present, there remains the possibility that there are other thermolabile toxic substances in the diseased plants. Further work in this connexion should prove profitable.

SUMMARY

1. Determination of pathogenicity

The pathogenicity of three fungi to cabbage, lettuce and wheat seedlings was investigated both in experiments in soil, and under sterile conditions. R.solani(S) caused severe damping-off of cabbage, but failed to damp-off lettuce seedlings. R.solani(L) was severely pathogenic to lettuce, but did not damp-off cabbage seedlings.

C.praticola parasitized both cabbage and lettuce, causing rapid damping-off. Wheat was parasitized only to a very limited extent by each fungus. Specificity of parasitism was clearly defined, and was not altered by environmental conditions.

2. Effect of host exudates

A = On fungus growth

a) Root exudates from cabbage stimulated the growth of each fungus by 30 - 40 %, and those from lettuce did so by 120 - 150 %. Wheat root exudates were hardly stimulatory. Both pathogenic, and non-pathogenic fungi were therefore stimulated to about the same extent by each root exudate, and there is therefore, no correlation between the stimulation of fungus growth by exudates and pathogenicity.

b) Exudates from roots, hypocotyls and cotyledons had almost the same stimulatory effects as exudates from roots alone. This stimulation too, cannot be correlated with pathogenicity.

c) Exudates collected in the first week from each host were stimulatory, but this dropped sharply in the second and third weeks. It appears that

that the biggest stimulatory effects were obtained with younger seedlings.

B - On the formation of infection cushions

The addition of host exudates to washed epidermis strips had no effect on the number of infection cushions formed by any fungus, or on the size and development of these cushions. Host exudates are therefore non-specific in their mode of action, which is not correlated with pathogenicity.

3. Behaviour of the fungi on the host surfaces and on other surfaces

A - Host surfaces

a) Each fungus produced infection cushions on the hypocotyls, but not on roots, cotyledons and leaves of cabbage and lettuce seedlings.

They also produced infection cushions on wheat coleoptiles, and

C.praticola formed them on wheat leaf sheaths.

b) The number and degree of complexity of the infection cushions was variable, but they were generally more abundant and better developed on susceptible host surfaces.

c) Each fungus grew along the lines of junction of the epidermis cells, and also longitudinally, along cell faces, parallel to these lines of junction.

d) Each fungus became orientated on dried epidermis strips, and also on strips treated with organic solvents, as well as water, to remove any host exudate that might have been present.

e) Host surfaces were covered with thin films of plastic materials to destroy the surface configuration. No orientation of the hyphae were observed under these conditions. The surface configuration appears,

therefore, to be important in this respect.

B - Non-living surfaces

a) 'Contact cushions' were formed by R.solani(S) on cellophane, and by both R.solani(L) and C.praticola on glass and polystyrene surfaces. These were not identical in appearance with infection cushions. The surface configuration varied, and this appears to influence the appearance of the structures produced. No stimulatory substances were concerned in their formation.

b) The hyphae of each fungus orientated on true, as well as on inverse replicas of the host surfaces on which the hyphae normally orientate. Because no stimulatory substances could be present on the replicas, it was concluded that orientation was independent of them, and depends on the surface configuration, and the fungus concerned.

4. Examination of host surfaces under the electron microscope

The surfaces of the host plants were examined under the electron microscope, and it was found that each of them had longitudinal ridges on the cuticular faces of the cells. These could account for the longitudinal orientation of the hyphae, the growing points of which could have been directed along the adjacent grooves. It casts doubts on the possibility of substances of host origin bringing about the orientation of the hyphae.

5. Penetration

Penetration always took place from infection cushions. The extent of penetration was, however, variable in different host-parasite

combinations. Each fungus penetrated hypocotyls, but not roots, cotyledons or leaves of cabbage and lettuce, and penetrated coleoptiles but not roots of wheat. In the host-parasite combinations that did not result in damping-off, the extent of penetration from infection cushions was very erratic.

6. Invasion of the host tissues

The speed, extent and direction of invasion of the host tissues was variable. Pathogenic fungi invaded susceptible host tissues laterally, rapidly progressing towards the stele, whereas non-pathogenic invaded at a slower rate in both lateral and longitudinal directions.

7. Sclerotia

The presence of sclerotia both on the surfaces, and inside the tissues of the hosts, appeared to have little bearing on pathogenicity.

8. Hypersensitive reactions

Host cells were observed to be discoloured in advance of penetrating hyphae, and were in some instances caused by a hypersensitive reaction. Fungus growth was stopped in some, but not all lesions in non-susceptible hosts. The result of the hypersensitive reaction was fungistatic and not fungicidal. This, however, could not completely account for the specificity of parasitism.

9. Effect of host decoctions and homogenates on fungus growth

Mild inhibitors of fungus growth appear to be present in decoctions and homogenates of host tissue. These were non-specific in their action, and were not sufficiently potent under the conditions of bio-assay, to

be significant in the specificity of parasitism.

10. The action of cell wall degrading enzymes

A - Enzymes produced on synthetic substrates

- a) Each fungus produced PME, PG and cellulase when grown in culture on appropriate synthetic substrates.
- b) Enzyme activity varied, depending on the substrate used for fungus growth.
- c) There was no good correlation between the growth of the different fungi on a particular synthetic medium, and the enzyme activities of these fungi.
- d) There was no correlation between pathogenicity, and the activity of any of the enzymes present in the culture filtrates

B - Enzymes present in diseased host tissues

- a) Each fungus produced PME, PG, and cellulase when they parasitized cabbage and lettuce seedlings.
- b) Enzyme activity (for each of the enzymes), of the extracts of the diseased seedlings bore no relationship to that of the culture filtrates of the fungi grown on synthetic substrates.
- c) There was no correlation between the activity of each enzyme, in the extracts in infected seedlings, and pathogenicity.
- d) The presence of pectic substances in the tissues of cabbage and lettuce hypocotyls was demonstrated by histochemical methods.

C - Ability of fungus extracts to macerate host tissues

- a) The culture filtrates of the fungi growing on synthetic substrates macerated cabbage and lettuce seedlings, but these effects were unrelated with pathogenicity.
- b) Extracts of the plants infected with the fungi, macerated cabbage and lettuce seedlings, but the effects were non-specific, and could not be directly correlated with pathogenicity. There was an indication that a pathogenic fungus produced extracts of higher enzyme activity than than did a non-pathogenic fungus, but this might have been due to the better growth of the non-ogenic fungus, rather than the basis of the specificity of parasitism.

11. Toxins

- a) Extracts of the host plants infected with the fungi were toxic to the growth of cabbage and lettuce seedlings.
- b) The toxic principle was non-specific, and its action could not be correlated with pathogenicity, neither could be distinguished as separate from cell wall degrading enzymes.
- c) The toxic principles were found to be thermolabile.

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