MECHANISMS OF PATHOGENICITY OF THREE FUNGI WHICH CAUSE DRY ROT IN POTATO TUBERS

By

ABDELKADER KEBABTI

A thesis presented for the degree of Doctor in Philosophy

in the

Faculty of Science at the University of Glasgow

Division of Environmental and Evolutionary Biology
Institute of Biomedical and Life Sciences
University of Glasgow
March 1996

ACKNOWLEDGEMENTS

The work described in this thesis was carried out at the Brian Research Laboratories of the Department of Botany, University of Glasgow.

The author wishes to express his gratitude and his sincere thanks to the following:

The Head of Department Professor R. G. Cogdell and Dr. C. T. Wheeler for placing the facilities of the Department at the disposal of the author during the period of the research.

Dr. D. D Clarke, who supervised this work. His advice, encouragement and guidance through the experimental research and for all the facilities providing to complete this work.

Dr. P. J. Dominy, Dr. K. J. Murphy and Mr J. Curral for their help and advice in statistical analyses.

Mrs. M. Gallacher, Miss. A. Adam and all the staff at the Brian Laboratory, Garscube and in the Department of Botany for the various help and useful discussions.

Mr I. Roberston for his work with the preparation and examination of the electron microscope material and Mr. N. T. Tait for the photographs included in this thesis.

The author would like to thank his wife for her constant encouragement and help throughout.

The Algerian Ministry of Education, University of Oran, (Institute of Sciences Naturals) for allowing my study in Glasgow University, and also providing the financial and moral support in the completion of this work.

Summary

Four isolates each of *F. coeruleum* and *F. avenaceum* and three isolates of *Cylindrocarpon destructans* were isolated from dry rot infected potato tubers.

Pathogenicity tests showed that *F. coeruleum* and *F. avenaceum* were strongly pathogenic to potato cvs Maris Piper and Record but *C. destructans* caused slow spreading lesions only. For all isolates of each species the higher the number of spores inoculated the faster the lesions established and the faster the lesions expanded within the tuber.

An analysis of the size of the dry rot lesions produced 24 days after inoculation by each isolate revealed significant differences in pathogenicity between isolates of each species in both cultivars. Isolates Fc1 and Fc2 of *F. coeruleum* were slightly more pathogenic than the other two isolates of *F. coeruleum* and isolates Fa1 and Fa2 of *F. avenaceum* were slightly more pathogenic than the other two isolates of *F. avenaceum*. Although all isolates of *C. destructans* were only weakly pathogenic Cd2 was the most pathogenic. For all three fungi cv Maris Piper was slightly more susceptible than cv Record.

Small but significant differences were found between isolates of each species in spore germination on the surface of tuber slices. Differences in the rates of germ tube growth between different isolates were significant for *F. coeruleum* and *F. avenaceum*, but not for *C. destructans*. The rates of lesion development into tuber slices were about the same for all isolates of *F. coeruleum* and for all isolates of *F. avenaceum*, but a significant difference was found between the isolates of *C. destructans*. The differences between the three species were supported by an analysis of chitin on the surface and in the internal tissues of the slices. Much larger amounts of chitin were present on the external surfaces of the slices than in the internal tissues for all three species and much more chitin was found with *F. avenaceum* and *F. coeruleum* than with *C. destructans*

An electron microscopic study of the colonisation of tuber tissues by both *F. coeruleum* and *F. avenaceum* showed that the middle lamellar region of the cell wall was relatively resistant to degradation compared to the primary wall and remained intact for a

considerable time after the tissue was infected. The resistance of the middle lamellar region to degradation explains the dry-rot nature of the lesions caused by *Fusarium* species.

All three species produced pectin and pectate degrading enzymes during the early stages of growth in tuber tissue medium and in living tuber tissue. The activities of pectin lyases and hydrolases were higher than those of pectate lyases and hydrolases, and lyase activities were greater than hydrolase activities. Both *endo* and *exo*-pectic lyase and hydrolase activities were found.

In *F. coeruleum,* enzyme production was found to be strongly inducible by substrate and inhibited by simple sugars. Strangely the pectic enzymes induced in synthetic media, containing pectin or polypectate substrates and in potato broth medium did not macerate potato tuber tissue. However, macerating activity was found in extracts from autoclaved tuber tissue and from living tuber tissue. It is probably that the main enzymes responsible for maceration of tuber tissue were enzymes other that pectinases, such as glucanases, or any of a number of other polysaccharide degrading enzymes such as galactanases, xylanases, arabanases or cellulases.

All three species produced toxic substances when growing in tuber tissue medium and in living tuber tissue. The activity of the toxic substances produced by *F. avenaceum* and *C. destructans* in living tuber tissue, or in autoclaved tuber tissue was only slightly reduced by boiling but the activity of that produced by *F. coeruleum* in living tuber tissue was almost completely destroyed. Clearly the toxins produced in living tuber tissue by *F. avenaceum* and *C. destructans* are different from those produced by *F. coeruleum.*

F. ceoruleum did produce heat stable toxins when grown in Baker's medium containing glucose as the sole carbon source but not when grown in Czapek-Dox medium with simple sugars or pectic substances as carbon sources and nor when grown in potato broth. but these toxins are likely to be artefacts of the culture condition since heat stable toxins were not found in other substrates including living tuber tissue.

The results of this study suggest that the isolates of all three species cause dry rot in potato tuber tissue by a complex interaction of various factors, including the ability to produce multiple forms of a range of cell wall degrading enzymes and toxins but that the mechanisms of pathogenicity of each fungus are different.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

CHAPTER I
INTRODUCTION

CHAPTER I

INTRODUCTION

1.1. Description of potato dry rot

Dry rot is a common storage disease of potato tubers (*Solanum tuberosum*). The first signs of infection are the development of small brown lesions internally which are visible through the surface of the tuber, and these lesions can increase in size quite rapidly even under dry storage conditions. Soon after lesion initiation, concentric wrinkles begin to develop over the surface, particularly towards the periphery of the diseased area, as the tuber tissue begins to shrink. The tuber tissue internally develops a brown rot, often with dark bands, blending at the margins into the healthy tissue. Pustules break through the skin covering the lesion and these pustules consist of fungal mycelium and sporodochia bearing spores and sometimes chlamydospores. At later stages of decay, cavities develop within the necrotic tissue inside the tuber which are lined with white, blue, pink, brown or beige mycelium the colour varying according to the causative fungus. Eventually, the tuber becomes totally infected, and it becomes hard and mummified, or is invaded by secondary soft rotting bacteria. Dry rot is generally not a disease of the growing crop but is the cause of serious losses of potato during storage, developing most rapidly under conditions of high humidity and at temperatures around 20°C. The disease can cause serious economic losses, particularly of seed tubers (Butler & Jones, 1955; Lapwood & Hide, 1971; Booth, 1971; Burton, 1989).

1.2. Brief history of potato dry rot in Britain

There is little information about the incidence of potato dry rot in Britain until the early years of the last century. However, it is likely to have occurred in tubers during storage from around 1600 when the potato was first introduced into Britain. Symptoms typical of dry rot were reportedly common in Britain prior to the outbreak of potato blight in the 1850's but in the half century following the introduction of *Phytophthora infestans* the report of such symptoms diminished for reasons which are not clear. However, from 1900 onwards, dry rot become an important disease again and remained so until the 1950's-1960's after which it declined again in importance. Its incidence is probably mainly related to the general level of susceptibility of the commonly grown cultivars and as cultivars changed so does the incidence of the disease. It is probable that

the reduced incidence of dry rot following the introduction of blight was due to the elimination of many of the older potato varieties because of their acute susceptibility to the blight fungus, while the surviving, more blight resistant cultivars, could have been resistant to dry rot also. In the 1900's, breeding for improved tuber quality appears to be have produced cultivars with high levels of susceptibility to dry rot (Butler & Jones, 1955) including the variety Doon Star, introduced in 1928. The current decline is again due to the increased acreage grown with more resistant cultivars and the reduction in the acreage grown of susceptible cultivars such as Doon Star and Arran Pilot. Improved control measures and improved storage conditions probably also have played a part.

1.3. The causal organisms

Attempts to establish the cause of dry rot were not really begun in Britain until the early years of this century. Earlier, in 1879, Reinke & Berthold reported that *Nectria solani* was associated with Die Zersetzung der Kartoffel (tuber decay) in Germany. In England, the dry rot of potato was stated by Massee (1904) and Lounsbury (1909) to be caused by *Nectria solani.* Pethybridge & Bowers (1908) in Ireland and Longman (1909), in England were the first to associate a species of *Fusarium* with dry rot but identified it as *F. solani.* Pethybridge & Lafferty (1917) were the first workers to show conclusively that the main fungus responsible for dry rot of potato tubers in England, Scotland and Ireland was in reality *F. coeruleum,* now recognised as a variety of *F. solani.* They also isolated *F. arthrosporiodes* from dry rot lesions and showed that this species could also cause such lesions when inoculated into healthy tubers, establishing that more than one species of *Fusarium* could cause the disease. Foister (1940) wrote a general account of the disease in Scotland and reported that the *F. coeruleum* which causes dry rot lesions of tubers in store is present in field soils and in the soil adhering to the tubers. According to Booth (1971), *F. solani* var. *coeruleum* is the main causal organism of potato dry rot world-wide. This organism is variously reported in the literature under the names *F. solani* var. *coeruleum* or *F. coeruleum.* Throughout this thesis, for simplicity, it will be referred to as *F. coeruleum.*

As indicated above, several *Fusarium* spp. have been recorded to cause the disease. Wollenweber (1913) in Germany reported that as well as *F. coeruleum*, *F. discolour* var. *sulphureum* (syn. *F. sulphureum*), *F. trichothecioides* and *F. ventricosum* were associated with dry rot lesions. Moore (1945) isolated *F. avenaceum* from rotted tubers collected during a survey of wastage in potato clamps in Great Britain, and

confirmed that this species was pathogenic and could produce dry rot lesions in potato tubers. According to Booth (1971) *F. avenaceum* has virtually a world-wide distribution occurring wherever enable crops are grown. However, it is chiefly a fungus of the temperate zones where it is often a severe parasite of overwintering cereals and many other crops.

Greer (1956) found that *F. coeruleum* was the most important cause of potato dry rot in Northern Ireland with *F. avenaceum* being of secondary importance.

Both *F. avenaceum* and *F. coeruleum* have been reported as wound parasites of potato tubers in many countries including the U.S.A. (Sherbakoff, 1915), Germany (Appel & Fuchs, 1912; Schmidt, 1928 Wollenweber & Reinking, 1935), France (Lansade, 1950), and Holland (Mooi, 1950).

In relatively recent years *F. sulphureum* has been recorded as a primary wound parasite of potato tubers and to cause dry rot lesions tubers in Great Britain (Boyd & Tickle, 1972). This species has been reported to be a common cause of dry rot in other parts of Northern Europe and particularly in North America.

Although *F. coeruleum, F. avenaceum* and *F. sulphureum* appear to be the main causes of dry rot, other species of *Fusarium* have been isolated from potato dry rot lesions and the species have been shown to be pathogenic, or slightly pathogenic, to potato tubers on reinoculation. For example Greer (1956) found that *F. tricinctum, F. sambucinum* and *F. culmorum* were occasionally responsible for tuber rots in Northern Ireland. In Finland Seppänen (1981a) found that *F. graminearum*, *F. sambucinum* var. *coeruleum*, and *F. trichothecioides* were strongly pathogenic to potato tubers while *F. sporotrichioides, F. solani*, *F. tricinctum*, *F. oxysporum*, *F. oxysporum* var. *redolens*, *F. acuminatum*, *F. culmorum* and *F. sambucinum* were moderate or weak pathogens. In France, Tivoli & Jouan (1981) isolated *F. arthrosporioides* and showed it to be very pathogenic to potato tubers while, *F. tricinctum*, *F. culmorum* and *F. graminearum* were slightly pathogenic. In Hungary, Hornok (1982) reported that *F. trichothecioides* was very pathogenic. In Algeria Kebabti (1986) isolated a number of *Fusarium* spp from seed potato tubers, including *F. solani, F. sulphureum* and *F. oxysporum* and found them to be very pathogenic. A number of other species *F. acuminatum*, *F. avenaceum*, *F. equiseti*, *F. equiseti* var. *bullatum* and *F. sambucinun* were moderately or weakly pathogenic. In South Africa Theron & Holz (1989, 1990) isolated *F. oxysporum*, *F. sambucinum*, *F. equiseti*, *F. acuminatum*, *F. graminearum*, *F. crookwellense* and *F.*

scirpi (syn *F. equiseti,* Booth, 1971) from dry rot lesions of potatoes and found that all of them were very pathogenic but that their pathogenicity varied. All the species of *Fusarium* which have been shown to cause dry rot lesions in potato are listed in Table 1.

Cylindrocarpon spp have also been isolated from tuber dry rot lesions by several workers (Salam, 1948; Greer, 1956) with *C. radicicola* (now *C. destructans*) being the most commonly recorded species. Tubers inoculated with *C. destructans,* in some cases showed no external symptoms of rotting while in others only a slight depression developed around the edge of the inoculation hole. However, internally, large cavities were formed which were lined with cinnamon coloured mycelium bearing spores, and the cavities were surrounded by light to dark brown or cinnamon coloured tissue. In some rots the spread of the fungus was restricted by a thin layer of cork, but around most cavities lay a thin zone of necrotic tissue. Lansade (1950) reported that *C. radicicola* was pathogenic to potato tubers but McKee (1952), who also isolated this fungus from rotted tubers, considered it to be a secondary invading saprophytic fungus.

1.4. Sources of inoculum and incidence of infection

1.4.1. Sources of inoculum

Small (1944a, 1944b) reported that *F. coeruleum* commonly occurs in field soil and is widespread in soils wherever potatoes are grown (Booth, 1971). It can survive in soil for at least 9 years after a potato crop and can remain viable in dried soil for at least 5 years. In contrast, *F. avenaceum,* although widespread in soil, rarely produces chlamydospores and loses viability in dry soil after as little as one year (Mckee and Boyd, 1952). Greer (1956) found that five isolates of *F. avenaceum* obtained from seed oats were pathogenic to potato tubers indicating that isolates are not specific for cereals or potatoes. However, Small (1944a, 1944b); Boyd & Logan (1967) and Boyd & O'Donnell (1968) suggested that infected seed tubers, not the soil, was the main inoculum source for progeny tubers. Adams & Lapwood (1983) suggested that seed tuber borne inoculum of *F. coeruleum* and *F. sulphureum* can be the important sources of infection for progeny tubers. However they found that inoculum of *F. coeruleum* was more readily transmitted to daughter tubers from infected than from tubers simply contaminated with spores on the surface, whereas with *F. sulphureum* it was the reverse.

1.4.2. Incidence of infection by different dry rot causing fungi

McKee & Boyd (1952) investigated the incidence of different dry rot fungi in different field soils. They found that 91% of lesions were caused either by *F. coeruleum*

Table 1. *Fusarium* species which, according to Booth (1971), have been isolated from potato dry rot lesions and have proved to be pathogenic or slightly pathogenic to potato tubers on reinoculation.

Species

Section Martiella

F. solani

F. solani var. *coeruleum* (*F. coeruleum*)

Section Sporotrichiella

F. tricinctum

Section Arthrosporiella

F. sporotrichoides

F. avenaceum (*F. roseum* var *avenaceum*)

Section Elegans

F. oxysporum

F. oxysporum var. *redolens*

Section Gibbosum

F. acuminatum

F. arthrosporioides

F. equiseti

F. equiseti var. *bullatum*

F. scirpi (*F. equiseti*)

Section Discolor

F. culmorum

F. graminearum

F. sambucinum

F. sambucinum var. *coeruleum*

F. sulphureum

F. trichothecioides

Section (Not assigned)

F. crookwellense

or by a few other *Fusarium* species of which the most common was *F. avenaceum*. In a separate study, McKee (1952) isolated several species of *Fusarium* from dry rot lesions but 93% of all isolates were of *F. coeruleum*, while only 6% of the lesions yielded *F. avenaceum* and less than 1% yielded *F. arthrosporioides* or *F. tricinctum*, *Cylindrocarpon* spp. were also found but *Cylindrocarpon* was most frequently associated with blighted tubers. McKee & Boyd (1952) reported that when diseased seed tubers, were planted, the rots could progress to kill the sprouts causing blanks in the rows but if tubers with small lesions were planted in conditions favouring growth they could produce normal plants. McKee (1954, 1955) showed that *F. coeruleum* possessed greater ability to initiate infection in wounded tubers than *F. avenaceum* and was able to cause infections earlier in the storage season.

The incidence of potato dry rots and their causal pathogens, were studied in tuber samples collected over the 3 years from 1979-1981 in Finland (Seppänen, 1983). Mechanical damage was very common and about 10% of all potato tubers were infected. Half of the tuber rots were dry rots caused by *Fusarium* spp., while a quarter were gangrene lesions caused by *Phoma foveata*. *F. avenaceum*, *F. coeruleum* and *Phoma exigua* var. *foveata* were the dominant pathogens, each causing about 25-30% of the total loss. None of the other *Fusarium* spp. isolated were of any real importance. In a similar survey in the United Kingdom, Jellis & Boulton (1984) found that in 1980 about 11.8% of all infected tubers sampled were infected with dry rots while in 1981 about 9.0% of all infected tubers sampled were infected with dry rot. Thus dry rots on average are responsible for about 10.0% of all tuber rots in potato. Some *Fusarium* spp. including some forms of *F. solani,* and *F. oxysporum* have been reported to infect the growing potato plant before harvest, infecting through the roots to induce foliage symptoms and varying degrees of tuber rot (Burton, 1989).

1.4.3. Effect of environmental factors on the incidence of dry rot

Many environmental factors influence the incidence of tuber infection by dry rot fusaria during storage. The effects of temperature in particular, have been investigated in great detail. Moore (1945); McKee (1954); Tivoli & Jouan (1981) and Seppänen (1981b, 1982b) found that the optimum temperature range for infection by *F. coeruleum* was between 15-20°C while that for *F. avenaceum* was between 20-25°C. These workers reported that at low temperatures *F. coeruleum* caused a faster rot than *F. avenaceum*. Boyd (1952d) found that the resistance of tubers inoculated with *F.*

coeruleum was greatly decreased by increasing the storage temperature. Both fungi are also favoured by a high relative humidity (Boyd, 1952d).

Different soil types may influence tuber susceptibility. Some differences in susceptibility were also observed between different stocks of the same variety, which were possibly due to the soil conditions under which the crops were grown. Nitrogen or potassium fertilisation of the growing crop can increase the susceptibility of tubers to *Fusarium* infection at later stages in store while a balanced NPK fertiliser application decreases liability to infection (Lapwood & Hide, 1971; Boyd, 1967). Dry rot is often more serious after dry than wet seasons, possibly because tubers are more liable to mechanical injuries when lifted from dry soils than from wet soils (Lapwood & Hide, 1971).

1.5. Varietal responses

1.5.1. Differences in resistance

In Great Britain, early varieties have been found generally to be more susceptible to dry rot than late varieties (Boyd, 1952a and 1952c). However, many workers, starting with Pethybridge and Lafferty (1917) have reported that there are wide differences in resistance between main crop cultivars to the different *Fusarium* spp. Cultivars also differ in their relative susceptibility to *F. coeruleum* and *F. avenaceum*. Of a number of cultivars tested, including Majestic, King Edward, Doon Star and Arran Pilot, King Edward was the most susceptible to *F. avenaceum* and Doon Star was the most susceptible to *F. coeruleum* (Moore, 1945; McKee, 1952).

Seppänen (1981c) tested 22 cultivars for their resistance to *Fusarium* species. Resistance to *F. coeruleum* varied and infection was progressive in some cultivars but became arrested by resistance reactions in other cultivars. Cvs Hankkijan Tuomas, Eigenheimer and Sirtima were the most resistant, followed by Sabina and Posmo, while Maris Piper, Bintje, Record and Ostara were among the group of cultivars which were very susceptible. In relation to *F. avenaceum*, cvs Sabina, Provita and Veto were the most resistant, while Maris Piper, Hankkijan Tuomas, Stina and Jaakko were intermediate in resistance and Bintje and Ostara were the most susceptible. *F. avenaceum* advanced four times more rapidly in the tubers of the most susceptible cultivars than in the most resistant. *F. sulphureum* was strongly pathogenic infecting the tubers of all cultivars easily and generally very similarly. However, cvs Ostara, Jaakko, Sabina and Eigenheimer were the most resistant followed by Hankkijan Timo, Sieglinde

and Hankkijan Tuomas. Jellis & Starling (1983) investigated fourteen cultivars for resistance to penetration and colonisation by *F. sulphureum* and found that differences in resistance to penetration were small compared to those reported for *F. coeruleum*, and were more apparent in one year than another. Cvs King Edward, Record and Pentland Squire were significantly less susceptible to penetration than any of the other cultivars tested. Tubers grown at different sites gave similar results. The medullary tissue of cultivars was generally more susceptible than the cortex. However, despite little differences between cultivars in resistance to penetration there were large differences in resistance to colonisation and these differences were consistent between years and sites. Wastie *et al.* (1989) examined the susceptibility of fifteen potato cultivars to dry rot caused by *F. sulphureum* and *F. coeruleum* over a period of 8 years. *F. sulphureum* was the more aggressive of the two species but there was little correlation between the rank order of susceptibility of the different cultivars to the two pathogens.

1.5.2. Changes in resistance in relation to tuber maturity

Boyd (1952b) found that the susceptibility of tubers to *F. coeruleum* was low at around lifting time but gradually rose during storage. Susceptibility was at its highest in immature tubers just after flowering, but this high susceptibility was followed by a rapid increase in resistance which reached a maximum when the haulm was dead and at the time of lifting. Boyd (1967) also found that resistance increased when the foliage was killed by herbicides as well as when it died naturally. The susceptibility of the immature tuber is not due to thinness of the corky phellem layer surrounding the tuber but to physiological or chemical conditions in the tuber.

Seppänen (1982a) studied the influence of the stage of tuber development on tuber resistance to *F. sulphureum* in different cultivars and found that the response was very similar to that found with *F. coeruleum*. During the earliest stages of tuber growth the tubers were very susceptible to infection, but their resistance increased during the course of the season. At harvest time, resistance was highest, and then decreased again during the course of the storage period. Planting time and vine killing had no significant effect on resistance.

1.6. Mechanisms of infection

1.6.1. Sources of inoculum and site of infection

Dry rot infection generally occurs during storage, from inoculum which is present in soil on the tuber surface. The fungus almost always gains entry through

wounds produced during lifting and grading, or during transport. Tivoli *et al.* (1986) studied how the nature of the wound affected the different *Fusarium* spp. They found that *F. sulphureum* caused extensive lesions regardless of wound size, in contrast to *F. coeruleum* and *F. avenaceum* which caused lesions only when the wounds were greater than two millimetre in depth. The most rapid rots develop from deep incised wounds because of the greater susceptibility of the medullary tissue. MeKee (1954) reported that tubers were less readily infected through clean-cut than through scarified wounds.

Occasionally, infection occurs through lenticels or buds, although not while the tubers are still attached to the plant. Within the store, infection can spread from diseased to adjacent healthy undamaged tubers. Infection can also occur through lesions caused by other pathogens, such as powdery scab, blight and gangrene. Infections can also occur through sprouts damaged during handling and such infections caused serious losses of seed tubers in the Netherlands in 1950 (Mooi, 1950).

1.6.2. Fungal growth within the tuber

Infection occurs from spores germinating on the surface of wounds. Tivoli *et al.* (1986) found that at low inoculum levels certain *Fusarium* spp can be highly aggressive. For example *F. sulphureum* and to a lesser extent *F. coeruleum* and *F. graminearum,* could initiate a rot from a few spores, whereas *F. arthrosporioides* and *F. culmorum* could only initiate rots when a high inoculum level was used.

Histological studies of spreading lesions caused by *F. coeruleum,* showed that the hyphae could initially grew through the intercellular spaces without causing a marked reaction in the adjacent host cells. The tissues did not show browning at the surface until 3-7 days after inoculation. This suggests that *F. coeruleum* can grow initially as a hemibiotroph. However, the cells do eventually die and then *F. coeruleum* grows as a necrotroph in the dead tissue. The hyphae of *F. avenaceum,* on the other hand never form any association with living cells, always killing the cells with which they come into contact and then developing necrotrophically on the dead tissue (McKee, 1954). In some cases, lesions caused by *F. avenaceum* may become restricted soon after penetration, when the lesion is still quite small. Restriction of *F. coeruleum* lesions may also occur, but generally only when they are quite large and established well into the tuber. Restriction of lesion expansion in tuber tissue infected by both fungi is accompanied by the deposition of suberin on the host's cell walls, surrounding the infected tissues. Suberization of the peripheral cell walls around small lesions was probably stimulated by

the products of necrosis from host cells which had reacted violently to the infection, rather than by the direct action of the fungal mycelium, since at that stage fungal mycelium was present only in small amounts. Potato tubers may be susceptible to *F. coeruleum* but resistant to *F. avenaceum*, because the latter fungus always grows intracellularly so that its growth is more likely to be inhibited by factors within the cell (McKee, 1954, 1955).

1.6.3. Host reaction to infection

1.6.3.1. Wound healing

Since infection generally occurs through wounds, the processes of wound healing, including suberisation and periderm formation, can have a profound effect on the processes of infection (Smith & Smart, 1955; McKee, 1954, 1955, 1959; Müller, 1957; Lyon, 1989).

Wound healing can be stimulated by various treatments. For example, treatment with gibberellins and other hormones have been reported to induce or stimulate enzymes involved in wound healing. The suberisation of the cells at the wounded surface is one of the first steps in wound healing. A phellogen is then initiated below the suberised surface, and layers of periderm cells are cut off externally.

1.6.3.2. Relationships between the processes of wound healing and infection

The formation of cork, or even the formation of the initial suberised surface over a wound is not immediate and infection can develop readily from an inoculum in a fresh wound and for several days thereafter. Boyd (1952c) found a relationship between the rate at which wounds healed in particular cultivars and resistance to infection by *F. coeruleum.* Resistance to infection was greater when a short interval elapsed between wounding and inoculation than when the wound was inoculated immediately after wounding. This resistance was related to an increase in the intrinsic ability of the cells near the wound to resist infection rather than to the development of any physical barrier, such as a suberised layer over the wound surface, or to wound periderm formation. Tivoli *et al.* (1986) reported that the resistance which developed during wound healing to *F. culmorum* and *F. arthrosporioides* was very rapid and nearly complete by 2-4 days. However, resistance to *F. coeruleum* and *F. sulphureum* developed slowly, requiring about 8 days to develop, while resistance to *F. graminearum* required about 15 days. They suggested that the wound healing reaction can modify the relative ranking order for cultivars; a cultivar classified as resistant in the absence of healing becoming itself

grouped with the less resistant ones after wound healing, probably because of a rate of wound healing lower than that of the other cultivars.

1.6.3.3. Biochemical basis of resistance

The resistance of wound tissues may be due to the mechanical and chemical composition of the cell walls or to the release of inhibitory compounds from the damaged cells. (Boyd, 1952c; Wellving, 1976; Leach & Webb, 1981). The spread of the rot may also be retarded by inhibitory substances which are produced by the peripheral tuber tissue at the point of infection as a result of induction by diffusate from the invading fungus. Simmons (1973) excluded from the list of possible inhibitors those substances not specifically induced by the infection but normally produced by damaged tissues, including the glycoalkaloids.

Lyon (1989) reported that the complex polymers, lignin and suberin, which are components of the cell walls of potato wound tissue may prevent infection. These compounds rapidly accumulate over a wound surface and their main function is probably to seal the tissues against water loss but they may also act as a physical and chemical barrier to penetration by pathogens. Many fungal pathogens possess enzymes able to degrade cutin (Soliday *et al.*, 1984) or suberin (Zimmermann and Seemüller, 1984) and such enzymes may be involved in mechanisms of infection. Lignin-like compounds have been reported to be produced in potato tubers and leaves (Friend *et al.*, 1971; Friend, 1973; Friend, 1976) and the deposition of these compounds in the cell walls may also produce enzyme inhibitory barriers.

The tissues also naturally contain significant amounts of inhibitory compounds such as phenols (Farkas & Kiraly, 1962) and steroid glycoalkaloids (Allen & Kuc, 1968) mainly in the vacuoles in undamaged cells. McKee (1954, 1955) indicated that the most toxic of the steroidalkaloids was the alkaloid solanine. The concentration of solanine in the region of a wound, and the alkalinity developed in infected tissues suggests that this alkaloid could play a role in the restriction of fungal infection in potato tubers. The fact that α-solanine, like other alkaloids, is concentrated largely in the vacuole in living cells means that it is released into the apoplast on cell death. Solanine and chaconine can also accumulate in response to wounding (Kuc, 1966; Ishizaka & Tomiyama, 1972). Steroid glycoalkaloids such as α-solanine and α-chaconine which are produced in potato tissue are antifungal (Allen & Kuc, 1968; Allen, 1970; Sinden *et al.*, 1973). McKee (1959) found that the spores of *F. coeruleum,* when exposed to a concentration of 30 mg

solanine l^{-1} become shrunken and their contents dense and uniform, in contrast to the vacuolate appearance of unaffected spores. Spores of *F. avenaceum* and *F. culmorum* behaved similarly. However, macrospores from mature cultures were more tolerant of solanine than those from young cultures. Furthermore, macrospores of *F. avenaceum,* and *F. culmorum* were found to have a sensitivity to solanine similar to that of *F. coeruleum* spores. McKee (1959) also found that chaconine was about twice as toxic as solanine to spores of *F. coeruleum* and solanidine was less toxic to spores than solanine. He suggested that the lower toxicity of solanidine was due to its poor solubility. Demissine and tomatine were more toxic to *F. coeruleum* spores than was solanine. Other workers have suggested that steroid glycoalkaloids are factors in the resistance of potato and tomato tissues to fungal pathogens. Tomatine from tomato and solanine from potato have been shown to be very toxic to many fungi *in vitro* (Fontaine *et al.*, 1948; Allen & Kuc, 1968; Arneson & Durbin, 1968). Zoospores of *P. infestans*, which consist of naked protoplasts, in the presence of solanine come to rest prematurely, suddenly increase greatly in size and disintegrate. The viability of spores of *Streptomycin scabies* however was not affected by solanine (McKee, 1959). Also, young cultures of bacteria were not affected by several hours of exposure to 2000 mg. solanine l^{-1}.

Kosuge (1969) and Friend (1985) reported that phenolics can also play an important role in the general defence mechanisms of plants to infection since phenolic compounds, particularly in their oxidised state, are highly inhibitory to many micro-organisms. This inhibitory activity is probably largely due to their ability to denature proteins, and thus inhibit any extra-cellular enzyme activities of the parasite. Mullen & Bateman (1971, 1975a, 1975b) found that the activity of cell wall degrading enzymes in extracts from freshly harvested tuber dry rots caused by *F. avenaceum* were very low and this was apparently due to inactivation of these enzymes by oxidised phenols. The recovery of the cell wall degrading enzymes could be enhanced by extracting the tissue with 2-mercaptoethanol or by freezing them for about 2-10 months, which prevented the rapid oxidation of phenolics. Cole (1958) and Cole & Wood (1961) found that phenolic substances were present in healthy tissues and rotted tissues of apple infected with *Sclerotinia fructigena*, *Botrytis cineria* and *P. expensum* and that extracts of healthy apple tissue reduced the activity of the polygalacturonase in culture filtrates of *S. fructigena.* The substances responsible for this inhibition were tentatively identified as

leuco-anthocyanins which changed to other compounds following the action of polyphenoloxidase.

Waites *et al.* 1978 reported, in relation to resistance to *F. coeruleum,* that the chlorogenic acid concentration in tubers of the cv Homeguard was between 2 to 3 times the value in the susceptible cv. Graigs Alliance. It is however, unlikely that chlorogenic acid was a resistance factor since these workers found that low concentrations of chlorogenic acid actually stimulated the growth of *F. coeruleum in vitro.*

Phytoalexins are low-molecular-weight antimicrobial compounds that are synthesised by, and accumulate in, plants after exposure to micro-organisms (Paxton, 1980; Kuc, 1982)) and are regarded as factors limiting the growth of pathogens in many plants (Müller, 1940; Ebel, 1986). A number of compounds with the properties of phytoalexins have been recorded for potato. The most important of them being terpenoid compounds (Kuc, 1973). Price *et al.* (1976) reported the presence of rishitin, phytuberin, diacetylphytuberin and solavetivone in eight different cultivars of potato tuber inoculated with *F. avenaceum, P. infestans* and *P. exigua var. foveata.* The most consistent response to infection by the three fungi was the formation of solavetivone which was produced by all eight cultivars inoculated with *F. avenaceum* and *P. exigua* and seven of the cultivars inoculated with *P. infestans.* The considerable quantitative differences in the levels of the various sesquiterpenoid phytoalexins which accumulated in potato tubers inoculated with *F. avenaceum, P. infestans* and *P. exigua var. foveata* are difficult to explain (Price *et al.*, (1976). For none of the pathogens could the level of any of the compounds produced be related to the degree of susceptibility of the cultivar because there were no significant differences in susceptibility observed between the cultivars examined. However, Corsini *et al.* (1977) who examined the relationship between rishitin accumulation and *Fusarium* resistance in potato breeding clones found that it was produced more rapidly in resistant clones than in susceptible clones. However, they concluded that resistance is determined by more than one factor. Rishitin and phytuberin are phytotoxic, causing the death of plant protoplasts and isolated cells (Lyon & Mayo, 1978). An unidentified sesquiterpene glucoside has been reported to occur in potatoes infected with *P. exigua* (Malmburg & Theander, 1980) while glycosides of rishitin, hydroxysolanascone and hydroxysolavetivone have been detected in tobacco.

1.7. Mechanisms of pathogenicity

One of the significant features of dry rot lesions is that the host's cell walls appear to remain relatively intact. This feature distinguishes dry rot diseases from wet rots caused by such potato pathogens as *Pythium ultimum* or *Erwinia atroseptica*. However, the reasons why the cell walls are not degraded in tuber dry rot diseases are not clear. Several factors may be involved and since some of these may be concerned with the structure, or changes in the structure, of the potato cell wall this account will begin with an examination of the structure of the potato cell wall.

1.7.1. The role of cell wall degrading enzymes in pathogenicity

1.7.1.1. The structure of potato tuber cell walls

Burton (1989) has given an account of the structure and composition of the cell walls of potato tubers. The walls consist of hemicelluloses and cellulose embedded in a pectin matrix. This matrix also includes some proteinaceous and phenolic compounds. The wall is laid down on each side of a layer of pectin substances which are deposited on the cell plate. The cell plate is the structure which forms between adjacent cells at the time of cell division and the pectin incorporated onto the cell plate thus forms the middle lamella and remains as a cementing component between the primary walls of adjacent cells. The pectin also merges into the primary cell wall on either side of the middle lamella. The main components of the primary cell wall are cellulose and hemicelluloses which are arranged within the wall in a definite pattern held together by covalent bonds and hydrogen bonds. The primary wall is not solid but is permeated by interstitial spaces which contain water. Lignin only occurs to a minor extent in the potato tuber cell wall and when it does it encroaches on the water-filled spaces of the wall so that the water content of the wall may be reduced to about one fifth of that in unlignified parenchyma cells. Selvendran *et al.* (1987) gave the proportions of the cell wall constituents in typical potato parenchymatous cells as water 70%, cellulose 10%, pectin substances 12%, hemicelluloses 6% and glycoproteins 2%. Burton (1989) reported that the main sugar residues of the polysaccharides in unlignified cell walls of the potato tuber, as analysed by Ring & Selvendran (1981) are glucose 31.5%, galactose 28.4%, uronic acids 27.0%, arabinose 4.6%, 6-deoxyhexoses (including rhamnose) 2.0%, xylose 1.7%, and a trace of mannose.

Pectic polymers (rhamnogalacturonans) are the major constituents of the potato cell wall and as indicated above predominate in the intercellular region or middle lamella.

This is a zone of particular importance in parasitism as its degradation has profound effects on adhesion between cells and the pectic substances are exploited as source of carbon by many pathogens which grow between cells. Rhamnogalacturonan comprises chains of α-1,4-linked galacturonic acid interspersed with 1,2 linked rhamnose (Fig 1). The carboxylic groups of the galacturonic acid units may be methylated or cross-linked by calcium, and this cross linkage can have a considerable effect on cell wall properties and the susceptibility of the molecule to enzyme attack. To this backbone chain are linked highly branched α-1,3- and α-1,5 araban and linear β-1,4-galactan side chains but many other linkages can exist between the monomers (Darvill *et al.*, 1980).

Jarvis *et al.* (1981a) reported that the cell walls of potato tubers could be fractionated by successive extractions with various reagents to give a slightly degraded pectic fraction with 77% galacturonic acid and a further, major pectic fraction with 38% galacturonic acid. These two pectic fractions made up 52% of the cell wall. Analysis of both pectic fractions showed that they contained a range of molecular types, with a high arabinose:galactose ratio as well as much galacturonic acid in the most extractable fractions. From methylation data, the main side-chains were found to be 1,4-linked galactans and 1,5-linked arabinans, with smaller quantities of covalently attached xyloglucan. Extraction with NaOH-borate removed a small amount of hemicellulose and some cellulose. The main hemicelluloses were a galactoxyloglucan, a mannan or glucomannan and an arabinogalactan.

1.7.1.2. The structure of the cell wall in relation to infection

Pagel and Heitefuss (1989) reported that high levels of total pectin and non esterified pectin were correlated with resistance to infection by the tuber soft rot and black leg causing bacterium *Erwinia carotovora* subsp. *atroseptica*. Susceptible cultivars had higher levels of esterified pectins than resistant cultivars. McMillan *et al.* (1993) reported that the percentage esterification of pectin was generally greater in the tuber cortex than in the medullary tissue and, in contrast to the work of Pagel and Heitefuss, that esterification was 2.4 and 1.6 times greater in the medulla (69.4%) and cortex (94.1%) respectively of a cultivar resistant to *E. carotovora* subsp *atroceptica* than in the corresponding tissues of other genotypes of lower resistance. Levels of calcium in tubers were greater in the cortex than in the medulla but were not different between genotypes. Maceration was generally more rapid if pectin methyl esterase was also present, McMillan *et al.* (1993) also suggested that there is an association between tuber

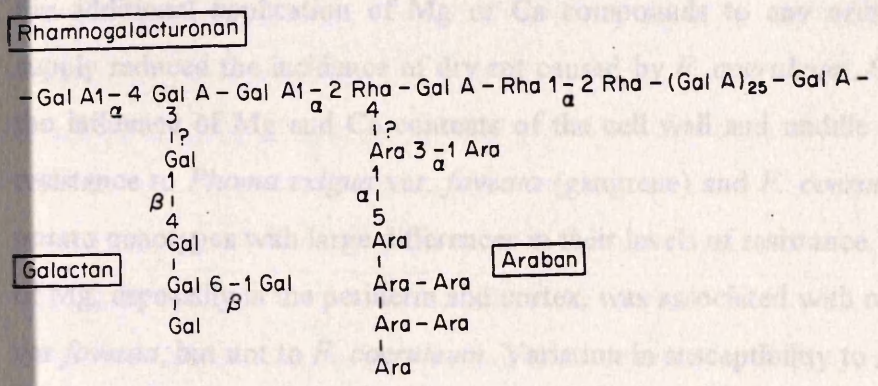


Figure 1. Structure of 'pectic' polymers rhamnogalacturonan and covalently linked neutral polysaccharides. GalA, galacturonic acid; Rha, rhamnose; Gal, galactose; Ara, arabinose. (Cooper, 1984).

resistance to erwinias and pectin esterification which, since it is a performed resistance characteristic, would operate under the anaerobic conditions in which bacterial soft rot disease usually develops. Bateman & Millar (1966) reported that the improved structural integrity, due to cross-linkages between the carboxylic groups of the pectic chains via divalent cations, will make the cell wall less susceptible to degradation. Both Mg and Ca ions form such linkages and their concentration might thus have a role in resistance. A high content of Ca was found to be associated with resistance of the tuber tissue to *Erwinia carotovora* (McGuire & Kelman, 1984, 1986). Langerfield (1983) found that the additional application of Mg or Ca compounds to any ordinary N-P-K fertiliser supply reduced the incidence of dry rot caused by *F. coeruleum*. Olsson (1988) studied the influence of Mg and Ca contents of the cell wall and middle lamella in relation to resistance to *Phoma exigua* var. *foveata* (gangrene) and *F. coeruleum* (dry rot) in two potato genotypes with large differences in their levels of resistance. An increased content of Mg, especially in the periderm and cortex, was associated with resistance to *P. exigua* var *foveata*, but not to *F. coeruleum*. Variation in susceptibility to *P. exigua* var *foveata* was greater in samples from cv. Bintje than cv. Elsa. In cv. Bintje, variation in susceptibility to *F. coeruleum* was less pronounced than that to *P. exigua* var *foveata*, while in cv. Elsa the variation was greater but still bellow the level of attack in cv. Bintje. The effect of Mg was greater than that of Ca and the results indicate that the ratio of Mg:Ca might be of importance in resistance to *P. exigua*. This effect can be explained by the pectin-bound Mg which gives a firmer cell wall and middle lamella than pectin-bound Ca. The positive influence of Mg on resistance to *P. exigua* var *foveata*, apparently due to predisposition, was evident in both cvs Bintje and Elsa. An increased uptake of Mg had, however a greater effect upon resistance to gangrene for the susceptible cv. Bintje than for the relatively resistant cv. Elsa. However, the hypothesis that the Mg-bridges bound to pectic substances form part of a resistance mechanism did not seem valid for *F. coeruleum*. Other results indicate that factors other than pectin-structure must exert a more decisive influence on susceptibility of the tuber tissue to colonisation by *Fusarium* spp.

1.7.1.3. The role of cell wall macerating enzymes in tissue degradation

Enzymes capable of degrading plant cell wall polysaccharides are considered to play a significant role in pathogenesis (Bateman & Millar, 1966; Albersheim *et al.*, 1969) and the type of cell wall degrading enzymes produced by the pathogen may also be of

significance in dry rot development. However, little work has been done on the involvement of such enzymes in the development of dry rots and only in a few instances has their role been investigated in relation to dry rots of potato tubers caused by *F. roseum ' avenaceum'* (Mullen & Bateman, 1971; Mullen & Bateman, 1975a; Mullen & Bateman, 1975b). There have been limited studies in relation to other dry rot disease, e.g. in relation to dry rots of apple such as the brown rots caused by *Sclerotinia fructigena* and *S. laxa* (Cole, 1958; Cole & Wood, 1961), to dry rot of bean caused by *F. solani* f. sp *phaseoli* (Bateman, 1966) and to diseases in beans and a variety of other crops which often exhibit dry rot symptoms caused by *Rhizoctonia solani* (Bateman, 1963a, 1963b; Bateman *et al.*, 1969).

One particular group of cell wall degrading enzymes, the pectic enzymes, has attracted considerable interest in relation to pathogenicity, particularly in relation to their involvement in soft rot and wilt diseases. They alone, as a group, can be responsible for tissue maceration.

1.7.1.4. The degradation of rhamnogalacturonans of the middle lamella and of the primary wall by pectic enzymes

The enzymatic degradation of the rhamnogalacturonan chain involves a variety of enzyme types. A classification of the enzymes involved is given in Table 2. The rhamnogalacturonan chain is degraded (Fig 2) by hydrolases which yield galacturonic acid or its polymers, and by lyases which yield polymers and monomers with an unsaturated bond between carbons 4 and 5 at the non-reducing end of the broken chain (Fig 2). The hydrolases and lyases also exist in different forms showing specificity for regions of the chain in which the carboxyl groups are either methylated or free. Hydrolases usually have acidic pH optima (pH 4-5) whereas lyases are most active at high pH (pH 8-10) and have a requirement for Ca^{2+} (Cooper *et al.*, 1978). Bateman, (1966) also reported that the production of pectic lyases and hydrolases may be favoured by alkaline and acid environments respectively. He showed that the patterns of production of pectic enzymes by *Fusarium solani* f. sp *phaseoli* in two different media were different and speculated that the differences could be related to the pH of the cultures. He suggested that the types of enzyme produced in culture filtrates might be a reflection of their pH stability.

Hancock (1966) showed that the pH of alfalfa tissue infected with *Colletotrichum trifolii* increased and this could favour the activity of pathogen produced

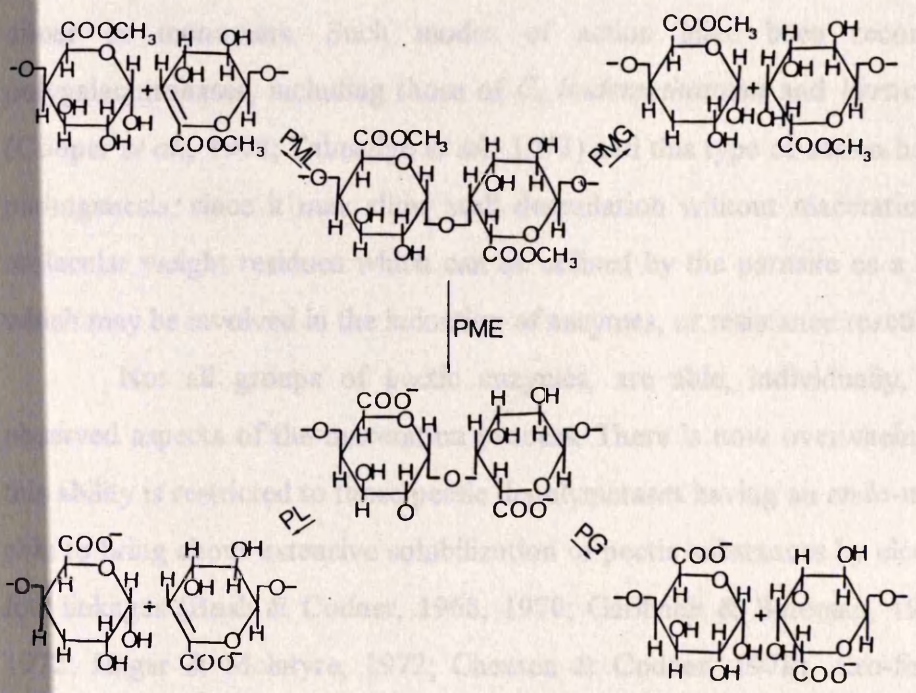
Table 2. Classification of pectin and pectic acid splitting enzymes (Goodman *et al.*, 1986).

Pectin (Polymethylgalacturonide)	Demethylated (polygalacturonide)
(a) Hydrolases	
Pectin hydrolase	Pectate hydrolase (PG)
Polymethylgalacturonase (PMG)	Polygalacturonase (PG)
Endo PMG	Endo PG
Exo PMG	Exo PG
(b)Lyases	
Pectin lyase	Pectate lyase
Polymethylgalacturonate lyase (PML)	Polygalacturonate lyase (PGL)
Endo PML	Endo PL
Exo PML	Exo PL

(c) Pectic methyl esterase

Pectic methyl esterase (PME) — demethylates polymethylgalacturonide → polygalacturonides

Figure 2. Mode of action of pectic enzymes.



PMG, polymethylgalacturonase (pectin hydrolase); PML, polymethylgalacturonate lyase (pectin lyase); PME, pectin methyl esterase; PG, polygalacturonase (pectate hydrolase); PL, pectate lyase (Chatterjee & Vidaver, 1986).

polygalacturonate trans-eliminase (pectate lyase), which has a pH optimum of between 8.5 and 9.0.

Endo forms and *exo* forms of hydrolases and lyases are also recognised. *Endo*-enzymes cleave the chain at random to release polymers of various lengths and cause up to a 50% decrease in substrate viscosity after cleavage of only about 1% of the glycosidic bonds. In contrast, *exo*-forms release monomers from the ends of the chains in sequence and must cause 20-40% hydrolysis to effect a 50% decrease in viscosity (Cooper, 1983). Forms capable of multiple attack, combining the two modes of action, have also been reported. These forms attack chains at random first, then later release dimer or monomers. Such modes of action have been recorded for various polygalacturonases, including those of *C. lindemuthianum* and *Verticillium albo-atrum* (Cooper *et al.*, 1978; Talmadge *et al.*, 1973) and this type of action has implications for pathogenesis, since it may allow wall degradation without maceration yet release low molecular weight residues which can be utilised by the parasite as a carbon source, or which may be involved in the induction of enzymes, or resistance reactions.

Not all groups of pectic enzymes, are able, individually, to reproduce all observed aspects of the maceration process. There is now overwhelming evidence that this ability is restricted to those pectic depolymerases having an *endo*-mode of attack and able to bring about extensive solubilization of pectic substances by cleavage of relatively few linkages (Bush & Codner, 1968, 1970; Garibaldi & Bateman, 1971; Archer et al., 1972; Hagar & McIntyre, 1972; Chesson & Codner, 1978). *Exo*-forms of pectolytic enzymes do not appear to cause maceration (Mclendon, 1964; Hancock & Miller, 1965). Although the most effective macerating enzymes isolated from dry rot lesions of *F. coeruleum* was *endo*-polygalacturonate lyases and hydrolases (Sturdy, 1973). Hancock (1968) reported that *endo*-pectate lyase was the main pectolytic enzyme to increase in concentration during pathogenesis by *F. solani* f. sp. *cucurbitae* infected squash hypocotyl tissues, but not polygalacturonase but, pectin lyase was also present. He also found that half of the galacturonic acid containing polymers had been removed from the walls of infected host tissue, and the degree of polymerisation of the residual pectic substances in infected tissue was approximately one-half that of pectic substances extracted from healthy tissue. Bateman (1966) found that *F. solani* f. sp. *phasioli* produced pectic enzymes degrading cell walls hydrolytically and by lyase activities.

Papavizas & Ayers (1966) investigated *F. solani* ff. sp. *phaseoli* and *pisi* and found that they both produced polygalacturonate lyase activity.

Pectin methyl esterase (PME) is produced by most plant pathogens and it is also present bound to the cell wall in healthy plant tissue. It hydrolyses the methyl ester groups of the uronic acid residues in the pectin chain forming uronic acid groups and methanol (Drysdale and Langcake, 1973; Bateman & Basham, 1976). PME of higher plants and bacteria have pH optima within the range pH 7.0-8.5, while those of many fungi have pH optima within the range pH 4.0-6.0 (Chesson, 1980). This difference in pH optima between plant and fungal PMEs has been used to differentiate the PME of host and fungal parasite origins *in vivo* (Langcake *et al.*, 1973).

Since PME converts pectin or polymethylgalacturonate to polygalacturonates it can act in concert with and facilitate cell wall breakdown by polygalacturonases or polygalacturonate lyases and so facilitate cell wall breakdown when these are the only depolymerases present. In *Clostridium multifermentans,* PME and polygalacturonate lyase exist as a bound complex and this complex facilitates rapid desterification and depolymerization (Sheiman *et al.*, 1976). The significance of PME in pathogenicity has however been questioned by studies involving mutant fungi. Thus strains of *Fusarium oxysporum* f. sp *lycopersici* which lack the ability to produce PME were found to be as pathogenic as the wild type (Mann. 1962; McDonnell, 1962).

1.7.1.5. Other enzymes involved in pathogenesis

Both *F. avenaceum*, and *F. coeruleum* produce cell wall degrading enzymes other than pectic enzymes and these enzymes could be involved in tissue maceration in infected tuber tissues *in vivo.* In particular, *F. coeruleum* produces a number of galactonases which individually cause maceration *in vitro* (Sturdy, 1973).

Mullen & Batemam (1971, 1975a, 1975b) reported that *F. roseum 'avenaceum'* produced in addition to *exo* polygalcturonase, *endo* polygalacturonase, a number of polysaccharide degrading enzymes including *endo*-ß-1,4 galactanase, *endo*-xylanase, two arabanases and *endo*-cellulase as well as *exo*-polygalacturonase and an *endo*-polygalacturonide lyase. These enzymes were capable of degrading the glycosidic bonds in the major polysaccharide constituents of the cell wall of potato tubers.

Friend & Knee (1969) examined the carbohydrates of cell walls prepared from tuber discs of a variety of potato which was susceptible to *P. infestans*. The rot caused in tubers by *P. infestans* is essentially a dry rot. All the polysaccharide fractions in control

discs increased with time but the increase was slower in the pectic fraction with a more rapid increase in the extraction residue in discs infected with *P. infestans*. These differences were related to the monosaccharide composition of hydrolysates; there was no increase in galactose, found predominately in the pectic fraction, but a rapid increase in glucose which is confined almost exclusively to the extraction residue. Part of the increase in glucose was due to an accumulation of hyphal wall of *P. infestans* which contained mainly an alkali-insoluble glucan. Galactanase activity, which was demonstrated in infected discs, could account for the divergence of galactose content from that of the controls. The enzyme degraded potato pectin to release a proportion of galactose; short oligosaccharades probably occurred among the products of the reaction. *In vitro* the enzyme rapidly reduced the viscosity of potato and lupin pectin solutions, with a slow concomitant release of reducing groups. In addition, it released total carbohydrate more rapidly than it released galactose from potato cell walls, results which indicate that the enzyme is an *endo*-glycanohydrolase. However, it had only a limited effect on the cohesion of the cells in potato tuber tissue (Knee & Friend, 1970).

Potato cell wall polysaccharide degradation by *P. infestans* was studied by Jarvis *et al.* (1981b). Culture filtrates of *P. infestans* contained two polygalacturonases, four galactanases and two pectin esterases. An *endo*-polygacturonase and an *endo*-(1,4)-β-D-galactanase were partially purified and used to degrade potato cell walls. The polygalacturonase released less than 6% of the cell wall carbohydrate, even after galactanase treatment, or removal of the more soluble pectin fraction. Its limited degradative activity may be connected with the biotrophic habit of the blight fungus. The galactanase detached up to 23% of the cell wall, including uronic acid when calcium ions had been removed beforehand. Arabinose was also released, showing that there are arabinogalactan side-chains in the pectin. Galactanase degradation of a soluble pectin fraction left 63% of the arabinose attached to large rhamnogalacturonan fragments with only a little galactose remaining. Many arabinan chains are thus perhaps attached directly to rhamnogalacturonan.

Van Etten & Bateman (1969) reported that *S. rolfsii* grown on an autoclaved bean hypocotyl medium produced enzymes which readily degraded pectic galactan (β-1,4 galactopyranose polymer), galactomannan (β-1,4 mannopyranose polymer with monomeric β-1,6 galactopyranose side branches) and xylan (β-1,4-xylopyranose polymer). The pH optima for the degradation of all three substrates were all below pH

5.0 and an analysis of the reaction products indicated that *S. rolfsii* produced *exo*-galactanase, *endo*-mannanase, α-1,6-galactosidase and *endo*-xylanase. These enzymes were also present in *S. rolfsii* infected bean hypocotyl tissue, but they were absent from or in very low concentrations in healthy hypocotyls.

1.7.1.6. The role of cell wall macerating enzymes and cell death

Dry rot diseases are typically caused by necrotrophic parasites and so cell death is a feature of the developing lesion. A role for cell wall macerating enzymes in cell death as well as tissue maceration was first indicated by DeBary (1886); and its possible importance was supported by the researches of Jones (1909) and Brown (1915). Many workers (Wood, 1967; Hall & Wood, 1970, 1973; Basham & Batemen 1975a and 1975b) now suggest that cell death in soft rot diseases is due, indirectly, to the degradation of pectic components of the cell walls. It is suggested that the inability of the weakened cell walls to support the protoplast causes the plasmalemma to stretch and undergo conformational changes under turgor pressure. The protoplasts eventually burst when the limit of elasticity is reached and this is the direct cause of cell death. Support for this view comes from the observation that if tissue is plasmolysed before treatment with pectic enzymes, cell protoplasts are not killed but retain the ability to accumulate neutral red even though the tissue is macerated (Tribe, 1955; Fushtey, 1957; Basham & Bateman 1975a). Furthermore, pectic enzymes do not lyse isolated protoplasts from cucumber (Tseng & Mount, 1974) or onion (Pillet, 1973).

However, Kenning & Hanchey (1980) observed ultrastructural changes in the cytoplasm of bean hypocotyl cells infected with *Rhizoctonia solani* before any changes could be observed in the cell walls, indicating that the ultrastuctural alterations associated with this disease are not solely the consequence of weakening of cell walls and bursting of protoplasts (Misaghi, 1982).

Mount *et al.* (1970) have suggested that pectic enzymes can damage plant cells by direct interaction with the host plasmalemma, and that the sites of interaction between membranes and pectic enzyme may be masked when the plasmalemma contracts away from the wall during plasmolysis. Protoplast death could be the result of the activity of hydrogen peroxide or active oxygen species which are produced by other enzymes released from the cell wall by pectolytic enzyme activity. For example Lund (1973) showed that *Erwinia carotovora* infected cauliflower floret tissue contained high peroxidase activity and Mussel (1973) and Strand *et al.* (1976) have shown that purified

polygalacturonases from *V. albo-atrum* and *F. oxysporum* release proteins with peroxidase activity from all fractions prepared from potato tuber tissue, carrot xylem parenchyma, and etiolated cotton hypocotyls.

In conclusion, for soft rot diseases it has not been possible to establish whether host cell death results from the direct or indirect effects of pectolytic enzyme activity and there is, certainly no evidence to link enzyme activity with cell death in diseases characterised by dry rots.

1.7.2. Phytotoxins as pathogenicity factors

One group of factors which may be important in causing cell death in necrotrophic dry rot diseases are phytotoxins. Phytotoxins may be defined as substances, excluding enzymes, hormones and genetic (nucleic acids) determinants, which are produced by micro-organisms and which are injurious to higher plants. They are usually organic compounds of relatively low molecular weight, which are mobile within plants and which are active at low (physiological) concentrations (Manners, 1993; Goodman *et al.*, 1986). Turner (1984) reported that known toxins are of various chemical types and include peptides, glycoproteins, polysaccharides, organic acids, fatty acids and their derivatives, polyketides and terpenoids. Their modes of action are varied and often incompletely known or even completely unknown (Durbin, 1981; Daly & Deveral, 1983; Knoche & Duvick, 1987; Graniti *et al.*, 1989).

The ability of a pathogen to produce a toxin in culture does not mean that the same toxin will be produced within the plant. It must also be remembered that a toxin may be produced at one stage of a disease but not another (Manners, 1993).

Turner (1984) stated that the evidence for the involvement of a toxin in the cause of disease is usually obtained in several stages. The first stage involves demonstrating that sterile filtrates from cultures of a particular pathogen reproduce part or all of the disease symptoms, and this is followed by the purification of a toxic substance. In order to assay for the presence of the toxin during the purification procedure it is necessary to have a bioassay which is based on part of the natural symptom complex and which involves the use of whole plants or cuttings. The next stage involves showing that the purified compound causes part or all of the symptoms of the disease when applied to the host plant. The final stage is the demonstration that the compound is produced in the infected tissue in amounts which bear a meaningful

relationship to the development of the disease. Further evidence for the direct involvement of a toxin can be obtained using non-toxigenic mutants of the pathogen.

Most classification schemes for toxins, are based on the biological activity of the compounds rather than on their chemical structure (Goodman *et al.*, 1986). One commonly used system classifies toxins into host-specific toxins and non-host-specific toxins.

1.7.2.1. Host-specific toxins

Host-specific toxins are toxins which exhibit host specificity in that the sensitivity or insensitivity of the plant to the toxin parallels the susceptibility or resistance of the plant to the pathogen. Generally, the virulence of the pathogen varies with its capacity to produce the toxin and the toxin produces symptoms in the susceptible plant that are characteristic of the disease.

Victorin (HV-toxin) was the first toxin to be shown to have host-specific activity. This toxin is produced by *Helminthosporium victoriae.* The causal organism of foot and root rot and leaf blight of oat. It is highly toxic to those oat cultivars which are susceptible to *H. victoriae* but has little toxicity for resistant cultivars.

T-toxin (HMT-toxin) is produced by Race T of *H. maydis* the causal agent of southern corn leaf blight, one of the most serious diseases to occur in the recent history of plant pathology. *H. maydis* race T exhibits a high level of virulence on corn lines having Texas male sterile cytoplasm and these are the only plant lines which are sensitive to HMT toxin

A number of other host specific toxins have been reported to be produced by various pathogens, HC toxin by *H. carborum* a pathogen of corn (Scheffer & Ullstrup, 1965; Pringle & Scheffer, 1967a, 1967b, 1967c) PC toxin by *Periconia circinata,* PM toxin by *Phyllosticta maydis* and HS toxin by *H. sacchari* the causal agent of eye spot disease of sugarcane (Steiner & Byther, 1971; Strobel & Steiner, 1972; Strobel *et al.*, 1972; Livingston and Scheffer, 1981). There are also several reports of host-specific toxin production by different *formae speciales* of *Alternaria alternata* (*A. tenuis* (Gilchrist & Grogan, 1976; Templeton, 1972) which are pathogenic to Japanese pear (*Pyrus serotina*), apple (*Malus sylvestris*), citrus (*Citrus reticulata* and *C. jambhiri*) and tomato (*Lycopersicon esculentum*). No host specific toxins have been described for any *Fusarium* spp.

1.7.2.2. Non-host-specific toxins

Goodman *et al.* (1986) has reviewed the role of non-host-specific toxins in pathogenicity. Such toxins have been extensively studied in relation to diseases caused by *Fusarium* spp. Fusarial wilts, which are characterised by leaf epinasty, plugging and browning of xylem vessels, tissue wilting and necrosis appear to be the result of a complex interaction between several toxins, enzymes, and hormones. Each of the toxins, when tested alone, is able to produce only a portion of the disease syndrome. The wilt toxins are also non-specific and cause some physiological changes which are not attributable to the pathogen. For example, fusaric acid, one of the most extensively studied wilt toxins, decreases the respiratory activity of tomato stem tissues, whereas tissues naturally infected with *Fusarium oxysporum* exhibit a marked increase in respiration (Wu & Scheffer, 1962). Fusaric acid is produced by several species of *Fusarium,* mainly belonging to the group Elegans. *F. oxysporum,* in particular, causes wilt diseases of tomato, cotton, pea, banana and other plants. Chemically, fusaric acid is 5-n-butylpicolinic acid (Gäumann, 1957, 1958). Some of the wilt fungi produce other toxic metabolites some of which are closely related to fusaric acid, e. g. dehydrofusaric acid, which has a double bond at the end of the side chain, while others are entirely different compounds.

Fusaric acid is toxic to both higher plants and micro-organisms and interferes with their metabolism in various ways. Before the appearance of visible symptoms on the plant, such as necrotic spots on the leaf blade or shrivelling and drying of leaves, the water permeability of protoplasts and the water balance of the whole plant is altered and respiratory rates decrease (Kern, 1972). It is believed that the primary effect of fusaric acid *in vivo* is on cell permeability. A consequence of this is the disruption of the balance of inorganic ions in diseased plants and leakage of electrolytes (Gäumann, 1957). Fusaric acid inhibits polyphenoloxidase competitively at lower concentrations than it inhibits several other enzymes (Bossi, 1959). It may also interact with various phenol derivatives (Kern, 1972).

Fusaric acid has been detected in a number of plants after inoculation with their wilt pathogens. For example it has been detected in bananas (Page, 1959), flax, tomato and watermelon (Davis, 1967) and a positive correlation has been found between the severity of disease and the amount of fusaric acid present in the diseased species for several strains of *F. oxysporum* f. sp. *niveum* (Davis, 1969). Further more, Kern (1972)

reported that a pathogenic strain of *F. oxysporum* f. sp *pisi* produced considerable amounts of fusaric acid *in vitro* and *in vivo* whereas weakly pathogenic strains did not form fusaric acid either *in vitro* or *in vivo*.

However, despite some positive correlations between pathogenicity and the ability to produce fusaric acid *in vitro* and *in vivo* exceptions have also been reported (Kern, 1972). Studies with several uv-induced mutants of *F. oxysporum* f. sp. *vasinfectum* and of *F. oxysporum* f. sp. *lycopersici* (Panopoulos & Staskawicz, 1981) revealed no correlation between pathogenicity on tomato or cotton respectively, and the ability to produce fusaric acid in culture.

Other low molecular weight fusarial toxins reported from wilt inducing fusaria are phytonivein (a steroid) from *F. oxysporum* f. sp. *niveum*, phytolycopersin from *F. oxysporum* f. sp. *lycopersici,* and lycomarasmin from *F. oxysporum* ff. sp. *lycopersici*, *vasinfectum,* and *melonis* (Kern, 1972). Woolley (1948) reported that lycomarasmin is a tripeptide. It is also a chelating agent and its toxicity is increased by the presence of iron, but it is detoxified by copper, with which it forms a stable chelate. The iron chelate is translocated into the leaves, where the toxin is liberated, causing necrotic spots at the tips of the leaf blade. Lycomarasmin, when chelated with iron, induces an early increase in tomato leaf respiration (Neaf-Roth, 1959) and this compound may be the explanation of the increased respiration in *Fusarium*-infected plants before the appearance of symptoms. At later stages of infection the respiration rate decreases, and this reduction may be due to the action of fusaric acid.

Goodman *et al.* (1986) reviewed the role of non-host specific toxins produced by a number of *Fusarium* spp. other than *F. oxysporum.* Species of *Fusarium* from the group Martiella, including *F. solani, F. javanicum* and *F. martii* produce a number of phytotoxins in culture with a common naphthazarin structure (Kern & Neaf-Roth, 1965, and 1967; Kern, 1972; Kern *et al.*, 1972; Kern, 1978). Kern, (1978) reported the production of six naphthazarins with different chemical structures and biological activities *in vitro* by a number of *Fusarium* spp within the group Martiella including marticin, isomarticin, novarubin, fusarubin, norjavanicin and javanicin. Rapid production of marticin and isomarticin began about two days after inoculation, reaching maximum levels around 5 days, after which the concentrations fell sharply. Other toxins increased more slowly, reaching maximum concentrations much later in growth. Kern *et al.* (1970) reported that fusarubin and javanicin disrupt plant metabolism by inhibiting both

anaerobic and oxidative decarboxylation reactions. A very pathogenic strain of *F. solani* f. sp. *pisi* forms high levels of marticin after four days of shake culture whereas a slightly pathogenic strain formed considerable quantities of javanicin and fusarubin but only traces of marticin.

Baker *et al.* (1981) reported that an isolate of *F. solani* obtained from the fibrous roots of infected citrus, produced naphthazarin phytotoxins in culture. The toxic compounds included marticin, isomarticin, novarubin, fusarubin, norjavanicin, javanicin and anhydrofusarubin. Marticin and isomarticin were the most toxic substances to the plant and the least toxic to bacteria and fungi. At the other extreme were norjavanicin and novarubin, which are much more toxic to micro-organisms than to plants (Kern and Naef-Roth, 1967; Kern, 1972).

Similar correlations between toxins formed *in vitro* and pathogenicity were found with related *Fusarium* strains and species and in the genus *Neocosmospora* (Kern & Neaf-Roth, 1967; Kern, 1972). Marticin and other toxins produced by group Martiella have also been extracted from roots, stems and leaves of diseased pea plants in quantities sufficient to cause serious damage to the tissues.

Many fungi, including numerous *Fusarium* spp and *Cylindrocarpon* spp., produce trichothecenes. These compounds are toxic to plants, some at very low concentrations (Stoessl, 1981). Wyllie & Morehouse (1977) reported that trichothecene producing species of *Fusarium* are common and widespread in nature. Such species occur in soil and in decaying vegetation of all kinds, and many are parasites of wild and cultivated plants. Thus toxins could be isolated from soils and from various samples of fresh, stored, and overwintered cereals, from vegetable crops, fruits and from feed grains. The plant products, if consumed by man and animals, could cause severe illness and even death. Among these compounds are trichothecin, T-2 toxin, diacetoxyscirpenol, diacetylnivalinol, and triacetoxyscirpenediol and they have reviewed by Bamberg & Strong (1971) and Stoessl (1981). Stoessl (1981) has drawn attention to the apparent correlations between the animal toxicity of these compounds and phytotoxicity.

Brian *et al.* (1961) reported that culture filtrates of several strains of *F. equiseti* were highly phytotoxic and several phytotoxic substances were isolated of which the most important was diacetoxyscirpenol. Desjardins and Plantner (1989) reported that a wild-type, trichothecene-producing strain of *F. sporotrichioides* is pathogenic on *Pastinacea sativa* (parsnip) roots and produces T-2 toxin *in planta*.

Zearalenone or F-2 toxin is also produced by other *Fusarium* species classified in different groups including *F. roseum* (Arthrosporiella), *F. tricinctum* (Sporotrichiella), *F. oxysporum* (Elegans) and *F. moniliforme*, (Liseola). Zearalenone and other trichothecenes are produced on maize and barley in storage, and when the grains are fed to animals they cause a condition commonly referred to as hypereostrogenism. The organism most frequently associated with the disease is *F. avenaceum* although *F. tricinctum* can also be found in the feedstuff. Other trichothecenes such as 12, 13-epoxytrichothecene are among the toxic secondary metabolites produced by *F. tricinctum*, *F. roseum*, *F. oxysporum*, *F. solani*, *F. nivale*, *F. lateritium*, *F. rigidiusculum* and *F. espipheria*. Also T-2 toxin is one of the toxins produced by *F. tricinctumi* which inhibits auxin-promoted elongation of hypocotyls (Stahl *et al.*, 1973). *F. moniliform* (*Gibberella fujikuroi*) in particular produces relatively large amounts of moniliformin a myco and phytotoxic metabolite as well as large numbers of gibberellins (Panapoulos & Staskawicz, 1981). Lafont *et al.* (1983) found several trichothecene toxins in potato tubers both naturally and experimentally infected with *F. sambucinum*. Elbenna *et al.* (1984) reported that *F. coeruleum* as well as *F. sambucinum* produced trichothecenes such as deoxynivalenol and HT-2 toxin in potato tubers and in liquid media. Trichothecenes were found in dry rotted tubers stored at both 4°C and at 15°C for up 71 days and after growth in culture at 23°C for up 21 days. Desjardins & Planttner (1989) reported that diacetoxyscirpenol and other trichothecene toxins are produced by both *F. sambucinum* and *F. sulphureum*. These compounds are potent phytotoxins and inhibit protein synthesis in eukaryotes (Latus *et al.*, 1987; Steyn *et al.*, 1978). Desjardins & Planttner (1989) found most strains of *F. sambucinum* they tested were able to rot tuber slices of potato and produce 15-monoacetoxyscirpenol and 4,15-diacetoxyscirpenol as well as other minor trichothecenes in infected tubers. Thus the production of trichothecene toxins may be a common trait of *Fusarium* species causing dry rot in potato tubers that these toxins may be produced in potatoes naturally infected with this fungus in the field or in storage. Whether or not they are involved in pathogenicity depends upon the specific stage of the infection when they are produced. To be important in the mechanisms of infection they must be produced at, or soon after, germination. Ueno *et al.* (1973) reported that *F. solani* produced trichothecene when grown in a nutrient medium consisting of glucose, sucrose, peptone and yeast extract, either in shake culture or by jar fermentation at 24-27°C. Ueno *et al.* (1975) reported

that the growth of *F. solani* was maximum on day 3 after incubation by which time the glucose in the medium had been completely consumed. The production of the trichothecene began soon after inoculation and the maximum amounts were present 2 days after inoculation after which the levels declined. Thus trichothecene could be involved in the processes of infection.

1.8. The prevention and control of tuber dry rots

1.8.1. Optimum storage conditions

Since, as was reported earlier, infections invariably develop through wounds, careful handling to minimise all mechanical damage is one of the most important measures that can be taken to prevent potato dry rot. Wounding and bruising at any stage of the handling of the crop, from lifting to storage and during grading for sale after storage, should be avoided. Potato tubers should be mature when lifted. After the vines have been killed, lifting should be delayed for several weeks to allow the tuber skins to set. Potatoes should not be harvested on cold, frosty mornings because they bruise very easily under such conditions. After harvest, tubers should be stored at a relatively high temperature, as near as 20°C as possible, and in a moderate humidity for several days to allow wound healing to occur. Wound healing is much more rapid at around 20°C than at low temperatures. On average, the wounded surface suberizes in about 1-2 weeks at 2.5-5°C, but in about 4 days at 10°C, and 1-2 days at 20°C. Complete healing of the superficial layer of cells could take 3-6 weeks at 5°C, 1-2 weeks at 10°C, but only 3-6 days at 20°C. Once the wounds in the tuber have been allowed to heal the storage temperature can be reduced to the optimum storage temperature of about 5°C. Many modern potato stores are air conditioned and provide these optomum conditions. Chemical treatment of seed potato tubers can give good control. Dusting after lifting and before storage, with TCNB (tecnazene), which is a volatile compound, used to be a common treatment. It was effective for clamped and bulk stored tubers, but it would retard sprouting (Lapwood & Hide, 1971). Seed tubers which had been treated with TCNB especially of early varieties, which are particularly susceptible to dry rot, were removed from store and boxed for at least 6 weeks before planting to allow any residual TCNB to disperse so as to allow the sprouts to grow. Washing the tubers after lifting can remove some of the inoculum and when combined with an organo-mercury dip before drying and storing in boxes this procedure could also give satisfactory control of the disease (Lapwood & Hide, 1971). However, the concentration of the organo-mercury

was critical and too high a dose gave sprout damage. Both these procedures have been superseded with the development of safer chemicals. Now, spraying with a solution of 1200 ppm thiabendazole (TBZ) or benomyl after washing is more commonly employed (Leach, 1975; Leach & Nielsen, 1975; Hide & Cayley, 1980). Control by such materials can vary with the pathogen, 0.1% carbendazim, for example, being very effective against *F. coeruleum* but is less effective against other species, although it still gives appreciable control of *F. sulphureum* (Burton, 1989). Combinations of fungicides and antibiotics have been tried in the U.S.A. but are not usually recommended (Rich, 1983).

1.8.2. The production of resistant varieties

Growing resistant varieties offers an environmentally friendly and cost effective method for controlling disease. Methods for screening potato genotypes for resistance to those *Fusarium* spp. which cause tuber dry rots have attracted considerable interest in many countries. Many workers have investigated different methods of inoculation for screening purposes with variable results (Boyd, 1952c; McKee, 1954; Seppanen, 1981c, 1982a; Jellis, 1975; Jellis & starling, 1983; Leach & Webb, 1981; Langerfeld, 1983; Tivoli *et al.*, 1986; Wastie *et al.*, 1989; Wastie & Bradshow, 1993).

Since *Fusarium* spp. are wound parasites the type of wound produced to insert the inoculum can affect results. Furthermore, susceptibility of the tuber to mechanical damage can influence infection, although such susceptibility is not assessed in inoculation procedures. Wellving (1976) studied the relationship between resistance and wounding and found that potato clones, when injured mechanically, had wide genotypical differences in ability to resist penetration and invasion by *Fusarium* spp. and he suggested that both wound resistance and biochemical resistance to the pathogens should be considered when testing genotypes for resistance to potato dry rot.

However, there is little evidence for the existence of very high levels of resistance to *Fusarium* dry rots in cultivated or wild species of *Solanum* and complete resistance is not known. Thus it is difficult, currently, to breed for high levels of resistance.

Nonconventional plant breeding programs which involve the induction of increased variation in plant populations may provide a way forward. The use of chemical or other mutagens, such as radiation to generate increased variation is one possibility. Another possibility would be to select among the great range of variants that are present naturally among populations of microspores or which appear to be present naturally

among cell cultures produced from whole plants. This latter form of variation is known as somaclonal variation.

Earle (1982) reported that the most difficult obstacle to the production of useful resistant material in a conventional breeding program was the screening of progeny from crosses and the development of stable resistant lines of good quality. This obstacle is likely to be greatest when multiple genes control resistance traits rather than single major genes. She reported that screening for resistance among anther cultures may prove helpful. Culture of pollen grains gives either haploid plantlets or haploid callus from which haploid plantlets can be obtained (Maheshwari *et al.*, 1980). Recessive traits are exposed in such haploid plants or in the diploids obtained by chromosome doubling of the haploid plants. Favourable combinations of genes for disease resistance may therefore be selected for further breeding use more readily than from a normal sexually generated progeny where useful recessive genes could be masked in the heterozygous condition. Haploid techniques have been applied with success to tobacco (Nitsch & Nitsch, 1969), rape (Thomas & Wenzel, 1975), wheat (Ouyang *et al.*, 1973) and rice (Niizeki & Oono, 1968). The feasibility of this approach clearly depends on the status of anther culture technology for the plant material involved. This technique has been used with the Solanaceae (Johansson, 1986) and the yield of haploid plants per cultivated anther has usually been high.

Clarston (1973), very early on, demonstrated that cells and protoplasts can be screened in culture for resistance to pathogen toxins and that the plants regenerated from such cells often have an altered response to infection by that pathogen. Since those early days a great many claims have been made about the potential of cell-culture selections for the development of novel disease-resistant germ plasm (Daub, 1986). Daub has suggested that probably the best approach to developing systems for selecting for disease resistance in culture is to use pathogen toxins as the selecting agent. The use of toxins overcomes some of the problems which are involved when using the pathogen itself, since individual cells can be exposed easily and uniformly to toxins by dispersing the cells in a toxin solution or plating them on toxin-containing media. Culture filtrates containing specific toxins, have been used to select a range of resistant germ plasm from cell cultures. The host specific toxin, T toxin, from *Helminthosporium maydis* has been used to select maize lines for resistance to the fungus (Earle, 1982). Non-specific toxins in culture filtrates of *P. infestans* have also been used to select toxin resistant cells of

potato *in vitro* (Behnke, 1979). The leaves and stems of plants regenerated from unselected callus were damaged by the toxin but plants regenerated from resistant callus were not.

In recent years (Daub, 1986) research has been directed increasingly towards the isolation of mutants from plant tissue cultures without applying any selection. These efforts have been based on the observation that the process of growing plant cells in culture yields a high frequency of stable, heritable variants, which express traits useful in crop improvement. This variation has been termed somaclonal variation (Larkin, & Scowcroft, 1981).

Jellis *et al.* (1984) regenerated plants from isolated protoplasts of three potato cultivars Maris Piper, Foxton, and Feltwell and found specific improvements in resistances to common scab (*Streptomyces scabies*), potato virus Y (PVY), and potato leafroll virus (PLRV). In their 1982 scab trial, six protoclones from Maris Piper and 82 protoclones from Feltwell were classified as more resistant than their parents. In a second trial in 1983, three of the six Maris Piper clones and 41 of the 44 Feltwell clones proved of have superior resistance to scab. Of 70 Maris Piper clones tested for virus resistance, seven were resistant to PVY and 11 to PLRV and 32 and 31 of 288 Feltwell clones showed increased resistance to PVY and PLRV respectively.

Wenzel (1985) reported that somaclonal variants are superior to variations generated by mutation when selection pressure is applied during the *in vitro* phase. The common advantage the pathogen has over the host, namely the ability to shift rapidly to a virulent population, is offset somewhat by the fact that host cells can be about as numerous as the pathogen and that flexibility for change in the host cell population is about as great as it is in the pathogen population. However, to offset this common advantage of the pathogen requires a powerful in vitro screening system and a very large number of individual host cells.

1.9. The aims of the project

Different species of fungi, particularly of *Fusarium* cause dry rots in potato tubers and given cultivars show different degrees of resistance to particular fungal species. The aims of the project were.

1. To investigate the mechanisms of pathogenicity of 3 different dry rot causing fungi *F. coeruleum, F. avenaceum* and *C. destructans,* to determine if they each cause dry rot symptoms by the same or by different mechanisms.

2. To investigate the basis of the dry rot nature of the disease and determine why the fungi do not macerate the tissues since all are known to produce cell wall degrading enzymes in culture.
3. To determine the role of low molecular weight toxins in the mechanisms of pathogenicity.

CHAPTER II

GENERAL MATERIALS AND METHODS

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2.1. Fungal cultures

During 1989-1990 tubers of potato cvs Maris Piper and Record and several unknown cultivars were collected for the isolation of dry rot causing fungi. The fungi were isolated using conventional methods.

Tubers with dry rot lesions were surface sterilised in 10% Chloros (S.A.I.) solution for 5 min and then washed with sterilised water. Small pieces of the inner tissues were transferred, with a needle, into Petri dishes containing potato dextrose agar (PDA), potato sucrose agar (PSA) and oatmeal agar (see Appendix A for media formulae). In some cases small pieces of tuber tissue were first placed on moist filter papers in Petri dishes to stimulate hyphal growth on the surface before attempting to isolate onto agar media. The plates were incubated at 22°C ± 2°C in an incubator in the dark, or on the laboratory bench at about 20°C in a day/light cycle. After 3-5 days the mycelium had grown sufficiently to be isolated onto PDA, PSA or oatmeal agar. Each isolate was finally prepared as a single spore culture. For the purposes of identification all cultures were grown and tested for pathogenicity on potato tubers of cv. Maris Piper. Isolates of *Fusarium* spp were identified according to Booth (1971) and *Cylindrocarpon destructans* according to Barron (1972), on the basis of mycelium type, spore size, conidiophore, structure and type of growth and colour of culture. The identifications were confirmed by the International Mycological Institute, Bekeham Lane, Englefield Green Egham Surrey U.K.

Four isolates each of *F. coeruleum* and *F. avenaceum* and 3 isolates of *C. destructans* were obtained. The isolates of *F. coeruleum* and *C. destructans* were maintained on potato dextrose agar (PDA), while those of *F. avenaceum* were maintained on oatmeal agar. Cultures were stored in a cold room at 5°C. For all experiments a spore inoculum was obtained from 3 to 4 weeks old cultures.

2.2. Production of conidial inocula

A dense spore suspension consisting of a mixture of macro and microconidia was used as the inoculum in all experiments, and so an initial survey was carried out to determine the best conditions for spore production by each species. The following media

were tested: PSA, PDA, carrot potato dextrose agar (CPDA), oatmeal agar, soil extract agar and Czapek-Dox agar see Appendix A for media formulae.

Cultures were grown in each medium in Petri dishes or on slants in tubes and incubated at three different temperatures 22 ± 2°C or 15°C in the dark, and in a day/night cycle at room temperature of about 20°C for 2 to 4 weeks. PDA was found to support the best sporulation by isolates of *F. coeruleum* and *C. destructans* and oatmeal agar by isolates of *F. avenaceum* .

All inocula were prepared by washing spores off 3-week-old cultures of *F. coeruleum* and *C. destructans* or 4-week-old cultures of *F. avenaceum* with sterile distilled water. The spore concentration in the suspension was determined using a haemocytometer. The spore suspension was diluted with sterile distilled water to give the required spore concentration in the inoculum.

2.3. Potato cultivars

Tubers of potato cv Maris Piper and Record were obtained from the Glasgow Fruit Market and stored in a cold room at 5°C until required. The two cultivars were chosen for use because of their reported different susceptibilities to the dry rot fungus *F. coeruleum*, Maris Piper being more susceptible than Record (Jellis, 1975; Jellis & Starling, 1983; Wastie *et al.* 1989). Prior to inoculation, the tubers were surface sterilised in 10 % Chloros (SAI) for 15 min. After rinsing in tap water the tubers were dried with tissue paper or allowed to dry overnight at room temperature.

2.4. Inoculation of whole tubers

Tubers were surface sterilised for 15 minutes in 10% Chloros before inoculation. A cylindrical hole, 2 mm in diameter and 6 mm in depth was made by pushing a metal rod into the tuber half way between the rose and heel end. The rod was sterilised each time by immersion in alcohol and flaming. Tubers were inoculated by injecting 0.1 ml. of a spore suspension into the inoculation hole with a syringe.

2.5. Comparison of the pathogenicities of different dry rot fungi in whole tubers

After inoculation, the tubers were placed in plastic bowls on a layer of damp peat based compost with a layer of tissue paper between the tubers and compost. Each bowl was placed in a plastic bag for the first week and placed on shelves in a growth room at 20±1°C and about 75-85% relative humidity.

An inoculum was prepared for each isolate and adjusted to give a range of spore concentrations from $6x10^1$ to $6x10^5$ conidia ml^{-1}. Ten tubers of cv Maris Piper and 10 of cv Record were inoculated with each level of inoculum. The inoculated tubers were incubated as described above.

The size of the dry rot lesion developed in each tuber was recorded at intervals by measuring the diameter of the rotted zone visible on the surface of the tuber in two directions at right angles to one another. The average diameter was recorded for each tuber. The final size of the lesion inside the tuber was measured after cutting the tuber in half through the inoculation hole. The maximum depth and diameter of the lesion was measured. Control tubers were wounded and injected with sterile distilled water before incubating under the same conditions as the inoculated tubers.

2.6. Germination and the early stages of infection on tuber slices

2.6.1. Preparation of tuber slices and inoculation

Tubers were surface sterilised and then cut aseptically into slices 2-2.5 cm thick using a knife which had been sterilised by flaming. The slices were placed in containers lined with filter paper soaked with sterile distilled water to maintain a high relative humidity around the slices. The slices were then inoculated by spreading 0.5 ml of a spore suspension containing 6×10^6 conidia ml^{-1} over the cut surface. The containers were closed and incubated in the dark at 22° ± 2C. Control slices were treated with sterile distilled water and incubated under the same conditions.

2.6.2. Measurement of spore germination and germ-tube lengths on slice surfaces

The germinated spores were scraped from the slice surface into a small test tube containing 1 ml of sterile distilled water. After shaking the tube, a drop of suspension was transferred to a glass slide and stained with lactophenol cotton-blue. The numbers of germinated and ungerminated spores per field of view were counted. Germ-tube lengths of sporelings were measured using an ocular micrometer. Mean percentage spore germination and germ-tube length were determined from samples taken from three different tuber slices.

2.6.3. Development of dry rot lesions in slices

The development of dry rot lesions across the tuber slice was followed at daily intervals, up to 8 days after inoculation using a binocular microscope, Eight days after inoculation the maximum depth of the lesion in each slice was measured after cutting

them in half. The amount of fungal mycelium produced in the infected slices was determined by analysing for chitin as described below.

2.7. Chitin analysis

The method used was essentially that used by Ride and Drysdale (1972).

2.7.1. Reagents:

Solution A 5% (w/v) $NaNO_2$

5% $KHSO_4$

12.5% $NH_4SO_3NH_2$

0.5% 3-methyl-2-benzothiazolone hydrazone hydrochloride (MBTH).

Solution A was prepared daily.

Solution B 0.5% $FeCl_3$ (0.83g $FeCl_3$ $6H_2O$ dissolved in 100 ml water)

Solution C KOH (120g dissolved in 100 ml water)

Solution D 75% aqueous ethanol

Solution E 40 % aqueous ethanol

Solution F Celite suspension; obtained after mixing 1g Celite 545 with 20 ml of 75% ethanol and allowing to stand for 2 minutes .

The reagent solutions were stored in a cool place and discarded after 3 days.

2.7.2. Preliminary extraction of tuber tissue

Samples of tuber tissue, infected or control, (30 mg) were macerated in 15 ml of cold acetone and homogenised for 15 min with a pestle and mortar. The homogenate was then transferred to a glass centrifuge tube and centrifuged at 1500 rpm for 10 min. The supernatant was removed and the solid residue, after washing three times with 10 ml of cold distilled water was recentrifuged at 1500 rpm for 10 min.

2.7.3. Hydrolysis of chitin

The residue obtained after centrifugation was deacetylated and hydrolysed to convert chitin to chitosan by autoclaving in 3 ml KOH (solution C) at 130°C for 1h. After cooling, the alkaline solution was mixed with 8 ml of 75% ethanol and the tube placed in ice water for 15 min. Then, 0.9 ml of celite suspension was layered on top of the alkali solution and the tubes centrifuged as before. The supernatant was removed and the residue first washed twice with 40% ethanol and then three times with cold distilled

water. The final residue was made up to 1.5 ml with distilled water and assayed for chitosan .

2.7.4. Colorimetric assay for glucosamine

Five percent $KHSO_4$ and 5% $NaNO_2$ (1.5 ml each) were added to the chitosan suspension. The two reagents reacted to form HNO_2 which deaminated and depolymerised the chitosan (Thornton *et al.*, 1991). After the addition of the reagents the suspension was shaken using a vortex mixer for 15 min and then centrifuged at 1500 rpm for 2 min. Two samples (1.5 ml) were removed from the supernatant and to each sample was added 0.5 ml of a 12.5% solution of ammonium sulphate. The samples were shaken for 5 min, and then 0.5 ml of 0.5% MBTH was added and the mixture heated in a boiling water bath for 3 min. After cooling to laboratory temperature, 0.5 ml of 0.5 % $FeCl_3$ was added to each tube and the mixtures allowed to stand for 30 min for the blue colour to develop. The absorbance was measured at 650 nm in a spectrophotometer. A calibration curve was prepared using standard solutions of glucosamine hydrochloride. A linear relationship was found between absorbency at 650 nm and glucosamine equivalents over the range 4.5 µg - 40.5 µg glucosamine per 1.5ml sample (Fig 3). The chitin content of infected and uninfected tuber tissue was determined from the calibration curve.

2.8. Electron microscopy

2.8.1. Standard preparation of thin sections

Blocks of tissue (1-3mm^3) were cut from the edge of lesions and fixed in 3% gluteraldhyde in 0.2 M sodium cacodylate buffer, pH 7.2 at room temperature for 12-16 hours. After washing in four changes of the same buffer over 24 hours the tissue was post fixed in 1% osmium tetroxide in buffer for 3 hours at room temperature. Samples were washed in distilled water (3 changes over 30 min) and block stained in 2% aqueous uranyl acetate for 2 hours. The samples were then dehydrated through a graduated ethanol series (25%, 50%, 75% for 2h each and overnight in 100%), embedded in Spurr's resin and polymerised at 60°C for 24 hours. Sections of approximately 60 nm thickness were cut on an LKB III Ultramicrotome using a diamond knife and mounted on 300 mesh grids. Sections were stained with saturated uranyl acetate in 50% methanol for 20 minutes followed by Reynold's lead citrate for 5 minutes. They were examined using a Philips EM 301 electron microscope at 60 and 80 KV.

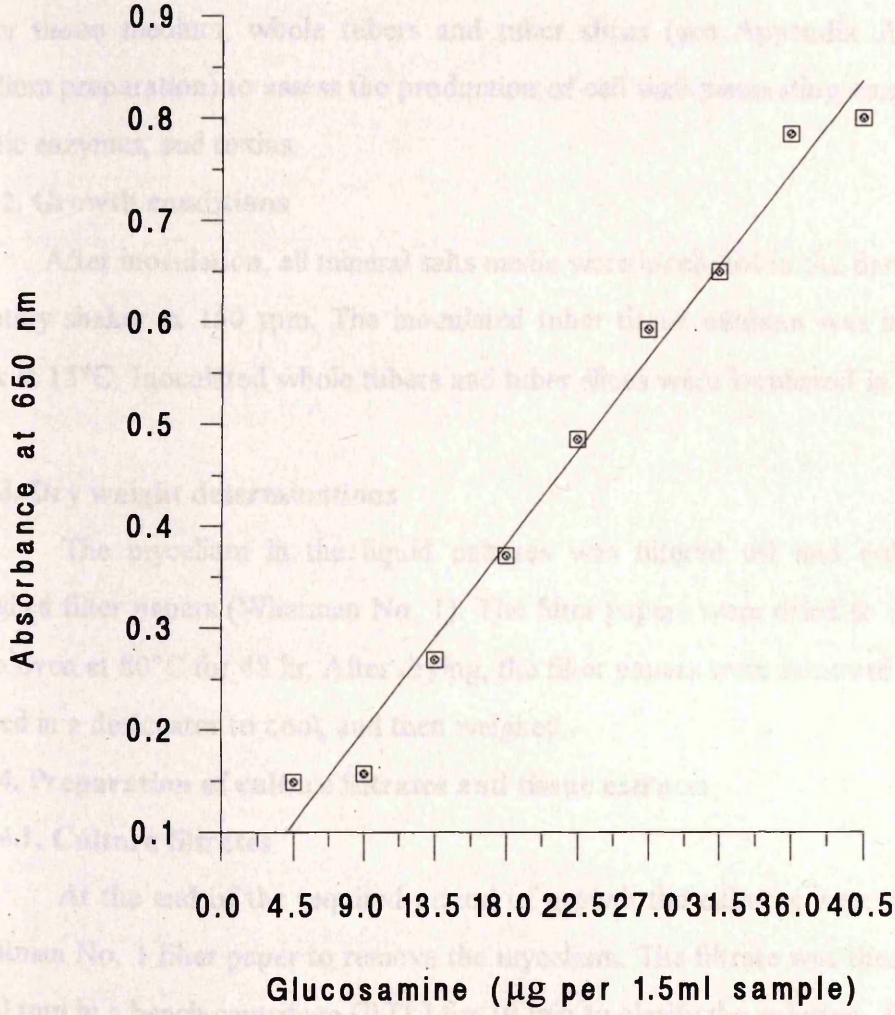

Fig. 3 Typical chitin assay calibration curve absorbance at 650nm against 0-40.5 µg glucosamine

2.9. Production of cell wall degrading enzymes and toxins by different isolates of *Fusarium* spp and *C. destructans*

2.9.1. Media

The isolates were cultivated on a variety of nutrient salts media, Czapek-Dox liquid medium, mineral salts liquid medium (Pringle & Scheffer, 1963), Baker *et al*'s (1981) medium and media derived from potato tubers including potato broth medium, tuber tissue medium, whole tubers and tuber slices (see Appendix A for details of medium preparation) to assess the production of cell wall macerating enzymes, including pectic enzymes, and toxins.

2.9.2. Growth conditions

After inoculation, all mineral salts media were incubated in the dark at 23±2°C on a rotary shaker at 150 rpm. The inoculated tuber tissue medium was incubated in the dark at 15°C. Inoculated whole tubers and tuber slices were incubated in the dark at 20° C.

2.9.3. Dry weight determinations

The mycelium in the liquid cultures was filtered off and collected on pre-weighed filter papers (Whatman No. 1). The filter papers were dried to constant weight in an oven at 80°C for 48 hr. After drying, the filter papers were removed from the oven, placed in a desiccator to cool, and then weighed.

2.9.4. Preparation of culture filtrates and tissue extracts

2.9.4.1. Culture filtrates

At the end of the required period of growth the cultures were filtered through Whatman No. 1 filter paper to remove the mycelium. The filtrate was then centrifuged at 4000 rpm in a bench centrifuge (BTL) for 10 min to clarify the solution. The pH value of the culture filtrate was determined and used for analysis after dialysing overnight against distilled water at 4°C or with undialysing extracts. The extracts were generally divided into a number of fractions and stored in a deep freeze until required.

2.9.4.2. Tissue extracts

Extracts were prepared from the rotted tissues produced after inoculating whole tubers or tuber slices and from the tuber tissue medium. The rotted tissues from whole tubers and tuber slices were first removed aseptically with a sterile knife. The rotted tissues were then suspended in cold sterile water, or in 0.25 M NaCl in a ratio of 1:1

(w/v). The suspension was homogenised in a Warring blender for three minutes and then filtered through several layers of cheese-cloth to remove mycelium and much of the potato tissue. The extract was finally centrifuged at 4000 rpm for 20 min to remove particulate matter. The resultant extracts were brown or black in colour and were either used immediately or stored in a deep freeze until required.

2.9.5. Assays of enzyme and toxic activities of culture filtrates and tissue extracts

2.9.5.1. Chemicals and solutions

Apple pectin (BDH Chemicals Ltd., Poole, England) and sodium polypectate (Sigma Chemicals Co., St Louis, USA) were washed to remove sugars and other low molecular weight compounds as described by Stephens (1974). The pectin or sodium polypectate (25g) was added to 150 ml of 70% ethanol containing 0.05 M HCl and stirred for 1 hr using a magnetic stirrer. The pectins were then collected by filtration through Whatman No. 1 filter paper and washed several times with 70% ethanol until the washings were free from detectable chlorine using $AgNO_3$ solution. The washed pectins were then placed on aluminium foil and dried in an oven at 70°C. Solutions of pectin or sodium polypectate for the enzyme assays, were prepared by slowly adding the solid to water at a temperature of 80-90°C while stirring vigorously on a magnetic stirrer.

The following buffers were used and prepared as described by Kamoun (1977).

(A) Sodium acetate (0.1 M)-acetic acid (0.1 M); range pH 3.8-5.6

(B) KH_2PO_4 (0. 1 M)-Na_2HPO_4. $2H_2O$ (0.1 M); range pH 5.0-8.0

(C) Tris (hydroxymethyl) aminomethane (0.2 M)-HCl (0.1 M); range pH 7.0-9.0

(D) Glycine (0.1 M)-NaOH (0.1 M); range pH 8.6-12.8

The neutral red solution, 0.001%, was made up in 0.9 M KNO_3 buffered with 0.1 M phosphate buffer at pH 7.5.

2.9.5.2. Tissue maceration and cell death assays

2.9.5.2.1. Preparation of tuber tissue discs

Cores of medullary tissue were cut from healthy tubers using a 6 mm diameter cork borer. Discs, approximately 0.4 to 0.6 mm thick, were cut with a bench microtome (LAB-LINE Instruments, INC. Melrose Park, ILL.). The discs were washed thoroughly with distilled water and then immersed in test solutions in a watch glass. For each experiment all the discs were prepared from the same tuber in order to obtain uniform tissue.

2.9.5.2.2. Tissue maceration

Tissue discs were immersed in 3 ml of culture filtrate in a watch glass either without modification or after adding buffer and adjusting to the pH required. After various periods of time, 5 discs were removed and assessed for cell separation. The degree of separation was estimated by rating the ease with which the discs could be pulled apart with a needle and spatula, on a scale (0-5) where a rating of 0 indicates fully coherent tissue comparable to that of the control, while 5 indicates complete loss of coherence.

2.9.5.2.3. Cell death

Cell death was assessed by the neutral red (plasmolytic) method described by Tribe (1955). Culture filtrates were used without modification or after adding buffer and adjusted to the pH required. In each experiment, tissue discs were treated by immersing in 3 ml culture filtrate in a watch glass. After different times of treatment 4 discs were transferred to 3 ml of neutral red solution, prepared freshly for each estimation. After 20 min the neutral red solution was replaced with 3 ml of the 0.9 M KNO_3 solution at pH 7.5. The percentage viability of the cells was determined using a microscope to count the numbers of cells which accumulated and retained the neutral red dye.

$$\text{Mean\% number of viable cells} = \frac{\text{Mean cells retaining dye in treated tissue} \times 100}{\text{Mean cells retaining dye in control tissue}}$$

Mean percentage cell death=100–Mean percentage viable cells

2.9.5.3. Pectin enzyme assays

2.9.5.3.1. Pectin methyl esterase (PME)

Pectin methyl esterase activity was determined by the cup plate assay method as described by Quantick *et al.* (1983).

Pectin (2.5 g) was dissolved in 245 ml distilled water and the pH adjusted to 6.0. Methyl red solution (5 ml), 0.025 g salicylanilide and 5.0 g agar were added and the mixture sterilised by autoclaving at 15 lbs pressure for 15 min. Twenty five millilitres were pipetted into 9.0 cm diameter Petri dishes and, after allowing it to set, 9.0 mm diameter agar plugs were removed with a sterile cork borer to produce a series of wells. Culture filtrate or extracts (3.5 ml) were added to 1.0 ml phosphate buffer at pH 6.0. The pH of the solution was adjusted with 0.1 M HCl as necessary. Water was then added to bring the volume of the solution up to 5.0 ml, and 0.5 ml of this solution was

pipetted into each well. The plate was then incubated at 25°C for 18 h. The liberation of pectic acid from the pectin, by the action of PME, was demonstrated by the development of a red zone around the well, and enzyme activity was determined by measuring the diameter of the zone.

2.9.5.3.2. Hydrolase and lyase assays

Pectic hydrolase and lyase activities were assayed by the thiobarbituric acid (TBA) method described by Ayers *et al.* (1966). The standard reaction mixture contained; 3.0 ml 1% w/v pectin or sodium polypectate, in 1.0 ml buffer 0.1 M Tris-HCl at pH 9.0 or acetate at pH 5.0. The solution was adjusted to the required pH with 0.1 M HCl or 0.1 M NaOH as required and water was added to bring the volume of the solution up to 5 ml, after which 4.0 ml culture filtrate or extract and 1.0 ml of 0.1 M $CaCl_2$ were added.

The mixture was incubated in a water bath at 28°C for 4 h at the end of which 0.6 ml of 9% (w/v) $ZnSO_4$. $9H_2O$, and 0.6 ml of 0.5 M NaOH were added. The mixture was then shaken and centrifuged at 4000 rpm for 15 min. Five millilitres of the reaction mixture was taken, and 3.0 ml of 0.04 M thiobarbituric acid (TBA), 1.5 ml of 1.0 M HCl and 0.5 ml of distilled water were added. After shaking, three tubes per sample were placed in a boiling water bath for 30 min. The red chromogen produced after cooling was measured in a spectrophotometer at 515 nm to determine hydrolase activity and at 550 nm to determine lyase activity. Blanks were prepared in the same way but using boiled culture filtrates or extracts.

2.9.5.3.3. Assay for *endo* enzyme activity

Endo enzyme activity was determined by measuring the change in viscosity of a pectic solution using a U tube viscometer BS/u IP71 size C (viscosity range cS 6-45). The viscometer was suspended in a water bath at 28°C. The reaction mixture contained 5.0 ml 2% (w/v) pectic substrate (pectin or sodium polypectate) in 2 ml acetate buffer at pH 5.0 or glycine buffer at pH 9.0. If necessary the pH of the solution was adjusted with 0.1 M HCl or 0.1 M NaOH. Water was then added to bring the volume of the solution up to 8.0 ml and finally 1.0 ml of 0.01 M $CaCl_2$ was added. One millilitre of enzyme solution at 28°C was added to the reaction mixture, mixed within the viscometer and the reduction in viscosity was measured at intervals. Enzyme activity was expressed as 1000/t where t is the time in minutes for 50% reduction in viscosity. All viscometers were calibrated against water which was taken as the level of viscosity of a pectic

solution after 100% reduction in viscosity. While the viscosity of 10 ml of reaction mixture incubated with boiled enzyme represented 0% reduction.

2.8.5.3.4. Assay for *exo* enzyme activity

Exo activity was assessed by determining the production of mono, di, tri and higher polymers of galacturonic acid using paper chromatography. The reaction mixtures contained: 1.0 ml of enzyme preparation and 4.0 ml 2% w/v (pectin or sodium polypectate) in 3 ml acetate buffer at pH 5.0 or glycine buffer at pH 9.0. The pH of the solution was adjusted with 0.1 M HCl or 0.1 M NaOH as required and water was added to bring the volume of the solution up to 10 ml. The reaction mixtures were incubated at 28°C in a water bath. After different times of incubation from 1 h to 24 h, extracts were spotted on chromatography paper (Whatman No. 1) and the chromatograms developed by ascending chromatography using the following solvent mixture; n-butanol/acetic acid/ water (2:1:1,v/v/v) for 8 h at about 22°C. The chromatograms were then dried overnight and sprayed with silver nitrate reagent. After spraying the chromatograms were dried at 90°C for 5 min when yellow - brown to dark colour spots were produced. The R_f's of the spots were compared with that of galacturonic acid.

2.10. Toxin purification

One litre of boiled culture filtrate or extract was extracted with an equal volume of ethyl acetate in a liquid-liquid extraction apparatus. The mixture was extracted at about 70°C for 2 h and the extract was concentrated in a rotary evaporator at a temperature of between 40-45°C to give a yellow brown gummy material. This material was dissolved in 1 ml ethyl acetate and then subjected to preparative thin layer chromatography (TLC) on 20 cm silica gel plates G-25 UV_{254}. The plates were developed using one of two solvent systems; n-butanol/acetic acid/water (4:1:5, v/v) and propanol/acetic acid/water (200:3:100, v/v/v), at about 22°C. The developed chromatograms were dried overnight and examined under ultraviolet light (366 and 254 nm). The R_f's of all fluorescent and non fluorescent bands were noted and each band was then scraped from the plates and extracted in 10 ml water, shaking for a few minutes to dissolve the components before filtering. The aqueous extracts were made up to 10 ml with water and this solution, refered to as the concentrated extract, was used for toxicity tests. A dilution series of this concentrated extract was made, and each was tested for toxic activity on tissue discs of potato.

CHAPTER III

A COMPARISON OF THE PATHOGENICITY OF DIFFERENT ISOLATES OF *F. coeruleum, F. avenaceum* AND *C. destructans* TO POTATO CVS MARIS PIPER AND RECORD

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3.1. Introduction

These experiments were carried out to compare the pathogenicity of the different isolates of the three species of potato dry rot causing fungi which were isolated and to look for differences in their modes of pathogenicity. The experimental procedures are described in Materials and Methods.

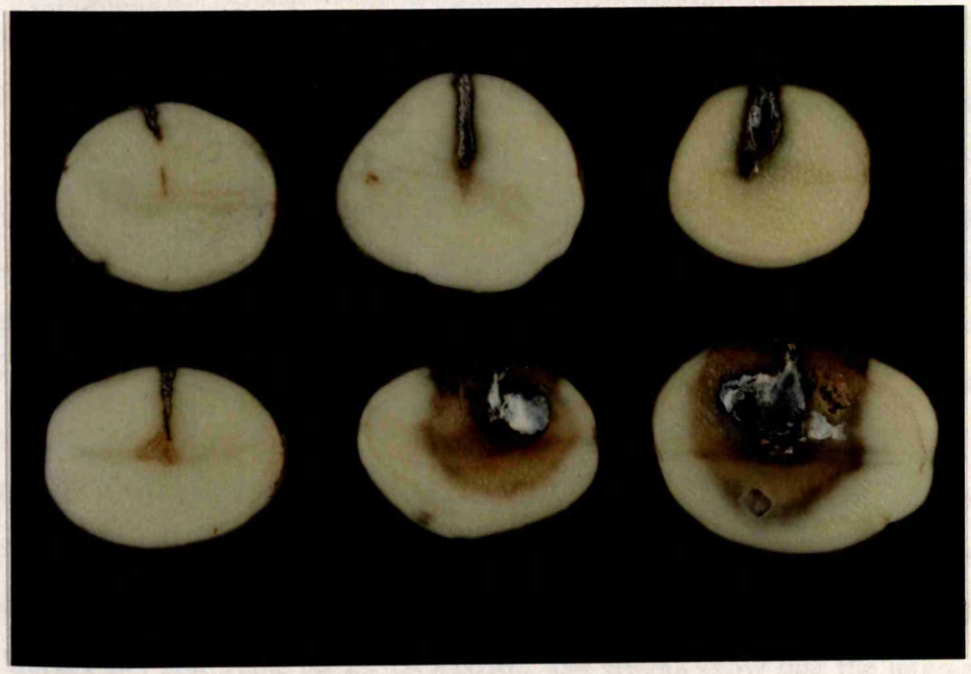
3.2. *F. coeruleum*

3.2.1. Description of lesions produced in cvs Maris Piper and Record

All four isolates of *F. coeruleum* caused dry rot lesions which were essentially similar to each other. One week after inoculation, dry rot was visible as small brown lesions around the edges of the inoculation holes. The rotted tissue was visible through the periderm, being coloured light to dark brown later on pustules of the fungus grew through the periderm and the surface of the lesion became covered with white, light, beige, or blue green mycelium. In the internal tissues of the tuber, lesion tissues were fairly moist and light brown at first but became darker brown and dry with age. Within the tubers the lesions spread equally in all directions with cavities developing in the centre around the inoculum hole. The cavities were lined with fungal mycelium. After long incubation periods most of the tubers became completely rotted, the tissues becoming wrinkled, dry and shrunken. With progressive shrinkage the tubers become very hard and covered with white to blue green mycelium (Plates 1 and 2) .

Plate 1. Dry rot lesion caused by *F. coeruleum*

1 2 3



4 5 6

Plate 2. Lesions produced internally in tuber tissue after inoculation with dry rot causing fungi:Tuber 1 control, tubers 2, 3 and 4 *C. destructans* isolates Cd1, Cd2 and Cd3, tubers 5 and 6 *F. coeruleum* isolates Fc1and Fc2

3.2.2. Comparison of the pathogenicities of four different isolates of *F. coeruleum* to cvs Maris Piper and Record

3.2.2.1. Assessment of relative pathogenicities from measurements of lesion expansion visible at the tuber surface

3.2.2.1.1. Experiments 1 and 2

Two experiments were carried out using all four isolates and both cultivars, one in March 1991 and the second in January 1992. Each experiment was set up as described in Materials and Methods.

In both experiments 24 days after inoculation all 10 tubers inoculated with each inoculum level had become infected.

In both cultivars in each experiment the increase in lesion size visible on the tuber surface was shown to be linearly correlated with time ($P \geq 0.001$) for all isolates.

The regression equations for lesion development by each isolate in the first experiment are given in Appendix Table 1a for cv Maris Piper and Table 1b for cv Record and for the second experiment are given in Appendix Table 1c for cv Maris Piper and Table 1d for cv Record.

The regression lines for the first experiment are plotted in Figure 4a for cv Maris Piper and in Figure 4b for cv Record and for the second experiment in Figure 4c for cv Maris Piper and Figure 4d for cv Record. The points at which the regression lines intercept the x axis broadly indicate the time taken for the inoculum to establish growth into the tissues away from the inoculution hole while the slopes indicate the rate of lesion expansion.

An analysis of the differences between the intercepts and the differences between the slopes for each isolate in each cultivar was carried out to determine if any of the differences between points of intercept and between slopes were statistically significant. The analyses for the first experiment are given in Table 3a for cv Maris Piper and Table 3b for cv Record, and those for the second experiment are given in Table 3c for cv Maris Piper and Table 3d for cv Record. The results show that the intercepts of the regression lines for the smallest and the largest inoculum levels were not significantly different for any of the isolates in either cultivar in either experiment. Thus growth of each isolate appears to be established into the tissues at about the same time in each cultivar regardless of the amount of inoculum used.

However, there were significant differences in slope between the regression lines for high and low inoculum levels of a number of isolates in both cvs Maris Piper and Record. Thus in general the rate of lesion expansion by each isolate was slower through the tuber from the small inoculum than that from the large inoculum.

3.2.2.1.2. Experiment 3

Although none of the differences between intercepts in experiment 1 and 2 were statistically significant, in almost all cases for all four isolates the intercepts were located along the axis in order of decreasing inoculum size. Since the lack of significance between intercepts found in experiments 1 and 2 could have been due to the limited number of data points, a third experiment was carried out in April 1993 to investigate in more detail the relationships between inoculum levels and both the time to initiate growth and the rate of lesion growth once growth had been initiated. In this experiment, only isolate Fc1 and cv Maris Piper were used and the size of the lesion visible at the surface of the tubers was recorded at 3 day intervals up to 42 days after inoculation.

The regression equations are given in Appendix Table 2 and the regression lines are plotted in Figure 5. The results again show that for each inoculum level lesion size increased linearly with time after inoculation. The results of the ANOVA, given in Table 4, show that the differences between the points of intercept for the regression lines of the largest and the smallest inoculum levels were significantly different ($P \leq 0.001$) as also were the differences between the slopes of the regression lines ($P < 0.001$). Thus growth was not only initiated significantly faster from the largest inoculum than the smallest inoculum but the rate of lesion expansion was significantly greater from the largest inoculum than from the smallest inoculum.

3.2.2.1.3. Assessment of relative pathogenicities from measurements of lesion diameter visible externally at the tuber surface 24 days after inoculation.

The final diameters of the lesions visible externally on the tubers in experiments 1 and 2 were recorded at the end of each experiment, 24 days after inoculation. The results are plotted in Figure 6 (a, b, c, d).

An ANOVA of the results for cv Maris Piper showed, for the first experiment (Table 5), significant differences ($P \leq 0.001$) in pathogenicity between the isolates but in the second experiment these differences were not found. In the first experiment (Figure 6a) isolate Fc1 appeared to be the most pathogenic, producing a slightly larger lesion

then the other isolates after 24 days. while isolates Fc2 and Fc3 were intermediate in pathogenicity with Fc4 being the least pathogenic.

The ANOVA for cv Record (Table 5) showed significant differences ($P \leq 0.001$) in pathogenicity between isolates in both experiments but the differences were largest in the first experiment (Figure 6b). Isolates Fc1 and Fc2 were more pathogenic than the other isolates in both experiments.

3.2.2.1.4. Assessment of relative pathogenicities from measurements of the sizes of lesions developed internally in the tubers 24 days after inoculation

The diameters of the lesions inside the tubers in experiments 1 and 2 were recorded at the end of each experiment, 24 days after inoculation. The results are plotted in Figure 7 (a, b, c d). The lesions produced by each isolate were essentially similar in size to the lesion visible at the tuber surface.

The ANOVA of the results for cv Maris Piper showed (Table 6) significant differences in pathogenecity between the isolates for both experiment. In the first experiment (Figure 7) isolate Fc1 was the most pathogenic with Fc2 and Fc3 producing intermediate sized lesions and Fc4 the smallest lesion. However, in the second experiment although Fc4 was the least pathogenic it was only slightly less than the others and Fc1, Fc2 and Fc3 were essentially similar in pathogenicity.

The ANOVA for cv Record (Table 6) showed for the first experiments, significant differences ($P \leq 0.001$) in pathogenicity between isolates, but in the second experiment these differences were not found. In general it appears (Figure 7c) that isolates Fc1, Fc2 and Fc3 were more pathogenic than isolate Fc4.

Figure 4 (a, b, c, d). Development of dry rot lesions visible on the tuber surface of cvs Maris Piper and Record after inoculation with four isolates (Fc1, Fc2, Fc3 and Fc4) of *F. coeruleum.*

Experiments carried out in March 1991 and January 1992. Tubers were inoculated with five different inoculum levels: 1 = $6x10^5$ spores, 2 = $6x10^4$ spores, 3 = $6x10^3$ spores, 4 = $6x10^2$ spores, 5 = $6x10^1$ spores. Each value is the mean of measurements on 10 tubers.

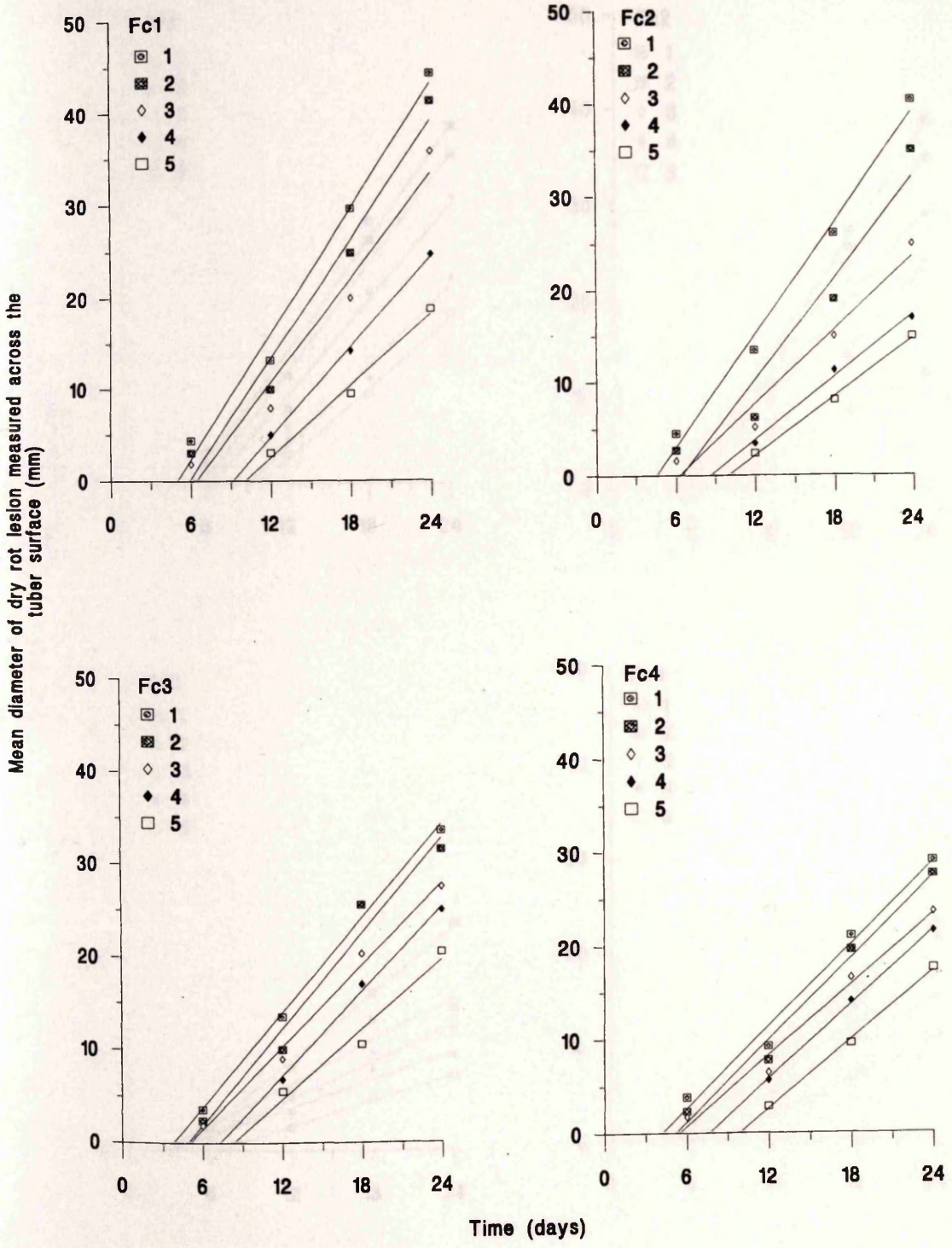


Fig. 4a First experiment (March 1991)
Cv Maris Piper

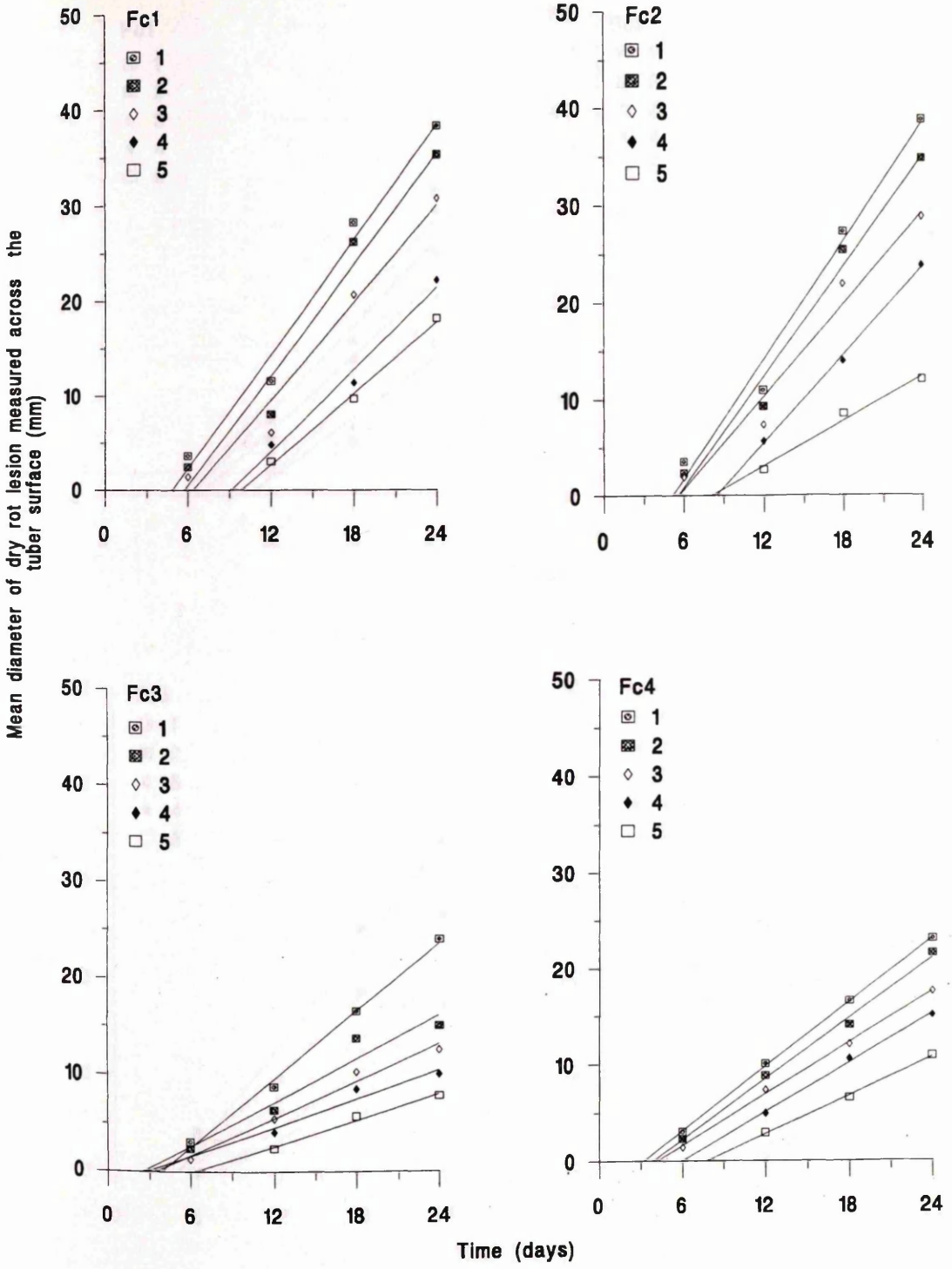


Fig. 4b First experiment March (1991)
Cv Record

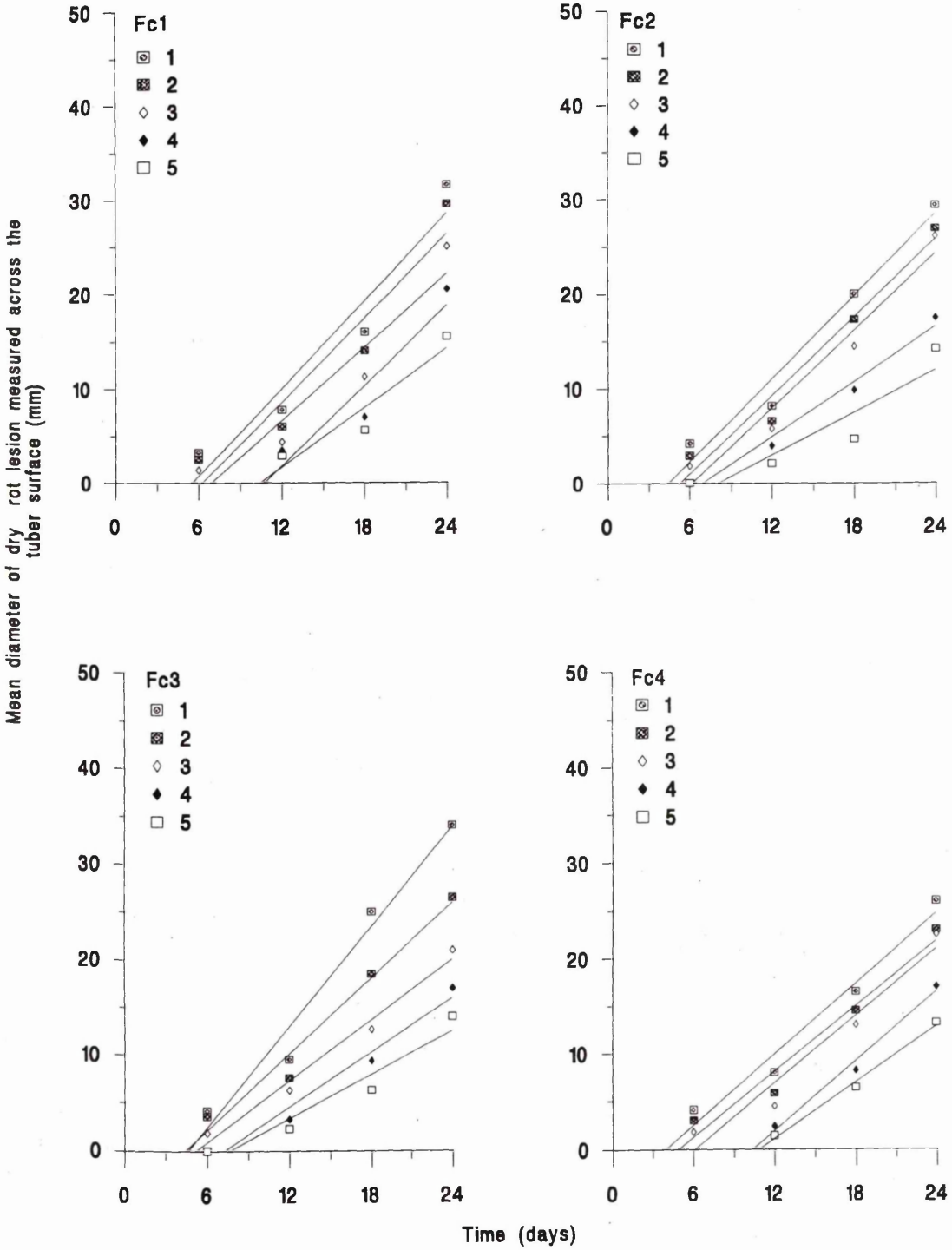


Fig. 4c Second experiment January (1992)
Cv Maris Piper

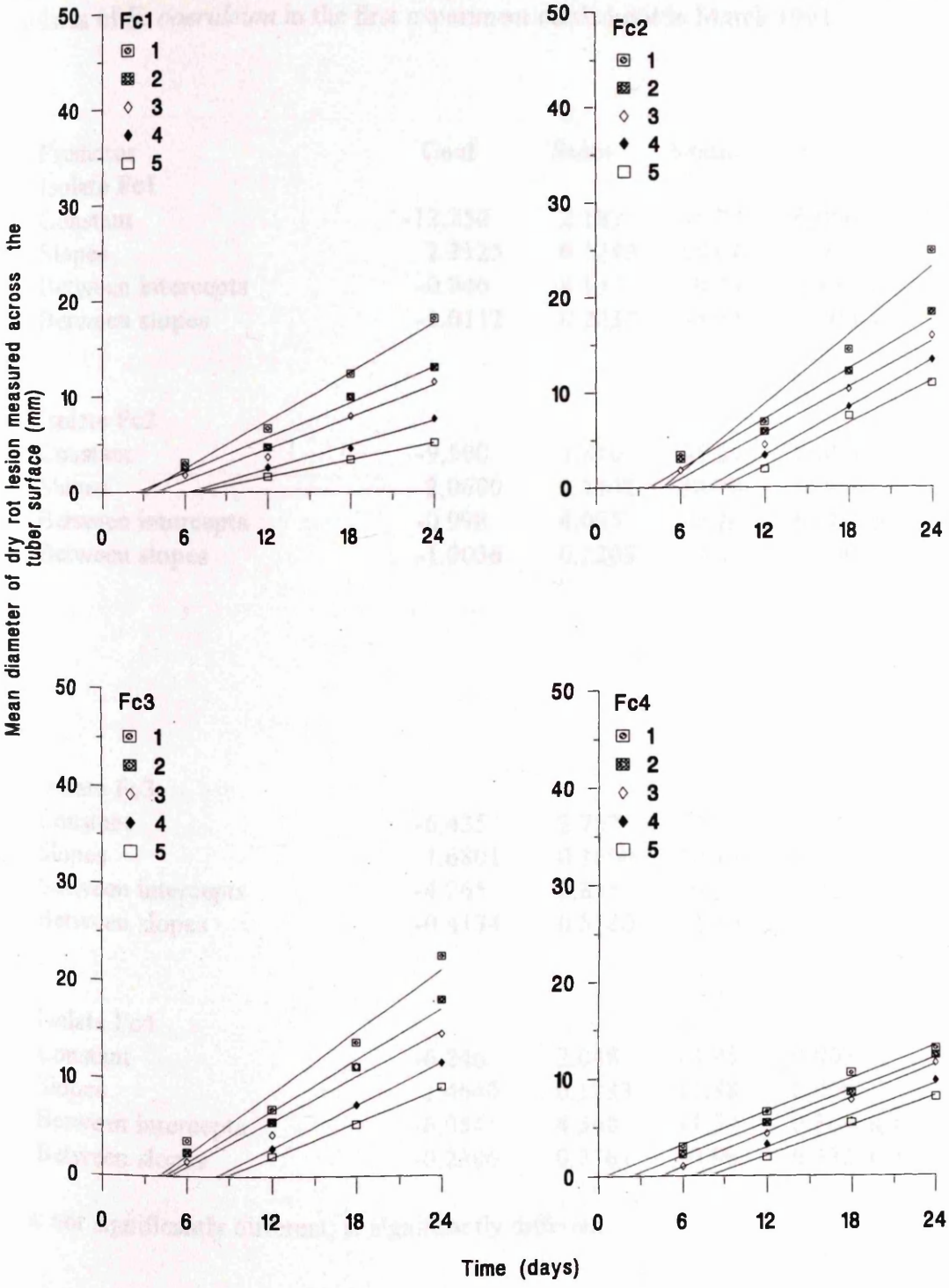


Fig. 4d Second experiment January (1992)
Cv Record

Table 3a. Analyses of differences between intercepts and slopes of regression lines of dry rot lesions development in cv Maris Piper from the smallest and the largest inocula of 4 isolates of *F. coeruleum* in the first experiment carried out in March 1991.

Predictor	Coef	Stdev	t-ratio	p	
Isolate Fc1					
Constant	-12.250	2.183	-5.73	0.000	
Slopes	2.3125	0.1243	18.61	0.000	
Between intercepts	-0.946	4.137	-0.23	0.820	n.s
Between slopes	-1.0112	0.2237	-4.52	0.000	s
Isolate Fc2					
Constant	-9.500	1.810	-5.25	0.000	
Slopes	2.0600	0.1102	18.70	0.000	
Between intercepts	-0.998	4.095	-0.24	0.808	n.s
Between slopes	-1.0056	0.2203	-4.56	0.000	s
Isolate Fc3					
Constant	-6.435	2.757	2.33	0.023	
Slopes	1.6801	0.1660	10.12	0.000	
Between intercepts	-4.265	5.855	-0.73	0.469	n.s
Between slopes	-0.4134	0.3180	-1.30	0.198	n.s
Isolate Fc4					
Constant	-6.246	2.048	-3.05	0.003	
Slopes	1.4640	0.1233	11.88	0.000	
Between intercepts	-6.054	4.348	-1.39	0.169	n.s
Between slopes	-0.2306	0.2361	-0.98	0.332	n.s

n.s: not significantly different; s: significantly different.

Table 3b. Analyses of differences between intercepts and slopes of regression lines of dry rot lesions development in cv Record from the smallest and the largest inocula of 4 isolates of *F. coeruleum* in the first experiment carried out in March 1991.

Predictor	Coef	Stdev	t-ratio	p	
Isolate Fc1					
Constant	-9.650	2.434	- 3.96	0.000	
Slopes	1.9950	0.1481	13.47	0.000	
Between intercepts	-2.883	5.779	- 0.50	0.620	n.s
Between slopes	-0.7323	0.3058	- 2.40	0.020	s
Isolate Fc2					
Constant	-10.665	2.35	-4.54	0.000	
Slopes	2.0566	0.1399	14.70	0.000	
Between intercepts	4.625	5.023	0.92	0.361	n.s
Between slopes	-1.2866	0.2697	-4.77	0.000	s
Isolate Fc3					
Constant	- 4.607	1.186	-3.89	0.023	
Slopes	1.17868	0.0705	16.70	0.000	
Between intercepts	1. 851	2.446	00.76	0.452	n.s
Between slopes	-0.7303	0.1327	-5.51	0.000	s
Isolate Fc4					
Constant	-3.484	1.561	-2.23	0.029	
Slopes	1.1075	0.0939	11.79	0.000	
Between intercepts	-1.728	3.314	-0.52	0.604	n.s
Between slopes	-0.4449	0.1800	-2.47	0.016	n.s

n.s: not significantly different; s: significantly different.

Table 3c. Analyses of differences between intercepts and slopes of regression lines of dry rot lesions development in cv Maris Piper from the smallest and the largest inocula of 4 isolates of *F. coeruleum* in the second experiment carried out in January1992.

Predictor	Coef	Stdev	t-ratio	p	
Isolate Fc1					
Constant	-9.194	2.314	3.97	0.000	
Slopes	1.5789	0.1377	11.47	0.000	
Between intercepts	-4.038	4.773	-0.85	0.401	n.s
Between slopes	-0.4229	0.2589	-1.63	0.107	n.s
Isolate Fc2					
Constant	-6.631	1.988	-3.33	0.000	
Slopes	1.4707	0.1197	12.29	0.000	
Between intercepts	-3.649	4.092	0.89	0.376	n.s
Between slopes	-0.4610	0.2244	-2.05	0.044	s
Isolate Fc3					
Constant	-8.218	1.920	-4.28	0.000	
Slopes	1.7567	0.1156	15.20	0.000	
Between intercepts	-1.862	3.952	-0.47	0.639	n.s
Between slopes	-0.7788	0.2167	-3.59	0.001	s
Isolate Fc4					
Constant	-5.000	2.023	-2.47	0.016	
Slopes	1.2467	0.1231	10.13	0.000	
Between intercepts	-5.876	4.413	-1.33	0.188	n.s
Between slopes	-0.2553	0.2400	-1.06	0.291	n.s

n.s: not significantly different; s: significantly different.

Table 3d. Analyses of differences between intercepts and slopes of regression lines of dry rot lesions development in cv Record from the smallest and the largest inocula of 4 isolates of *F. coeruleum* in the second experiment carried out in January 1992.

Predictor	Coef	Stdev	t-ratio	p	
Isolate Fc1					
Constant	-2.550	1.257	-2.03	0.000	
Slopes	0.8400	0.0764	10.98	0.000	
Between intercepts	-0.594	2.742	0.22	0.829	n.s
Between slopes	-0.5420	0.1491	-3.64	0.001	s
Isolate Fc2					
Constant	-5.802	1.831	-3.17	0.002	
Slopes	1.2142	0.1102	11.02	0.000	
Between intercepts	-1.798	3.767	0.48	0.635	n.s
Between slopes	-0.4521	0.2066	-2.19	0.032	s
Isolate Fc3					
Constant	-4.576	1.682	-2.71	0.009	
Slopes	1.0746	0.1006	10.68	0.000	
Between intercepts	-0.524	3.292	-0.16	0.874	n.s
Between slopes	-0.4913	0.1818	-2.70	0.009	s
Isolate Fc4					
Constant	-0.250	1.185	- 0.21	0.834	
Slopes	0.5633	0.0721	7.81	0.000	
Between intercepts	-3.800	2.435	- 1.56	0.123	n.s
Between slopes	-0.0550	0.1349	- 0.41	0.685	n.s

n.s: not significantly different; s: significantly different.

Figure 5. Development of dry rot lesions visible on the tuber surface of cv Maris Piper after inoculation with isolate Fc1 of *F. coeruleum.*

Experiment carried out in April 1993. Tubers were inoculated with different inoculum levels: 1 = $6x10^5$ spores, 2 = $6x10^4$ spores, 3 = $6x10^3$ spores, 4 = $6x10^2$ spores, 5 = $6x10^1$ spores. Each value is the mean of measurements on 10 tubers.

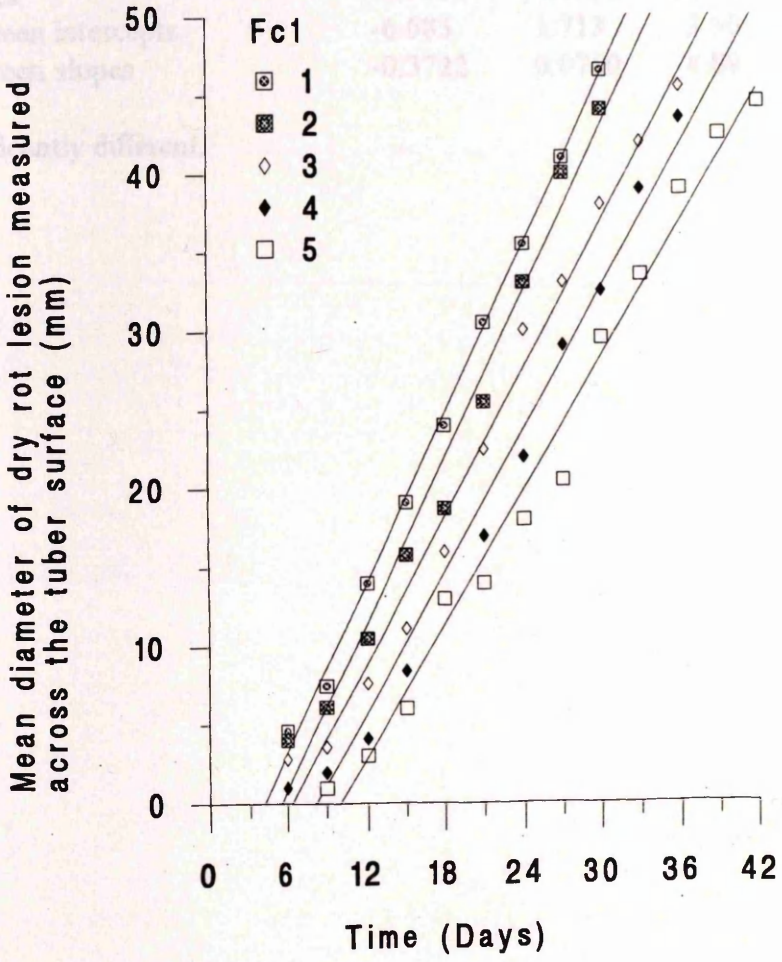

Fc1
1
2
3
4
5
Mean diameter of dry rot lesion measured across the tuber surface (mm)
50
40
30
20
10
0
0
6
12
18
24
30
36
42
Time (Days)

Table 4. Analyses of differences between intercepts and slopes of regression lines of dry rot lesions development in cv Maris Piper from the smallest and the largest inocula of isolate Fc1 of *F. coeruleum* in the third experiment carried out in April 1993.

Predictor	Coef	Stdev	t-ratio	p	
Constant	-7.611	1.249	6.09	0.000	
Slopes	1.7957	0.0634	28.31	0.000	
Between intercepts	-6.683	1.713	3.90	0.000	s
Between slopes	-0.3722	0.0760	4.89	0.000	s

s: significantly different.

Figure 6 (a, b, c, d). Dry rot lesions visible on the tuber surface of cvs Maris Piper and Record 24 days after inoculation with four isolates (Fc1, Fc2, Fc3 and Fc4) of *F. coeruleum*.

Experiment carried out in March 1991 and January 1992. Tubers were inoculated with different inoculum levels: 1 = $6x10^5$ spores, 2 = $6x10^4$ spores, 3 = $6x10^3$ spores, 4 = $6x10^2$ spores, 5 = $6x10^1$ spores. Each value is the mean of measurements on 10 tubers.

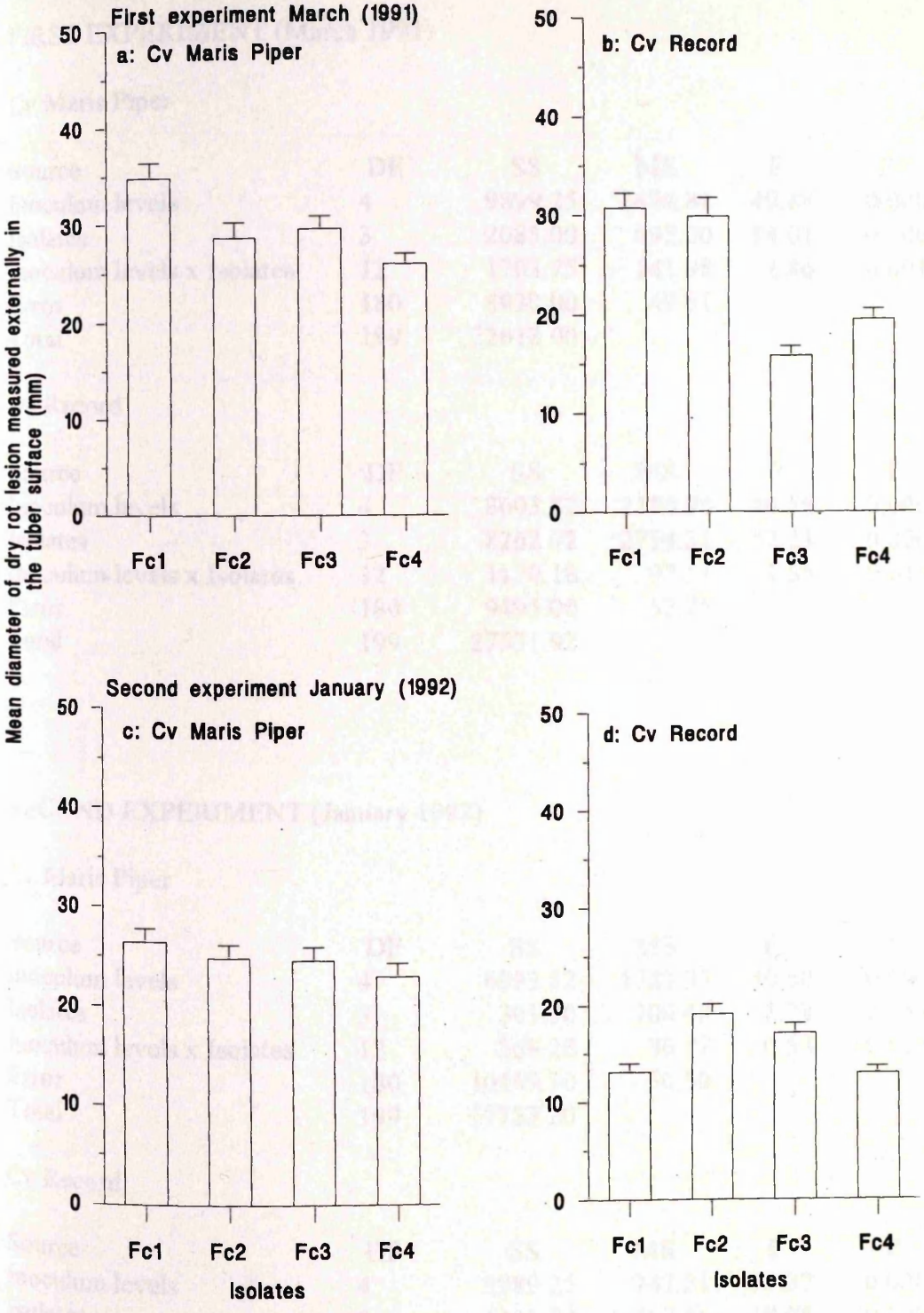

First experiment March (1991)
a: Cv Maris Piper
b: Cv Record
Second experiment January (1992)
c: Cv Maris Piper
d: Cv Record
Mean diameter of dry rot lesion measured externally in the tuber surface (mm)
50
40
30
20
10
0
Fc1
Fc2
Fc3
Fc4
Isolates

Table 5. Analysis of variance of dry rot lesion size visible externally on the tuber surface 24 days after inoculation with four different isolates of *F. coeruleum*.

FIRST EXPERIMENT (March 1991)

Cv Maris Piper

Source	DF	SS	MS	F	P	
Inoculum levels	4	9899.25	2474.81	49.88	0.000	s
Isolates	3	2085.00	695.00	14.01	0.000	s
Inoculum levels x Isolates	12	1703.75	141.98	2.86	0.001	s
Error	180	8930.00	49.61			
Total	199	22618.00				

Cv Record

Source	DF	SS	MS	F	P	
Inoculum levels	4	8603.82	2150.96	40.78	0.000	s
Isolates	3	8262.92	2754.31	52.21	0.000	s
Inoculum levels x Isolates	12	1170.18	97.51	1.85	0.044	n.s
Error	180	9495.00	52.75			
Total	199	27531.92				

SECOND EXPERIMENT (January 1992)

Cv Maris Piper

Source	DF	SS	MS	F	P	
Inoculum levels	4	6893.32	1723.33	30.50	0.000	s
Isolates	3	301.30	100.43	1.78	0.153	n.s
Inoculum levels x Isolates	12	369.28	30.77	0.54	0.883	n.s
Error	180	10169.30	56.50			
Total	199	17733.20				

Cv Record

Source	DF	SS	MS	F	P	
Inoculum levels	4	2989.25	747.31	30.37	0.000	s
Isolates	3	1401.14	467.05	18.98	0.000	s
Inoculum levels x Isolates	12	364.31	30.36	1.23	0.263	n.s
Error	180	4428.80	24.60			
Total	199	9183.50				

n.s: no significantly different; s: significantly different.

Figures 7 (a, b, c, d). Development of dry rot lesions internally in the tubers of cvs Maris Piper and Record 24 days after inoculation with four isolates (Fc1, Fc2, Fc3 and Fc4) of *F. coeruleum.*

Experiment carried out in March 1991 and January 1992. Tubers were inoculated with different inoculum levels: 1 = $6x10^5$ spores, 2 = $6x10^4$ spores, 3 = $6x10^3$ spores, 4 = $6x10^2$ spores, 5 = $6x10^1$ spores. Each value is the mean of measurements on 10 tubers.

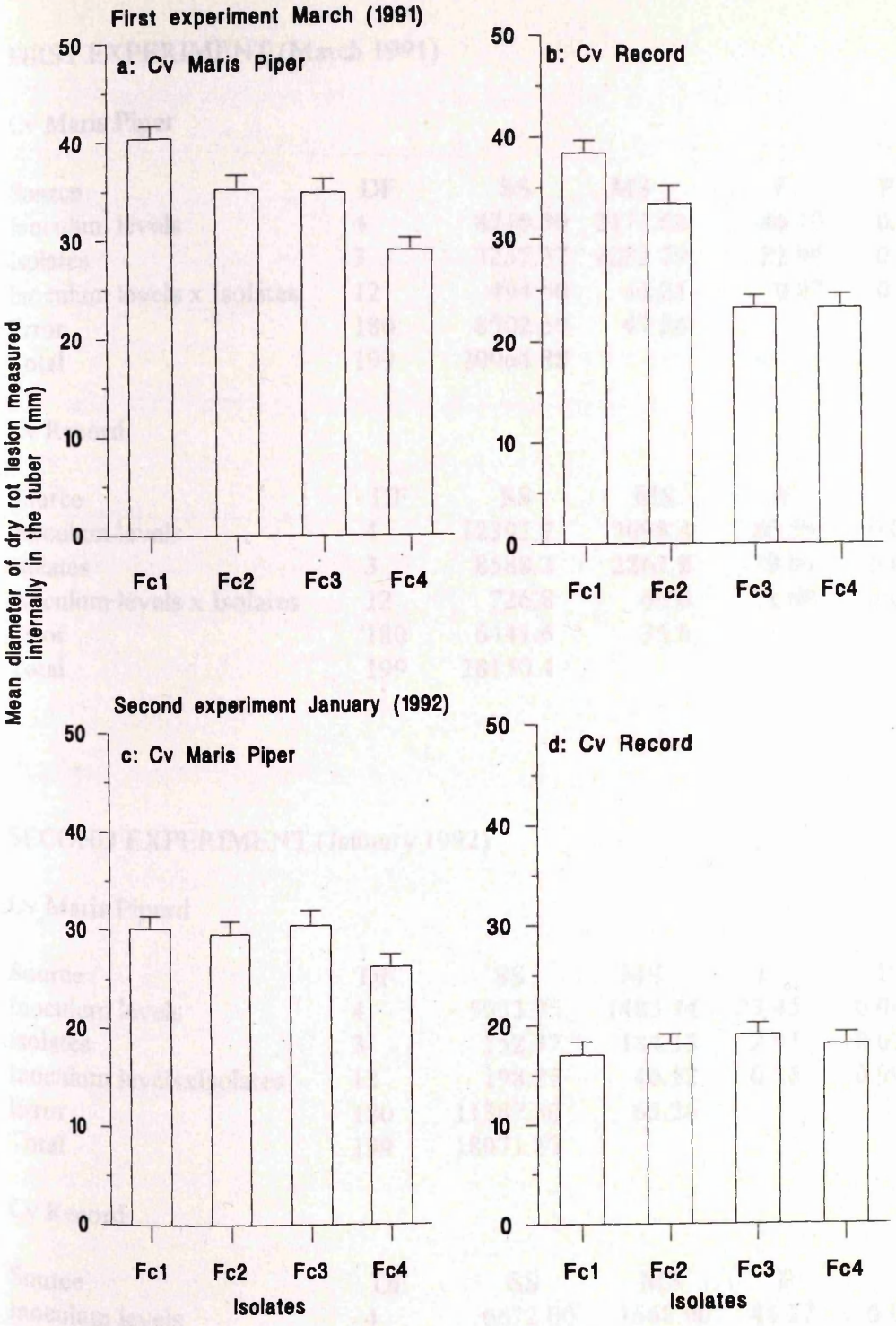

First experiment March (1991)
a: Cv Maris Piper
b: Cv Record
Second experiment January (1992)
c: Cv Maris Piper
d: Cv Record
Mean diameter of dry rot lesion measured internally in the tuber (mm)
50
40
30
20
10
0
Fc1
Fc2
Fc3
Fc4
Isolates

Table 6. Analysis of variance of dry rot lesion size measured internally in tubers 24 days after inoculation with four different isolates of *F. coeruleum*.

FIRST EXPERIMENT (March 1991)

Cv Maris Piper

Source	DF	SS	MS	F	P	
Inoculum levels	4	8710.50	2177.62	46.10	0.000	s
Isolates	3	3257.37	1085.79	22.99	0.000	s
Inoculum levels x Isolates	12	494.50	41.21	0.87	0.576	n.s
Error	180	8502.50	47.24			
Total	199	20964.88				

Cv Record

Source	DF	SS	MS	F	P	
Inoculum levels	4	12393.7	3098.4	86.58	0.000	s
Isolates	3	8588.3	2862.8	79.99	0.000	s
Inoculum levels x Isolates	12	726.8	60.6	1.69	0.072	n.s
Error	180	6441.6	35.8			
Total	199	28150.4				

SECOND EXPERIMENT (January 1992)

Cv Maris Piperd

Source	DF	SS	MS	F	P	
Inoculum levels	4	5933.75	1483.44	23.45	0.000	s
Isolates	3	552.37	184.13	2.91	0.036	s
Inoculum levelsxIsolates	12	198.25	16.52	0.26	0.994	n.s
Error	180	11387.50	63.26			
Total	199	18071.87				

Cv Record

Source	DF	SS	MS	F	P	
Inoculum levels	4	6672.00	1668.00	44.27	0.000	s
Isolates	3	76.37	25.46	0.68	0.568	n.s
Inoculum levels x Isolates	12	248.00	20.67	0.55	0.880	n.s
Error	180	6782.50	37.68			
Total	199	13778.88				

n.s: not significantly different; s: significantly different.

3.3. *F. avenaceum.*

3.3.1. Description of lesions produced in cvs Maris Piper and Record

All four isolates of *F. avenaceum* caused dry rot lesions which were essentially similar to each other. Four days after inoculation small brown lesions were first visible around the edge of the inoculation hole. From then on the lesions increased rapidly in size being clearly visible through the periderm with the colour of the diseased tissues becoming generally light or dark brown. In some tubers the inoculation hole was surrounded with white or pink mycelium. Internally, the lesions were fairly moist and light brown during the early stages of infection, but became darker brown and dry with time. As the lesions spread, cavities formed in some tubers which became lined with white and pink mycelium. The cavities were surrounded by a thin layer of dead cells, dark brown in colour and infested with mycelium. The infected tubers, usually became dry, shrunken, wrinkled and covered with white, pink or yellowish pustules of mycelium. After long periods of incubation most of the tubers become totally rotted.

3.3.2. Comparison of the pathogenicities of four different isolates to cvs Maris Piper and Record

3.3.2.1. Assessment of relative pathogenicities from measurements of lesion expansion visible at the tuber surface

3.3.2.1.1. Experiments 1 and 2

Two experiments were carried out using all four isolates and both cultivars, one in March 1991 and the second in January 1992. Each experiment was set up as described in Materials and Methods.

In both experiments, 24 days after inoculation all 10 tubers inoculated with each inoculum level had become infected.

In both cultivars in each experiment the increase in lesion size visible at the tuber surface was shown to be linearly correlated with time ($P \geq 0.001$) for all isolates.

The regression equations for the lesions developed by each isolate in the first experiment are given in Appendix Table 3a for cv Maris Piper and Table 3b for cv Record. The results for the second experiment are given in Appendix Table 3c for cv Maris Piper and Table 3d for cv Record.

The regression lines for the first experiment are plotted in Figure 8a for cv Maris Piper and in Figure 8b for cv Record and the results for the second experiment in Figure 8c for cv Maris Piper and Figure 8d for cv Record.

An analysis was carried out to determine if any of the differences between the points of intercept and between the slopes of the regression lines for each isolate in each cultivar were significant.

The analyses for the first experiment are given in Table 7a for cv Maris Piper and Tables 7b for cv Record, and those for the second experiment are given in Table 7c for cv Maris Piper and Table 7d for cv Record. The results show that the intercepts of the regression lines for the smallest and the largest inoculum levels were not significantly different for any of the isolates in either cultivar in either experiment. Thus growth appeared to be established into the tissues at about the same time in all cases regardless of the amount of inoculum used. However, there were significant differences in slope between the regression lines for high and low inoculum levels of a number of isolates in both cv Maris Piper and cv Record. Thus the rate of growth from the smallest inoculum of each isolate in most cases was significantly slower than that from the largest inoculum.

3.3.2.1.2. Experiment 3

Although none of the differences between intercepts were statistically significant in either experiment 1 and 2, in almost all cases the intercepts for all isolates were located along the x axis in order of inoculum size and, as with *F. avenaceum,* it is likely that lack of significance was due to the limited amount of data for each inoculum level.

Thus, a third experiment was carried out in April 1993 to investigate in more detail the relationship between inoculum levels and the time to initiate growth as will as the rate of lesion growth once it had been initiated. This experiment, was carried out using cv Maris Piper and one isolate, Fa1. The size of the lesion visible at the surface of the tubers was recorded at 3 day intervals up to 48 days after inoculation. The regression equations are given in Appendix Table 4 and the regression lines are plotted in Figure 9. Again an ANOVA of the results show that for each inoculum level lesion diameter increased linearly with time after inoculation ($P \geq 0.001$). The results, given in Table 8, show that the differences between the points of intercept for the regression lines of the largest and the smallest inoculum levels were significantly different ($p \leq 0.05$) as also were the differences between the slopes ($p < 0.01$). Thus not only was growth initiated significantly faster from the larger inocula than from the smallest inoculum but the rate of lesion growth was significantly greater from the larger inocula.

3.3.2.1.3. Assessment of relative pathogenicities from measurements of lesion diameter visible externally at the tuber surface 24 days after inoculation

The final diameters of the lesions visible externally on the tuber surfaces in experiments 1 and 2 were recorded at the end of each experiment, 24 days after inoculation.

An ANOVA of the results for cv Maris Piper and Record showed, for both experiments (Table 9) significant differences ($P \leq 0.001$) in pathogenicity between the isolates. Isolates Fa1 and Fa2 (Figure 10) produced bigger lesions than isolates Fa3 and Fa4 in 24 days in cv Maris Piper in both experiments. In cv Record (Figure 10) although in the first experiment isolate Fa2 produced a significantly bigger lesion than isolates Fa1, Fa3 and Fa4, in the second experiment the differences between all 4 were not very marked. The results in Figure 10 also show that larger lesions developed in cv Maris Piper than in cv Record in both experiments indicating that cv Maris Piper was slightly more susceptible then cv Record.

3.3.2.1.4. Assessment of relative pathogenicities from measurements of size of lesion developed internally in the tubers 24 days after inoculation

The diameters of lesions inside the tubers were recorded at the end of each experiment, 24 days after inoculation. The size of the lesions developed internally by each isolate were essentially similar to those visible at the tuber surface.

An ANOVA of the results for cv Maris Piper showed for both the first and the second experiment (Table 10) significant differences in pathogenicity between the isolates. Figure 11 a, b shows that isolates Fa1 and Fa2 were slightly more pathogenic than isolates Fa3 and Fa4. However, the ANOVA for cv Record (Table 10) showed significant differences in pathogenicity between isolates in the first experiment only with isolates Fa1 and Fa2 being slightly more pathogenic than the other two. These results are in broad agreement with the results derived from measurements of the external spread of lesions at the tuber surface.

Figure 8 (a, b, c, d). Development of dry rot lesions visible on the tuber surface of cvs Maris Piper and Record after inoculation with four isolates (Fa1, Fa2, Fa3 and Fa4) of *F. avenaceum.*

Experiments carried out in March 1991 and January 1992. Tubers were inoculated with five different inoculum levels: 1 = $6x10^5$ spores, 2 = $6x10^4$ spores, 3 = $6x10^3$ spores, 4 = $6x10^2$ spores, 5 = $6x10^1$ spores. Each value is the mean of measurements on 10 tubers.

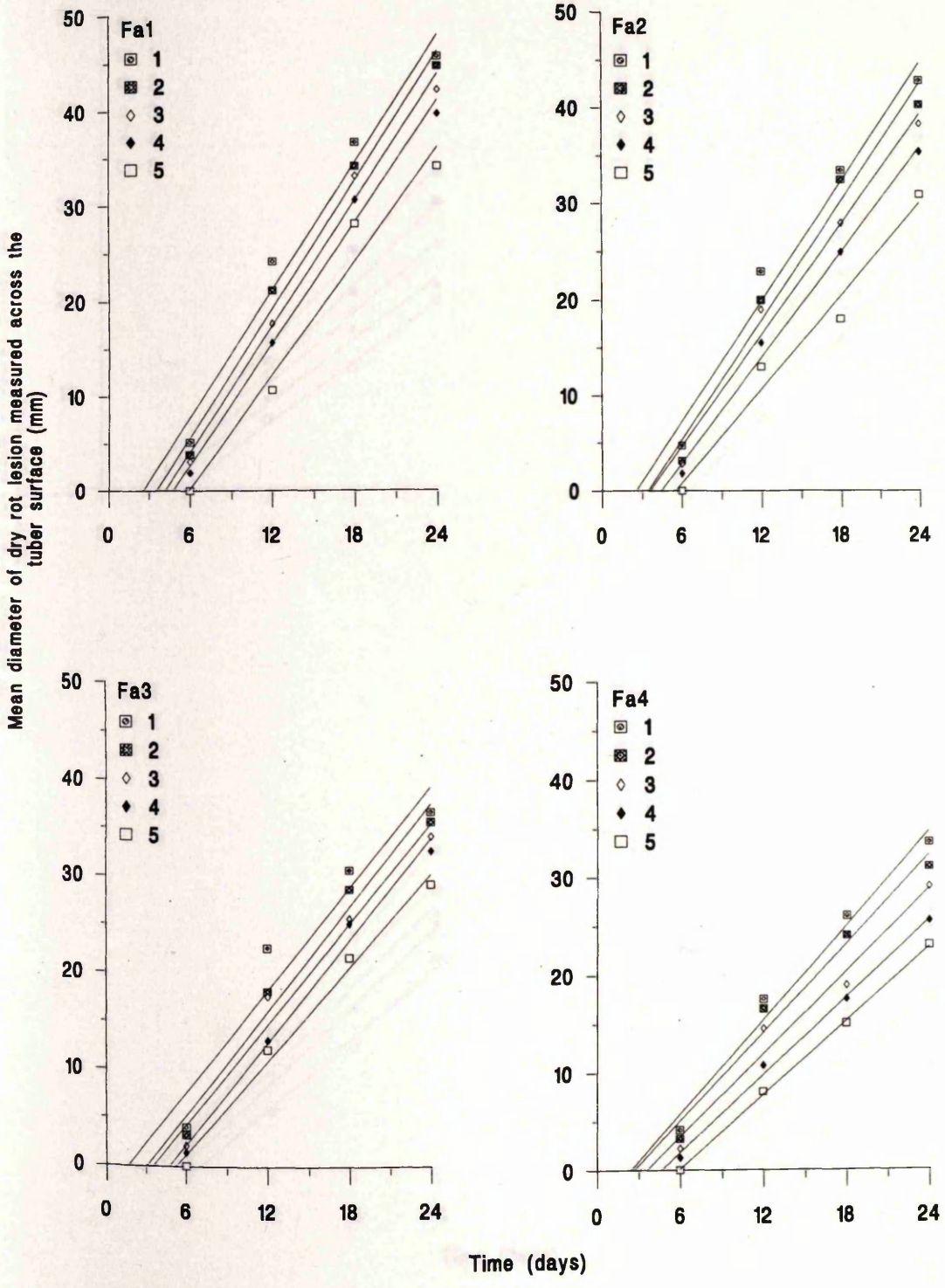


Fig. 8a First experiment march (1991)
Cv Maris Piepr

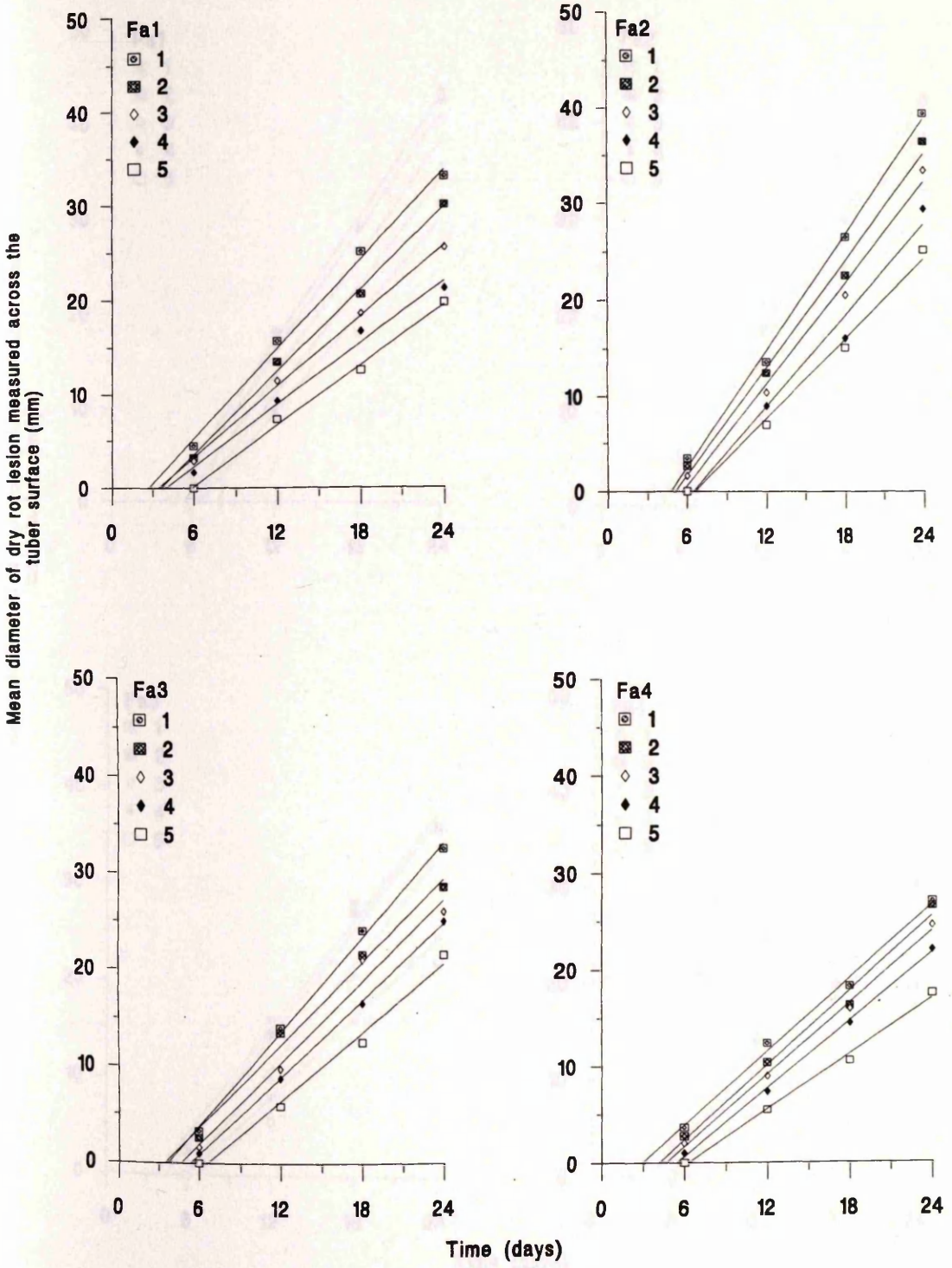


Fig. 8b First experiment March (1991)
Cv Record

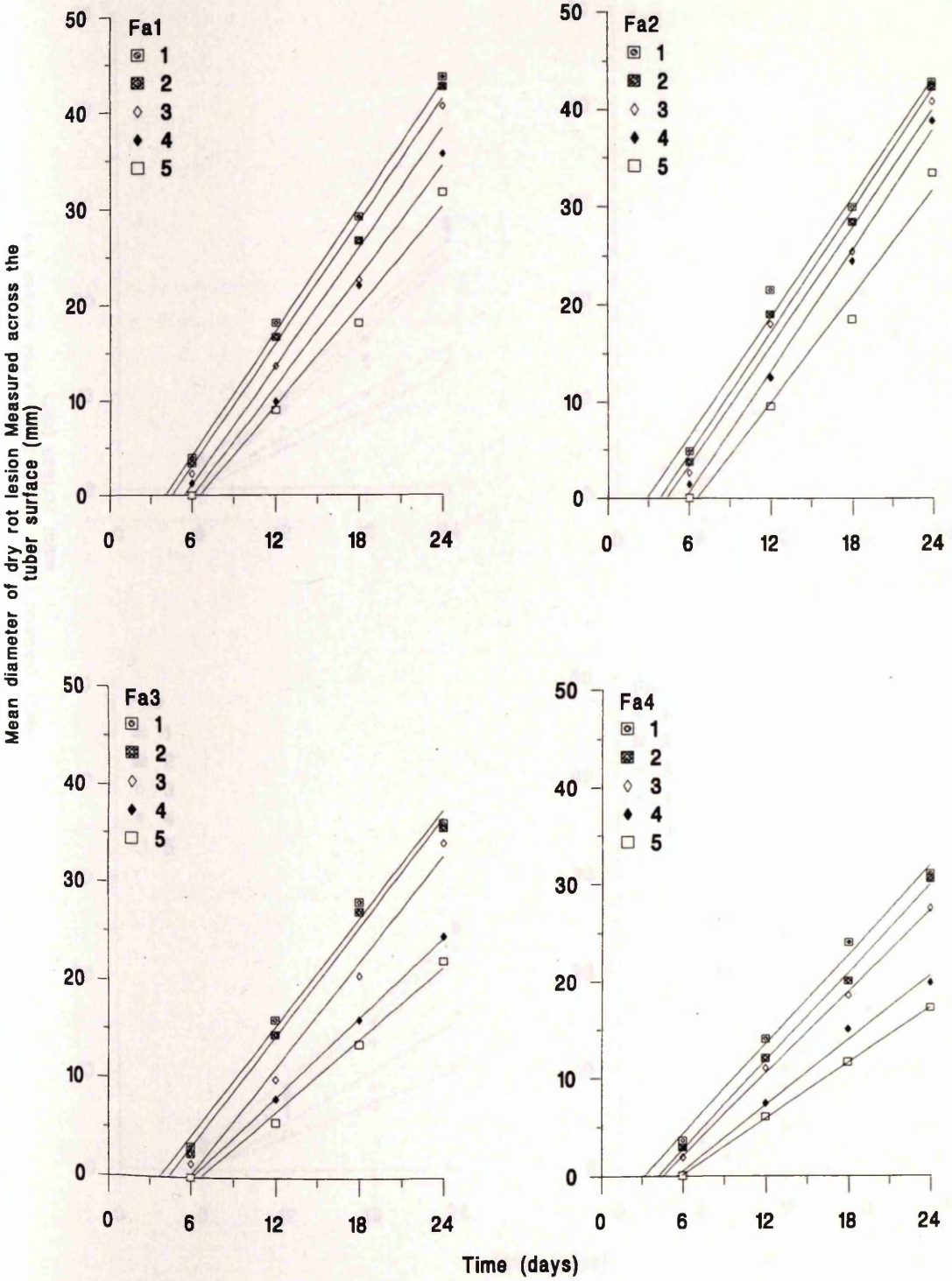


Fig. 8c Second experiment January (1992)
Cv Maris Piper

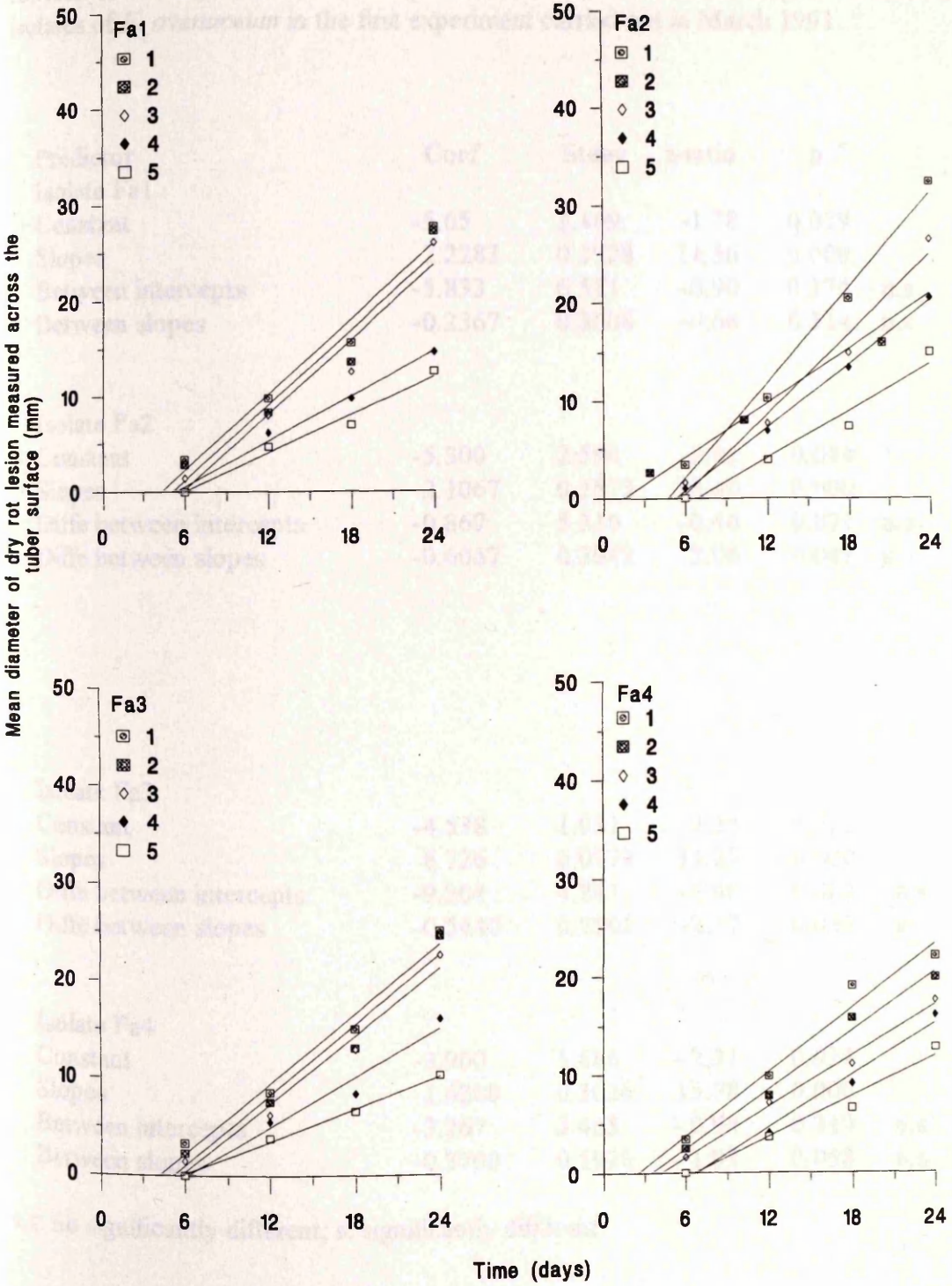


Fig. 8d Second experiment January (1992)
Cv Record

Table 7a. Analyses of differences between intercepts and slopes of regression lines of dry rot lesions developed in cv Maris Piper from the smallest and the largest inocula of four isolates of *F. avenaceum* in the first experiment carried out in March 1991.

Predictor	Coef	Stdev	t-ratio	p	
Isolate Fa1					
Constant	-5.65	3.169	-1.78	0.079	
Slopes	2.2283	0.1928	11.56	0.000	
Between intercepts	-5.833	6.511	-0.90	0.374	n.s
Between slopes	-0.2367	0.3608	-0.66	0.514	n.s
Isolate Fa2					
Constant	-5.300	2.584	-2.05	0.044	
Slopes	2.1067	0.1573	13.40	0.000	
Diffe between intercepts	-0.867	5.310	-0.16	0.871	n.s
Diffe between slopes	-0.6067	0.2942	-2.06	0.043	s
Isolate Fa3					
Constant	-4.538	1.931	2.35	0.022	
Slopes	8.726	0.0773	11.29	0.000	
Diffe between intercepts	-9.204	4.841	-1.90	0.062	n.s
Diffe between slopes	-0.5440	0.2502	-2.17	0.033	s
Isolate Fa4					
Constant	-3.900	1.686	- 2.31	0.024	
Slopes	1.6200	0.1026	15.78	0.000	
Between intercepts	-3.267	3.465	- 0.94	0.349	n.s
Between slopes	-0.3700	0.1920	- 1.93	0.058	n.s

n.s: no significantly different; s: significantly different.

Table 7b. Analyses of differences between intercepts and slopes of regression lines of dry rot lesions developed in cv Record from the smallest and the largest inocula of four isolates of *F. avenaceum* in the first experiment carried out in March 1991.

Predictor	Coef	Stdev	t-ratio	p	
Isolate Fa1					
Constant	-4.250	2.093	-2.03	0.046	
Slopes	1.5833	0.1274	12.43	0.000	
Between intercepts	-1.717	4.301	- 0.40	0.691	n.s
Between slopes	-0.5167	0.2383	- 2.17	0.034	s
Isolate Fa2					
Constant	-9.651	2.398	-4.04	0.000	
Slopes	2.0242	0.1443	14.02	0.000	
Between intercepts	-0.369	5.092	0.07	0.942	n.s
Between slopes	-0.6142	0.2766	-2.22	0.030	n.s
Isolate Fa3					
Constant	- 5.950	1.697	-3.51	0.001	
Slopes	1.6267	0.1033	15.75	0.000	
Between intercepts	-4.150	3.486	-1.19	0.238	n.s
Between slopes	-0.3267	0.1932	- 1.69	0.096	s
Isolate Fa4					
Constant	-3.750	1.501	- 2.50	0.015	
Slopes	1.2683	0.0913	13.89	0.000	
Between intercepts	-3.250	3.171	- 1.02	0.309	n.s
Between slopes	-0.2608	0.1741	- 1.50	0.139	s

n.s: not significantly different; s: significantly different.

Table 7c. Analyses of differences between intercepts and slopes of regression lines of dry rot lesions developed in cv Maris Piper from the smallest and the largest inocula of four isolates of *F. avenaceum* in the second experiment carried out in January 1992.

Predictor	Coef	Stdev	t-ratio	p	
Isolate Fa1					
Constant	-8.750	2.277	-3.84	0.000	
Slopes	2.1583	0.1386	15.57	0.000	
Between intercepts	-5.683	4.679	-1.21	0.229	n.s
Between slopes	-0.2750	0.2593	-1.03	0.293	n.s
Isolate Fa2					
Constant	-5.950	2.361	-2.52	0.014	
Slopes	2.0517	0.1437	14.28	0.000	
Between intercepts	-7.800	4.852	-1.61	0.113	n.s
Between slopes	-0.1767	0.2689	-0.66	0.513	n.s
Isolate Fa3					
Constant	-6.900	1.802	-3.83	0.000	
Slopes	1.8450	0.1097	16.82	0.000	
Between intercepts	-4.583	3.703	-1.24	0.220	n.s
Between slopes	-0.4367	0.2052	-2.13	0.037	s
Isolate Fa4					
Constant	-4.900	1.748	- 2.80	0.007	
Slopes	1.5200	0.1064	14.29	0.000	
Between intercepts	-0.133	3.593	- 0.04	0.971	n.s
Between slopes	-0.5867	0.1991	- 2.95	0.294	s

n.s: no significantly different; s: significantly different.

Table 7d. Analyses of differences between intercepts and slopes of regression lines of dry rot lesions developed in cv Record from the smallest and the largest inocula of four isolates of *F. avenaceum* in the second experiment carried out in January 1992.

Predictor	Coef	Stdev	t-ratio	p	
Isolate Fa1					
Constant	-5.600	1.531	-3.66	0.001	
Slopes	1.3066	0.0931	14.03	0.000	
Between intercepts	-1.750	3.145	0.56	0.580	n.s
Between slopes	-0.6483	0.1743	-3.72	0.000	s
Isolate Fa2					
Constant	-7.700	1.775	-4.34	0.000	
Slopes	1.6250	0.1081	15.04	0.000	
Between intercepts	-0.183	3..648	0.05	0.960	n.s
Between slopes	-0.700	0.2021	-3.46	0.001	s
Isolate Fa3					
Constant	-5.296	1.698	-3.12	0.003	
Slopes	1.2231	0.1022	11.97	0.000	
Between intercepts	-2.272	3.606	-0.63	0.531	n.s
Between slopes	-0.6811	0.1958	-3.48	0.001	s
Isolate Fa4					
Constant	-2.950	1.275	- 2.31	0.024	
Slopes	0.1083	0.0775	14.29	0.000	
Between intercepts	-0.722	2.781	- 0.26	0.796	n.s
Between slopes	-0.5157	0.1512	3.41	0.001	n.s

n.s: no significantly different; s: significantly different

Figures 9. Development of dry rot lesions visible on the tuber surface of cv Maris Piper after inoculation with isolate Fa1 of *F. avenaceum.*

Experiment carried out in April 1993. Tubers were inoculated with different inoculum levels: 1 = $6x10^5$ spores, 2 = $6x10^4$ spores, 3 = $6x10^3$ spores, 4 = $6x10^2$ spores, 5 = $6x10^1$ spores. Each value is the mean of measurements on 10 tubers.

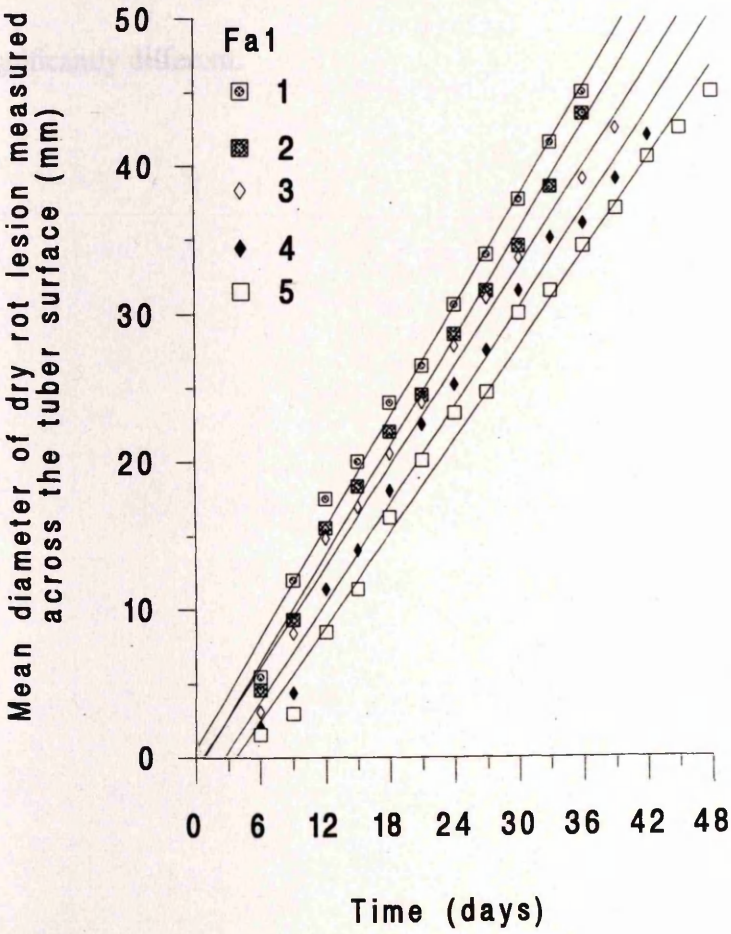

Mean diameter of dry rot lesion measured across the tuber surface (mm)
50
40
30
20
10
0
Fa1
1
2
3
4
5
0
6
12
18
24
30
36
42
48
Time (days)

Table 8. Analyses of differences between intercepts and slopes of regression lines of dry rot lesions developed in cv Maris Piper from the smallest and the largest inocula of isolate Fa1 of *F. avenaceum* in the third experiment carried out in Appril 1993.

Predictor	Coef	Stdev	t-ratio	p	
Constant	-0.698	1.368	-0.51	0.610	
Slopes	1.2412	0.0593	20.91	0.000	
Diffe between intercepts	-4.500	1.791	-2.51	0.013	s
Diffe between slopes	-0.1866	0.0761	-2.64	0.009	s

s: significantly different.

Table 9. Analysis of variance of dry rot lesion size visible externally on the tuber surface 24 days after inoculation with four different isolates of *F. avenaceum.*

FIRST EXPERIMENT (March 1991)

Cv Maris Piper

Source	DF	SS	MS	F	P	
Inoculum levels	4	2597.00	649.25	9.19	0.000	s
Isolates	3	4569.00	1523.00	21.55	0.000	s
Inoculum levels x Isolates	12	96.00	8.00	0.11	1.000	n.s
Error	180	12720.00	70.67			
Total	199	19982.00				

Cv Record

Source	DF	SS	MS	F	P	
Inoculum levels	4	3681.25	920.31	20.67	0.000	s
Isolates	3	2204.50	734.83	16.50	0.000	s
Inoculum levels x Isolates	12	236.75	19.73	0.44	0.944	n.s
Error	180	8015.00	44.53			
Total	199	14137.50				

SECOND EXPERIMENT (January 1992)

Cv Maris Piper

Source	DF	SS	MS	F	P	
Inoculum levels	4	4635.43	1158.86	29.81	0.000	s
Isolates	3	6610.46	2203.49	56.68	0.000	s
Inoculum levels x Isolates	12	236.09	19.67	0.51	0.909	n.s
Error	180	6997.60	38.88			
Total	199	18479.58				

Cv Record

Source	DF	SS	MS	F	P	
Inoculum levels	4	6446.57	1611.64	41.94	0.000	s
Isolates	3	1665.98	555.33	14.45	0.000	s
Inoculum levels x Isolates	12	321.67	26.81	0.70	0.753	n.s
Error	180	6917.20	38.43			
Total	199	15351.42				

n.s: no significantly different; s: significantly different.

Figures 10 (a, b, c, d). Dry rot lesions visible on the tuber surfaces of cvs Maris Piper and Record 24 days after inoculation with four isolates (Fa1, Fa2, Fa3 and Fa4) of *F. avenaceum*.

Experiment carried out in March 1991 and January 1992. Tubers were inoculated with different inoculum levels: 1 = $6x10^5$ spores, 2 = $6x10^4$ spores, 3 = $6x10^3$ spores, 4 = $6x10^2$ spores, 5 = $6x10^1$ spores. Each value is the mean of measurements on 10 tubers.

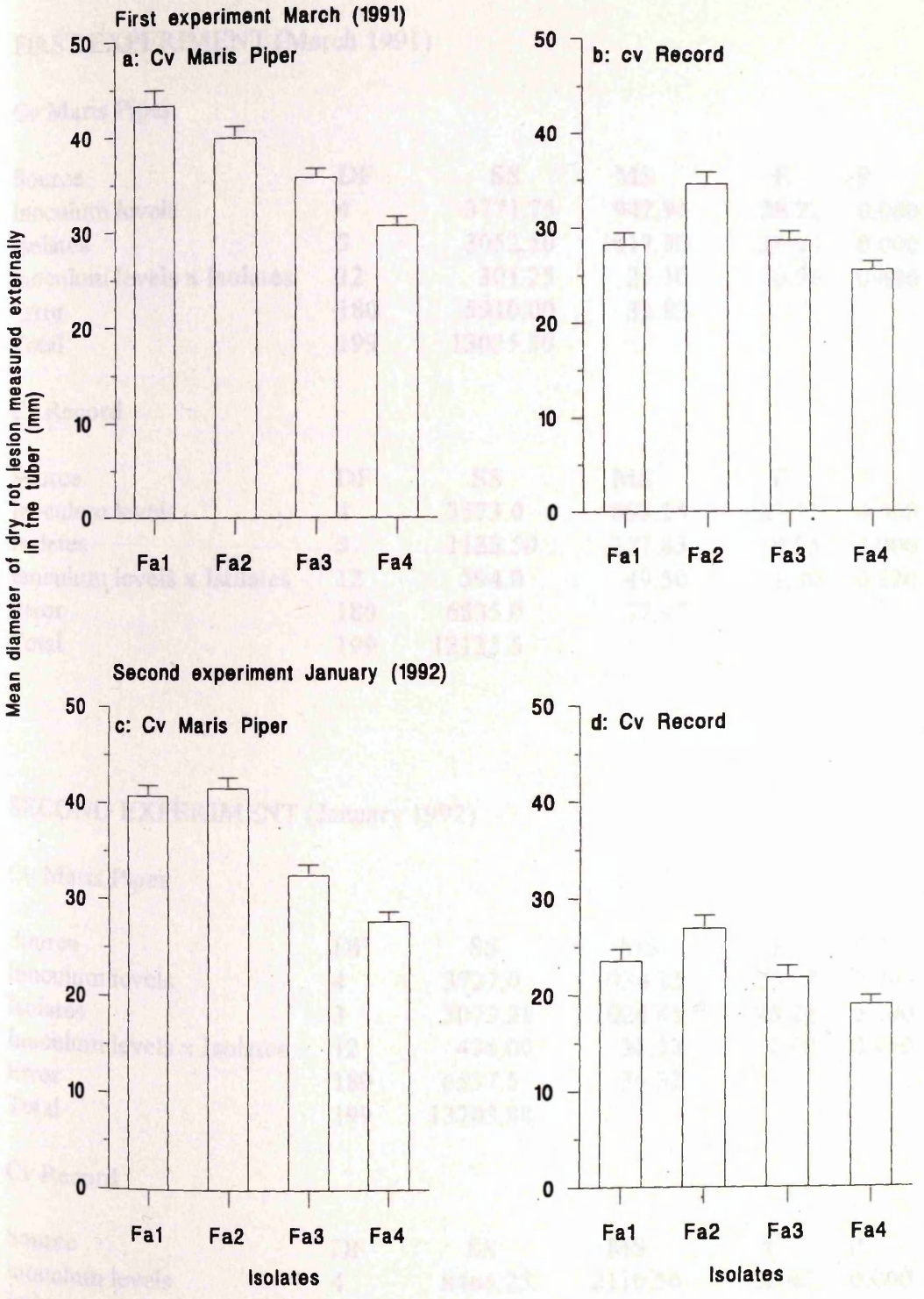

First experiment March (1991)
a: Cv Maris Piper
b: cv Record
Second experiment January (1992)
c: Cv Maris Piper
d: Cv Record
Mean diameter of dry rot lesion measured externally in the tuber (mm)
50
40
30
20
10
0
Fa1
Fa2
Fa3
Fa4
Isolates

Table 10. Analysis of variance of dry rot lesion size measured internally in tubers 24 days after inoculation with four different isolates of *F. avenaceum.*

FIRST EXPERIMENT (March 1991)

Cv Maris Piper

Source	DF	SS	MS	F	P	
Inoculum levels	4	3771.75	942.94	28.72	0.000	s
Isolates	3	3052.50	1017.50	30.99	0.000	s
Inoculum levels x Isolates	12	301.25	25.10	0.76	0.686	n.s
Error	180	5910.00	32.83			
Total	199	13035.50				

Cv Record

Source	DF	SS	MS	F	P	
Inoculum levels	4	3573.0	893.25	23.52	0.000	s
Isolates	3	1133.50	377.83	9.95	0.000	s
Inoculum levels x Isolates	12	594.0	49.50	1.30	0.220	n.s
Error	180	6835.0	37.97			
Total	199	12135.5				

SECOND EXPERIMENT (January 1992)

Cv Maris Piper

Source	DF	SS	MS	F	P	
Inoculum levels	4	3737.0	934.25	25.72	0.000	s
Isolates	3	3073.38	1024.46	28.21	0.000	s
Inoculum levels x Isolates	12	436.00	36.33	1.00	0.450	n.s
Error	180	6537.5	36.32			
Total	199	13783.88				

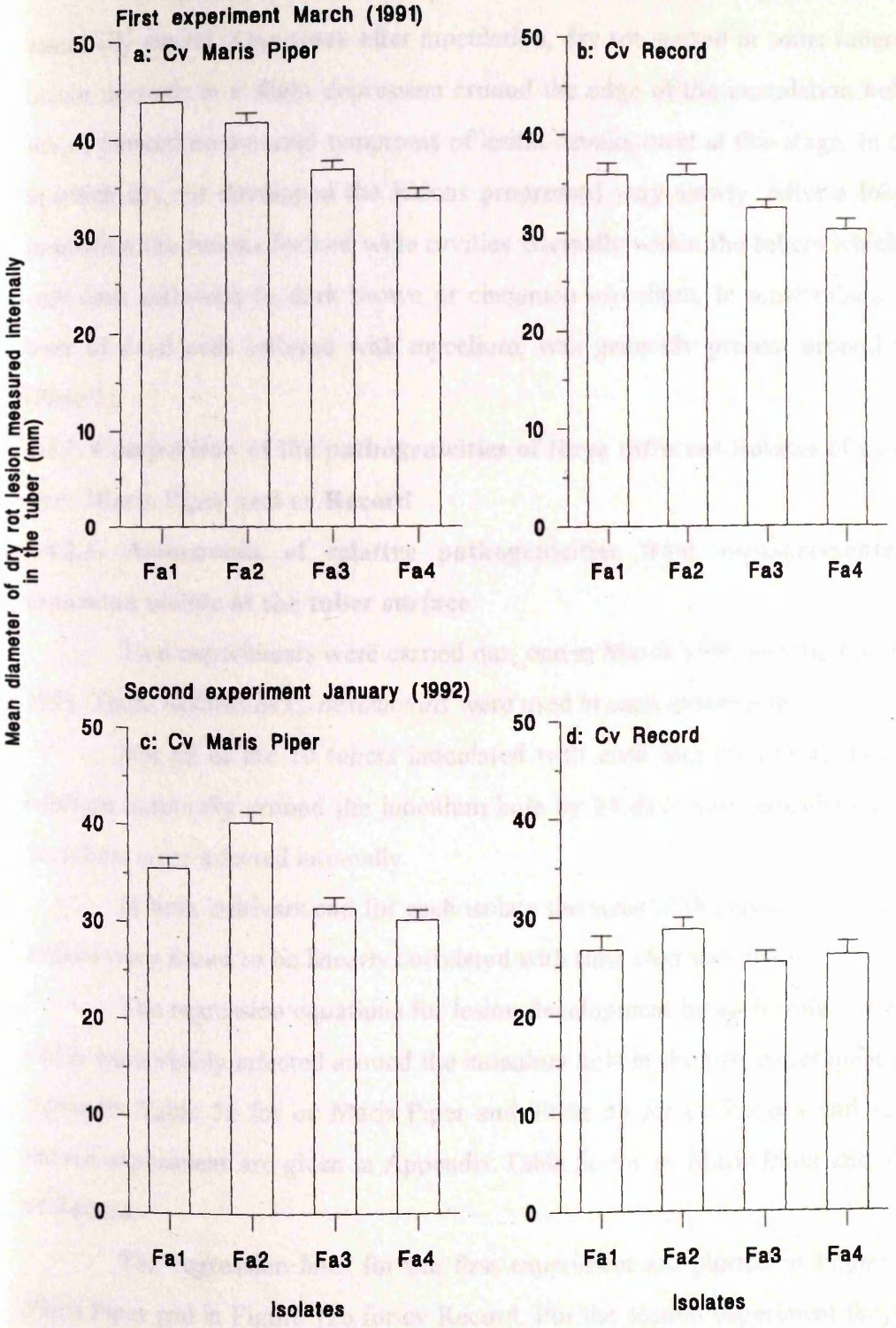
Cv Record

Source	DF	SS	MS	F	P	
Inoculum levels	4	8466.25	2116.56	56.40	0.000	s
Isolates	3	322.5	107.50	2.86	0.038	n.s
Inoculum levels x Isolates	12	443.75	36.98	0.99	0.465	n.s
Error	180	6755.0	37.53			
Total	199	15987.50				

n.s: no significantly different; s: significantly different.

Figures 11 (a, b, c, d). Development of dry rot lesions internally in the tubers of cvs Maris Piper and Record after inoculation with four isolates (Fa1, Fa2, Fa3 and Fa4) of *F. avenaceum*.

Experiment carried out in March 1991 and January 1992. Tubers were inoculated with different inoculum levels: 1 = $6x10^5$ spores, 2 = $6x10^4$ spores, 3 = $6x10^3$ spores, 4 = $6x10^2$ spores, 5 = $6x10^1$ spores. Each value is the mean of measurements on 10 tubers.


First experiment March (1991)
a: Cv Maris Piper
b: Cv Record
Second experiment January (1992)
c: Cv Maris Piper
d: Cv Record
Mean diameter of dry rot lesion measured internally in the tuber (mm)
50
40
30
20
10
0
Fa1
Fa2
Fa3
Fa4
Isolates

3.4. *C. destructans*

3.4.1. Description of lesions produced in cvs Maris Piper and Record

All three isolates caused dry rot lesions and the lesions produced by each were essentially similar. One week after inoculation, dry rot started in some tubers as a small brown necrosis in a slight depression around the edge of the inoculation hole but other tubers showed no external symptoms of lesion development at this stage. In those tubers in which dry rot developed the lesions progressed very slowly. After a long period of incubation the lesions formed wide cavities internally within the tubers which were lined with dark yellowish to dark brown or cinnamon mycelium. In most tubers, only a thin layer of dead cells infested with mycelium, was generally present around the cavities (Plate 2).

3.4.2. Comparison of the pathogenicities of three different isolates of *C. destructans* to cv Maris Piper and cv Record

3.4.2.1. Assessment of relative pathogenicities from measurements of lesion expansion visible at the tuber surface

Two experiments were carried out, one in March 1990 and the second in March 1991. Three isolates of *C. destructans* were used in each experiment.

Not all of the 10 tubers inoculated with each inoculum level showed signs of infection externally around the inoculum hole by 24 days after inoculation but most of the tubers were infected internally.

In both cultivars and for each isolate the sizes of the lesions visible at the tuber surface were found to be linearly correlated with time after inoculation.

The regression equations for lesion development by each isolate in those tubers which were visibly infected around the inoculum hole in the first experiment are given in Appendix Table 5a for cv Maris Piper and Table 5b for cv Record and results of the second experiment are given in Appendix Table 5c for cv Maris Piper and Table 5d for cv Record.

The regression lines for the first experiment are plotted in Figure 12a for cv Maris Piper and in Figure 12b for cv Record. For the second experiment they are plotted in Figure 12c for cv Maris Piper and Figure 12d for cv Record .

An analysis of the regression equations for each isolate in each cultivar was carried out to determine if any of the differences between isolates in relation to intercept or slope were statistically significant. The analyses of the first experiment are given in

Table 11a for cv Maris Piper and Table 11b for cv Record, and those from the second experiment in Table 11c for cv Maris Piper and Table 11d for cv Record. The analyses show that the differences between the intercepts for the smallest and the largest inoculum levels were not significantly different for any of the of three isolates in either cultivar in either experiment. Thus growth appears to be established into the tissues at about the same time in all cases regardless of the amount of inoculum used. There were significant differences between the slopes for the highest and lowest inoculum levels for a number of the isolates in both cvs Maris Piper and Record but some isolates showed no significant differences (Table 11 a, b, c, d). However, in general, the rate of growth of each isolate from a small inoculum was slower than that from a large inoculum.

3.4.2.2. Assessment of relative pathogenicities from measurements of lesion diameter visible externally at the tuber surface 24 days after inoculation

The final diameters of lesions visible externally on the tubers in experiment 1 and 2 were recorded at the end of each experiment, 24 days after inoculation. An ANOVA of the results for cv Maris Piper for the first and the second experiment (Table 12) showed no significant difference in pathogenicity between isolates while an ANOVA of the results for cv Record showed significant differences ($P \geq 0.001$) in pathogenicity between the isolates for the first experiment but not for the second experiment. In cv Record Cd2 was the most pathogenic in the first experiment. The sizes of the lesions produced by all 3 isolates in both the first and the second experiment were similar indicating that tuber resistance did not reduce during storage (Figure 13).

3.4.2.3. Assessment of relative pathogenicities from measurements of size of lesion developed internally in the tubers 24 days after inoculation

The diameters of the dry rot lesions developed inside the tubers in both experiments were recorded at the end of each experiment, 24 days after inoculation.

An ANOVA of the results for cv Maris Piper and record showed in both experiments (Table 13) significant differences in pathogenicity between isolates. The results, plotted in Figure 14 show that isolate Cd2 was slightly more pathogenic than isolate Cd1 and Cd3.

Figure 12 (a, b, c, d) Development of dry rot lesions visible on the tuber surface of cvs Maris Piper and Record after inoculation with three isolates (Cd1, Cd2 and Cd3) of *C. destructans.*

Experiments carried out in March 1990 and 1991. Tubers were inoculated with different inoculum levels: 1 = $6x10^5$ spores, 2 = $6x10^4$ spores, 3 = $6x10^3$ spores, 4 = $6x10^2$ spores, 5 = $6x10^1$ spores. Each value is the mean of measurements on 10 tubers.

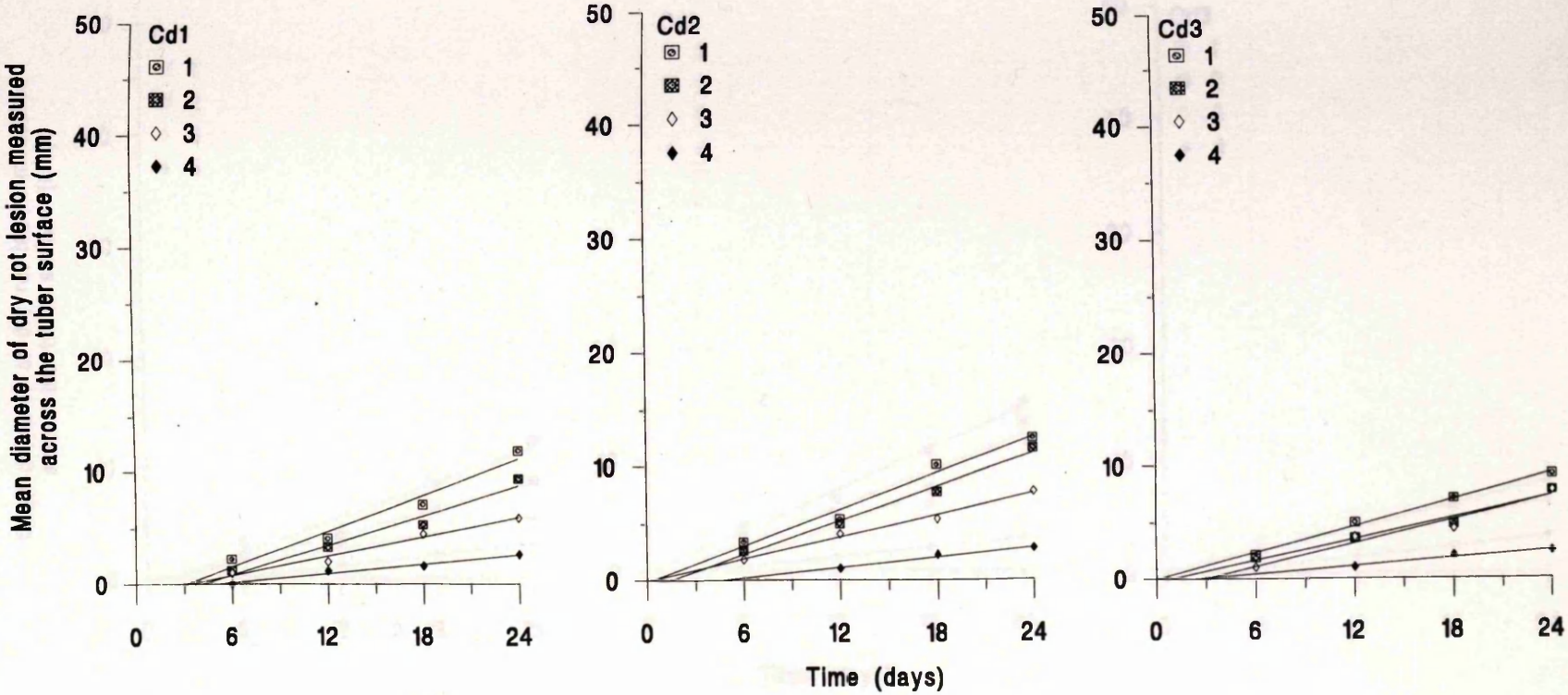


Fig. 12a First experiment March (1990)
Cv Maris Piper

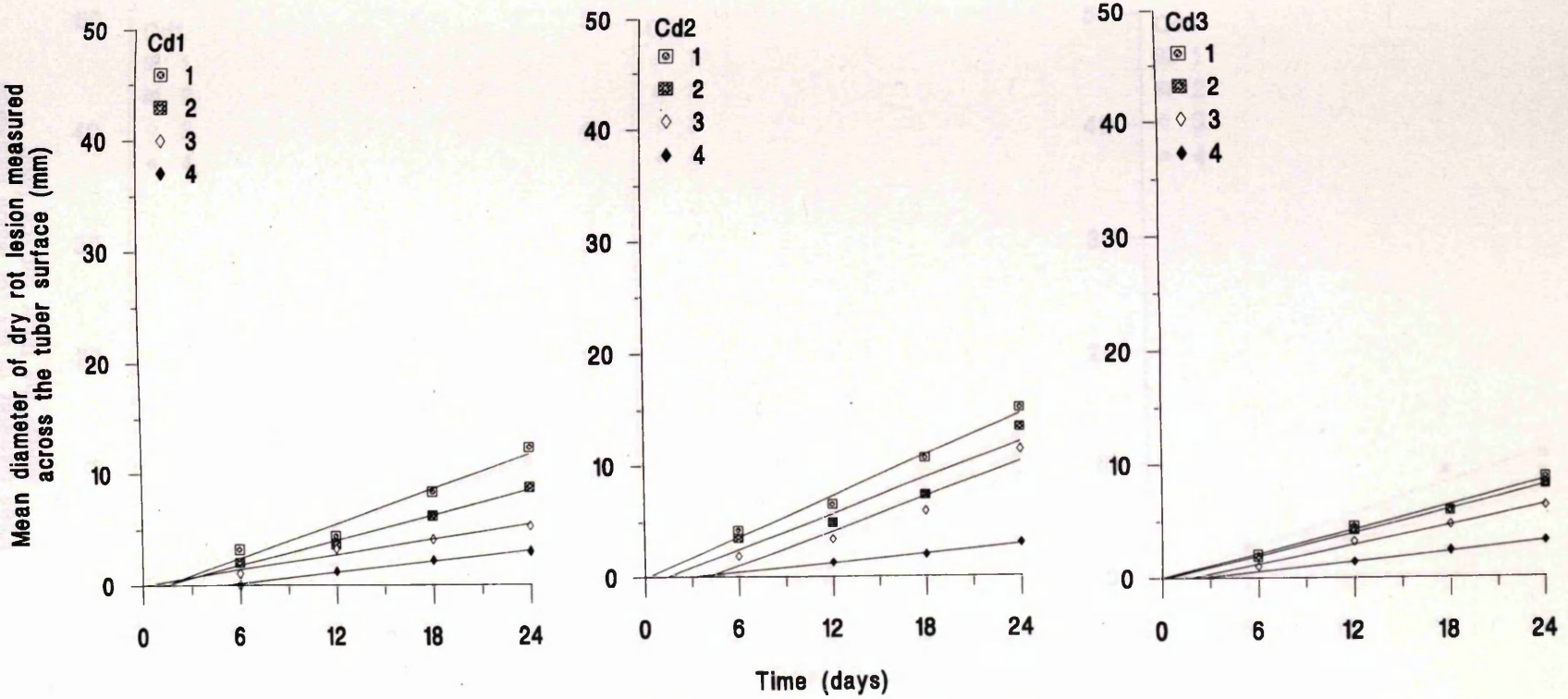


Fig. 12b First experiment March (1990)
Cv Record

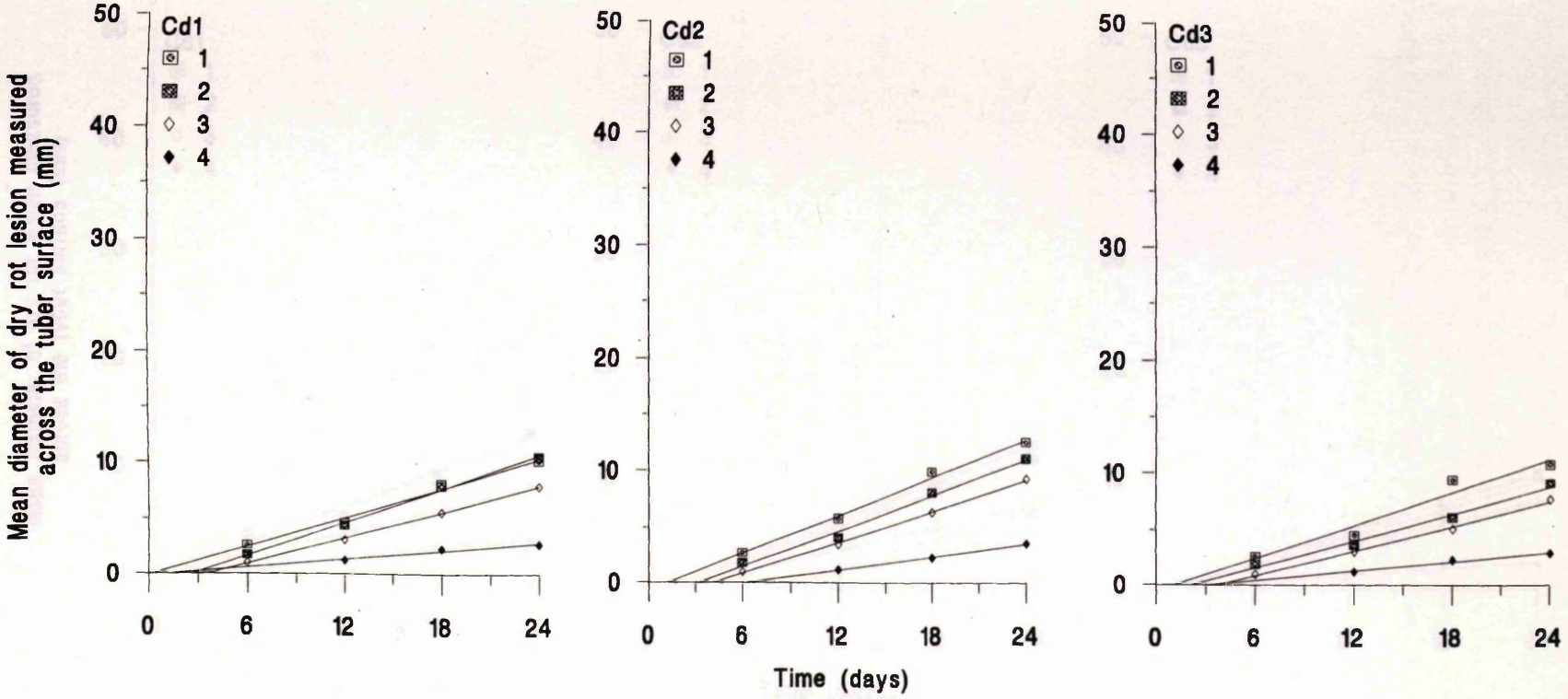


Fig. 12c Second experiment March (1991)
Cv Maris Piper

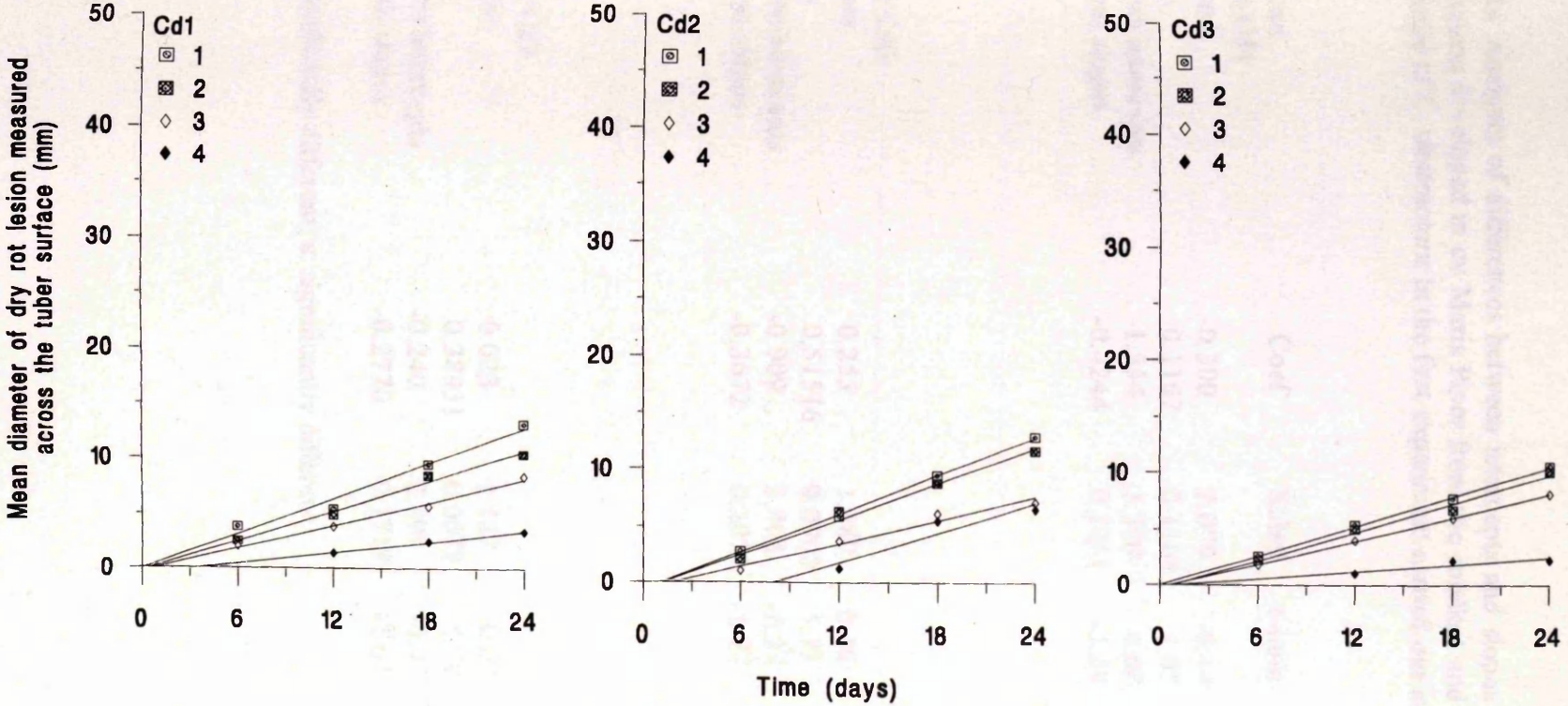


Fig. 12d Second experiment march (1991)
Cv Record

Table 11a. Analyses of differences between intercepts and slopes of regression lines of dry rot lesions developed in cv Maris Piper from the smallest and the largest inocula of three isolates of *C. destructans* in the first experiment carried out in March 1990.

Predictor	Coef	Stdev	t-ratio	p	
Isolate Cd1					
Constant	-0.300	2.070	-0.14	0.885	
Slopes	0.1167	0.1109	1.05	0.299	
Between intercepts	1.554	2.300	0.68	0.503	n.s
Between slopes	-0.4244	0.1251	-3.39	0.002	s
Isolate Cd2					
Constant	0.253	1.587	0.16	0.874	
Slopes	0.51516	0.0902	5.71	0.000	
Between intercepts	-0.909	3.904	-0.23	0.817	n.s
Between slopes	-0.3672	0.2079	-1.77	0.085	s
Isolate Cd3					
Constant	0.023	1.127	0.02	0.984	
Slopes	0.38931	0.0672	5.79	0.000	
Between intercepts	-0.240	2.299	-0.07	0.942	n.s
Between slopes	-0.2770	0.1719	-1.61	0.115	n.s

n.s: no significantly different; s: significantly different.

Table 11b. Analyses of differences between intercepts and slopes of regression lines of dry rot lesions developed in cv Record from the smallest and the largest inocula of three isolates of *C. destructans* in the first experiment carried out in March 1990.

Predictor	Coef	Stdev	t-ratio	p	
Isolate Cd1					
Constant	-0.988	1.030	-0.96	0.343	
Slopes	0.53538	0.0594	9.01	0.000	
Between intercepts	0.418	2.309	0.18	0.857	n.s
Between slopes	-0.3857	0.1241	-0.11	0.003	s
Isolate Cd2					
Constant	0.261	1.478	0.18	0.861	
Slopes	0.59684	0.0868	6.88	0.000	
Between intercepts	-0.829	3.896	0.21	0.832	n.s
Between slopes	-0.4496	0.2050	-2.19	0.033	s
Isolate Cd3					
Constant	0.7920	0.8544	0.93	0.360	
Slopes	0.31516	0.0493	6.33	0.000	
Between intercepts	-1.159	1.858	-0.62	0.537	n.s
Between slopes	-0.1652	0.1013	-1.63	0.111	n.s

n.s: no significantly different; s: significantly different.

Table 11c. Analyses of differences between intercepts and slopes of regression lines of dry rot lesions developed in cv Maris Piper from the smallest and the largest inocula of three isolates of *C. destructans* in the second experiment carried out in March 1991.

Predictor	Coef	Stdev	t-ratio	p	
Isolate Cd1					
Constant	-0.1523	0.9513	-0.16	0.873	
Slopes	0.44743	0.0549	8.14	0.000	
Between intercepts	0.030	2.058	0.01	0.989	n.s
Between slopes	-0.3248	0.1094	-2.97	0.005	s
Isolate Cd2					
Constant	-7.7822	0.9551	-0.82	0.417	
Slopes	0.56723	0.0565	10.04	0.000	
Between intercepts	-0.445	2.106	-0.21	0.833	n.s
Between slopes	-0.3686	0.1128	-3.27	0.002	s
Isolate Cd3					
Constant	-0.4885	91.94	0.53	0.598	
Slopes	0.48635	0.0550	8.84	0.000	
Between intercepts	0.187	2.209	0.08	0.933	n.s
Between slopes	-0.3511	0.1178	-2.98	0.005	s

n.s: no significantly different; s: significantly different.

Table 11d. Analyses of differences between intercepts and slopes of regression lines of dry rot lesions developed in cv Record from the smallest and the largest inocula of three isolates of *C. destructans* in the second experiment carried out in March 1991.

Predictor	Coef	Stdev	t-ratio	p	
Isolate Cd1					
Constant	-0.2870	0.9128	-0.31	0.755	
Slopes	0.49132	0.0528	9.30	0.000	
Between intercepts	0.872	1.911	0.46	0.651	n.s
Between slopes	-0.3231	0.1018	-3.17	0.003	s
Isolate Cd2					
Constant	-0.765	1.058	0.72	0.474	
Slopes	0.56532	0.0617	9.16	0.000	
Between intercepts	-2.869	2.237	-1.28	0.206	n.s
Between slopes	-0.0906	0.1197	-0.76	0.453	n.s
Isolate Cd3					
Constant	0.523	1.028	0.51	0.613	
Slopes	0.41257	0.0593	6.96	0.000	
Diffe between intercepts	-0.040	2.485	-0.02	0.987	n.s
Diffe between slopes	-0.3330	0.1296	-2.57	0.013	s

n.s: no significant differently; s: significantly different.

Table 12. Analysis of variance of dry rot lesion size visible externally on the tuber surface 24 days after inoculation with three different isolates of *C. destructans.*

FIRST EXPERIMENT (March 1990)

Cv Maris Piper

Source	DF	SS	MS	F	P	
Inoculum levels	3	819.13	273.04	18.02	0.000	s
Isolates	2	72.42	36.21	2.39	0.098	n.s
Inoculum levels x Isolates	6	36.90	6.15	0.41	0.873	n.s
Error	108	1212.34	15.15			
Total	119	2177.65				

Cv Record

Source	DF	SS	MS	F	P	
Inoculum levels	3	1004.82	319.76	22.09	0.000	s
Isolates	2	368.52	153.55	10.61	0.000	s
Inoculum levels x Isolates	6	116.64	19.44	1.34	0.249	n.s
Error	108	1129.32	14.48			
Total	119	2619.29				

SECOND EXPERIMENT (March 1991)

Cv Maris Piper

Source	DF	SS	MS	F	P	
Inoculum levels	3	908.789	305.276	51.30	0.000	s
Isolates	2	36.891	17.824	3.00	0.056	n.s
Inoculum levels x Isolates	6	10.224	1.704	0.29	0.942	n.s
Error	108	464.196				
Total	119	1420.100				

Cv Record

Source	DF	SS	MS	F	P	
Inoculum levels	3	1010.117	340.983	45.94	0.000	s
Isolates	2	10.297	3.492	0.47	0.626	n.s
Inoculum levels x Isolates	6	33.499	5.583	0.75	0.609	n.s
Error	108	593.771	7.422			
Total	119	1647.685				

n.s: no significantly different; s: significantly different.

Figures 13 (a, b, c, d). Dry rot lesions visible on the tuber surfaces of cvs Maris Piper and Record 24 days after inoculation with three isolates (Cd1, Cd2 and Cd3) of *C. destructans.*

Experiments carried out in March 1990 and 1991. Tubers were inoculated with different inoculum levels: 1 = $6x10^5$ spores, 2 = $6x10^4$ spores, 3 = $6x10^3$ spores, 4 = $6x10^2$ spores, 5 = $6x10^1$ spores. Each value is the mean of measurements on 10 tubers.

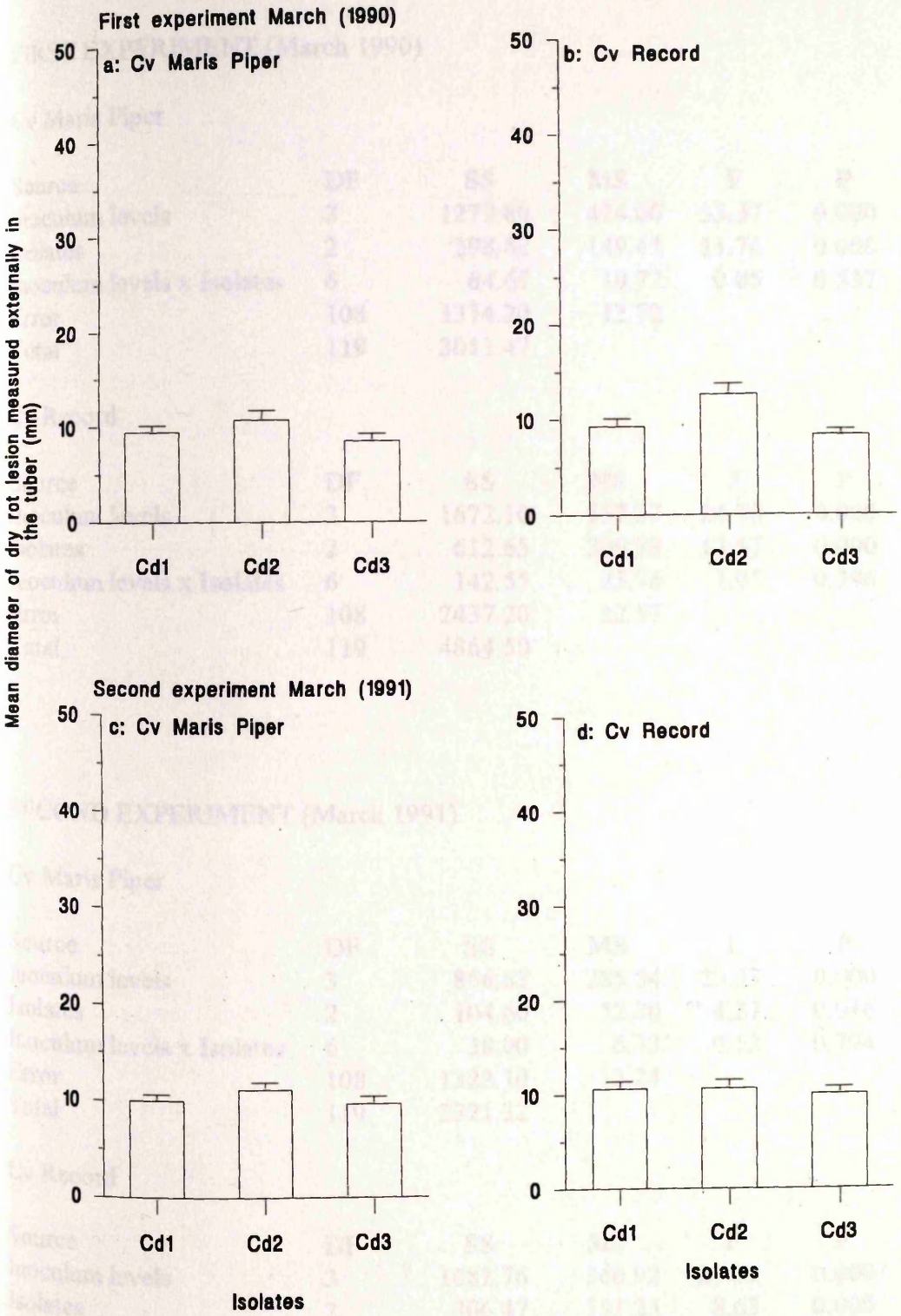

First experiment March (1990)
a: Cv Maris Piper
b: Cv Record
Second experiment March (1991)
c: Cv Maris Piper
d: Cv Record
Mean diameter of dry rot lesion measured externally in the tuber (mm)
50
40
30
20
10
0
Cd1
Cd2
Cd3
Isolates

Table 13. Analysis of variance of dry rot lesion size measured internally in tuber 24 days after inoculation with three different isolates of *C. destructans.*

FIRST EXPERIMENT (March 1990)

Cv Maris Piper

Source	DF	SS	MS	F	P	
Inoculum levels	3	1273.80	424.60	33.37	0.000	s
Isolates	2	298.82	149.41	11.74	0.000	s
Inoculum levels x Isolates	6	64.65	10.77	0.85	0.537	n.s
Error	108	1374.20	12.72			
Total	119	3011.47				

Cv Record

Source	DF	SS	MS	F	P	
Inoculum levels	3	1672.10	557.37	24.70	0.000	s
Isolates	2	612.65	306.33	13.57	0.000	s
Inoculum levels x Isolates	6	142.55	23.76	1.05	0.396	n.s
Error	108	2437.20	22.57			
Total	119	4864.50				

SECOND EXPERIMENT (March 1991)

Cv Maris Piper

Source	DF	SS	MS	F	P	
Inoculum levels	3	856.63	285.54	23.33	0.000	s
Isolates	2	104.60	52.30	4.27	0.016	s
Inoculum levels x Isolates	6	38.00	6.33	0.52	0.794	n.s
Error	108	1322.10	12.24			
Total	119	2321.32				

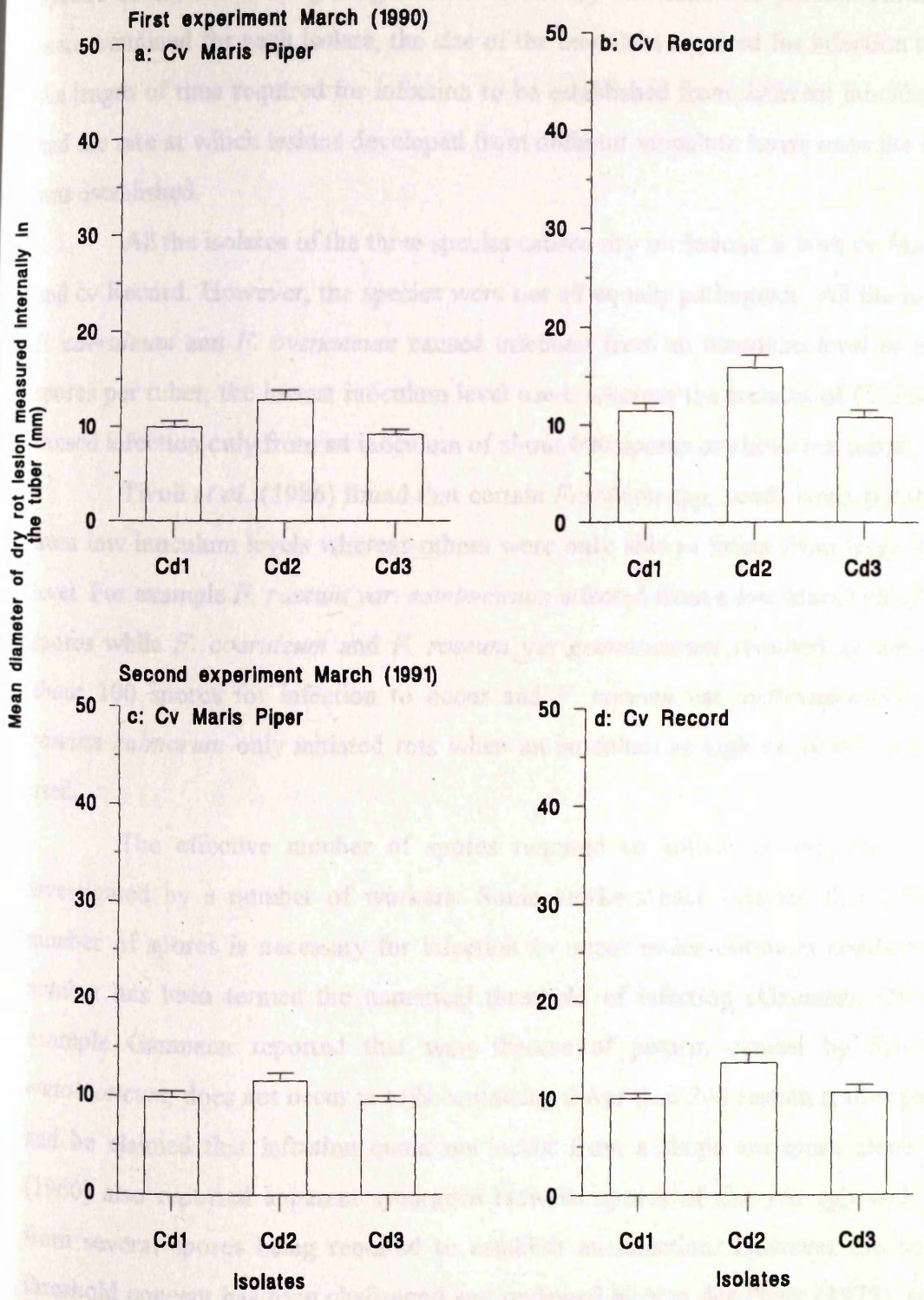
Cv Record

Source	DF	SS	MS	F	P	
Inoculum levels	3	1082.76	360.92	20.32	0.000	s
Isolates	2	306.47	153.23	8.63	0.000	s
Inoculum levels x Isolates	6	115.67	19.28	1.09	0.376	n.s
Error	108	1918.10	17.76			
Total	119	3422.99				

n.s: no significant differently; s: significant differently.

Figures 14 (a, b, c). Development of dry rot lesions internally in the tubers of cvs Maris Piper and Record 24 days after inoculation with three isolates (Cd1, Cd2 and Cd3) of *C. destructans*.

Experiment carried out in March 1991 and 1991. Tubers were inoculated with different inoculum levels: 1 = $6x10^5$ spores, 2 = $6x10^4$ spores, 3 = $6x10^3$ spores, 4 = $6x10^2$ spores, 5 = $6x10^1$ spores. Each value is the mean of measurements on 10 tubers.


First experiment March (1990)
a: Cv Maris Piper
b: Cv Record
Second experiment March (1991)
c: Cv Maris Piper
d: Cv Record
Mean diameter of dry rot lesion measured internally in the tuber (mm)
50
40
30
20
10
0
Cd1
Cd2
Cd3
Isolates

3.5. Discussion

These experiments compared the pathogenicity of different isolates of three species of dry rot causing fungi isolated from dry rot lesions in potato. Three factors were examined for each isolate, the size of the inoculum required for infection to occur, the length of time required for infection to be established from different inoculum levels and the rate at which lesions developed from different inoculum levels once the infection was established.

All the isolates of the three species caused dry rot lesions in both cv Maris Piper and cv Record. However, the species were not all equally pathogenic. All the isolates of *F. caeruleum* and *F. avenaceum* caused infection from an inoculum level of about 60 spores per tuber, the lowest inoculum level used, whereas the isolates of *C. destructans* caused infection only from an inoculum of about 600 spores or above per tuber.

Tivoli *et al.* (1986) found that certain *Fusarium* spp, could infect potato tubers from low inoculum levels whereas others were only able to infect from large inoculum level. For example *F. roseum var. sambucinum* infected from a low inoculum of about 5 spores while *F. coeruleum* and *F. roseum* var *graminearum* required an inoculum of about 100 spores for infection to occur and *F. roseum* var. *arthrosporiodes* and *F. roseum culmorum* only initiated rots when an inoculum as high as 10,000 spores was used.

The effective number of spores required to initiate an infection has been investigated by a number of workers. Some workers have claimed that a minimum number of spores is necessary for infection to occur under optimum conditions. This number has been termed the numerical threshold of infection (Gaumann, 1950). For example Gaumann reported that wart disease of potato, caused by *Synchytrium endobioticum,* does not occur in soils containing fewer than 200 resting spores per gram, and he claimed that infection could not occur from a single zoospore alone. Garret (1960) also reported apparent synergism between spores of *Botrytis* spp with hyphae from several spores being required to establish an infection. However the numerical threshold concept has been challenged and reviewed by Van der Plank (1975), since the theory requires a disease inoculum curve of a sort which has never been found for any fungus disease, and can not be derived from the data that Nutman & Roberts (1963) and others cite as evidence. It is now generally accepted that one spore is sufficient to establish an infection (Mckee, 1964; Deverall & Wood, 1961). For example Petersen

(1959) found no evidence of synergistic interaction between uredospores of *Puccinia graminis* at low inoculum levels and the number of infections stayed roughly constant, with increasing numbers of spores yielding a proportional increase in the number of infections, However, at high concentrations the spores appeared to act synergistically, helping one another to infect; but Petersen found evidence to suggest that at high concentrations spore germination, not infection, was stimulated. Wastie (1962) found that the ED_{50} for *B. fabae* on bean leaves was three to four spores while that for *B. cineria* was about 500, but the slope of the dose-response curves suggested that lesion development was initiated by the independent action of individual spores for both rather than by the synergistic action of a number. In all examples where it was claimed that a minimum number of spores was required to achieve infection, workers have confused a numerical threshold of infection with a dilution end point. A dilution end point, used extensively in virology, is the highest dilution of inoculum compatible with being reasonably sure of achieving infection. Thus, virologists are agreed that in ordinary conditions a tobacco plant must be inoculated with about a million particles of tobacco mosaic virus (the dilution end point) to ensure that the plant will become infected. However the disease-inoculum curve for tobacco mosiac virus is a ' one- hit ' curve and virologists are agreed that the actual infection is established from a single particle: i.e. only one of the million particles applied to the plant causes the infection and the rest take no part.

Gaumann (1950) was right in claiming that to be reasonably sure of infecting a potato plant with *Synchytrium endobioticum* the plant should be grown in soil containing at least 200 resting spores per gram. The actual infection however, is carried out by a single zoospore with either help nor hindrance from any other spore in the soil. The probability that any one particular zoospore will start an infection may be small, but if any one starts an infection, it does it alone.

The dilution end points for all isolates of the two *Fusarium* spp used in this study were lower than 60 spores per tuber, whereas those for the three isolates of *C. destructans* were much higher and around 600 spores per tuber. Clearly almost all spores of the two *Fusarium* spp were capable of initiating an infection whereas a much smaller proportion of those *C. destructans* were capable of doing so. However, even if only one spore was required to cause an infection, it is clear that the more spores that are inoculated the faster the lesions establish and the faster the growth of those lesions once

they are established. That the inoculum level affects the rate at which lesions establish is shown by the differences between the points of intercept for each inoculum level of each isolate, the smaller the inoculum the longer the time taken to establish infection. This result is not surprising since the larger the inoculum the larger the number of infections to establish a bridge head from the inoculation hole. In general, the isolates of *F. avenaceum* established growth faster than those of *F. caeruleum*, but surprisingly *C. destructans* established infection much faster than either of the two *Fusarium* spp. Other workers have reported that *F. avenaceum* establishes lesions faster than *F. caeruleum*. For example Mckee (1954) found that brown necrosis of tuber tissues was visible within a week after inoculation with isolates of *F. caeruleum*, but within 3 days with isolates of *F. avenaceum*.

Once growth was initiated into the tubers of both cultivars, by all three fungi lesion size increased linearly with time but the rate of increase was slower from low inocula than from high. The fact that growth rates were linear and that this rate can vary according to inoculum level suggests that the resistance of the tuber does not increase nor decrease as the lesion progresses. This result does not provide any evidence for the involvement of an active component in resistance to lesion growth, since tissue resistance appears to remain unchanged during the course of infection. Thus resistance is most likely determined by passive factors with the tissue acting more or less as an inert substrate for fungal growth. This result contrasts with that of Corsini *et al.* (1977) who studied the characteristics of *Fusarium* resistance in potato breeding clones and found that the terpenoid compound rishitin, was produced more rapidly in the more resistant clones, and suggested that resistance may be determined by both active and passive factors.

The mean sizes of lesions produced by both *F. caeruleum* and *F. avenaceum* at the end of each experiment were slightly larger in cv Maris Piper than in cv Record indicating that Maris Piper was slightly more susceptible than cv Record to both species. There were also consistent differences between the growth rates of the different isolates of both *Fusarium* species in each clone showing that the isolates within each species varied in aggressiveness. Not all workers have found that cv Maris Piper is more susceptible to dry rot disease than cv Record. Thus Seppanen (1981c) investigated the resistance of different cultivars of potato to *Fusarium* spp and found that both cvs Maris Piper and Record were moderately resistant to *F. avenaceum* but susceptible to *F.*

solani var. caeruleum. Jellis (1975) in a comparative study of the pathogenicity of one isolate of *F. caeruleum* found that Record was slightly more susceptible than Maris Piper, although Wastie *et al.* (1989) found, as in this work, that cv Maris Piper was more susceptible than cv Record to *F. caeruleum.*

The mean diameters of the lesions produced in cvs Maris Piper and Record by all the isolates of *F. caeruleum* and *F. avenaceum* were slightly larger at the end of the experiment carried out in March 1991 than at the end of the experiment carried out in January 1992. In contrast the lesions caused by isolates *C. destructans* were small and approximately the same size at the end in both experiments. Although it is difficult to draw conclusions from these experiments, since inevitably they were independent experiments, the results are consistent with a reduction in tuber resistance to both *F. coeruleum and F. avenaceum* during storage but not in resistance to *C. destructans.* Boyd (1952b) and Mckee (1952, 1954) reported that resistance to infection decreased as the storage season advanced. However it should be noted that the tubers used in each experiment were obtained from different sources and so the differences could be due to factors relating to the origin of the tubers rather than changes in resistance during storage.

CHAPTER IV

GERMINATION AND THE EARLY STAGES OF INFECTION IN TUBER SLICES

CHAPTER IV

GERMINATION AND THE EARLY STAGES OF INFECTION IN TUBER SLICES

4.1. Introduction

The experiments described in the last chapter showed that although *C. destructans* was a weaker pathogen than either *F. coeruleum* or *F. avenaceum* in terms of size of lesion produced it initiated growth into the tubers from all levels of inoculum much earlier than either of the *Fusarium* spp. Thus differences in growth allied to possible differences in the mechanisms of pathogenicity must develop after germination and the following experiments were carried out to investigate germination of the three species on the surfaces of tuber slices and to follow the early stages of tissue infection. These experiments were carried out to determine the stage at which the differences in the mode of infection by *C. destructans* were established relative to *F. caeruleum* and *F. avenaceum.* The details of the experiments are given in Materials and Methods.

4.2. Spore germination

The first germ-tubes were observed to emerge from spores of all three species about 3 h after inoculation and once germination began, the number of germinated spores of each species continued to increase rapidly, with the majority of spores having germinated by 12 h after inoculation. After 12 h the germ tubes became so entangled that it was not possible to count germinated spores or measure germ-tube length. The results are plotted in Figure 15 (a, b, c).

An analysis of variance was carried out on the results for each species to determine if the differences in germination were statistically significant. The result are presented separately for each species.

4.2.1. *F. coeruleum*

The ANOVA (Table 14a) shows significant differences ($P \leq 0.001$) in percentage germination between isolates with isolates Fc3 and Fc4 having slightly higher germination than isolates Fc1 and Fc2.

Figures 15 (a, b, c). Time course of germination on the wound surfaces of tuber slices of cv Maris Piper of different isolates of (a) *F. coeruleum,* (b) *F. avenaceum* and (c) *C. destructans.*

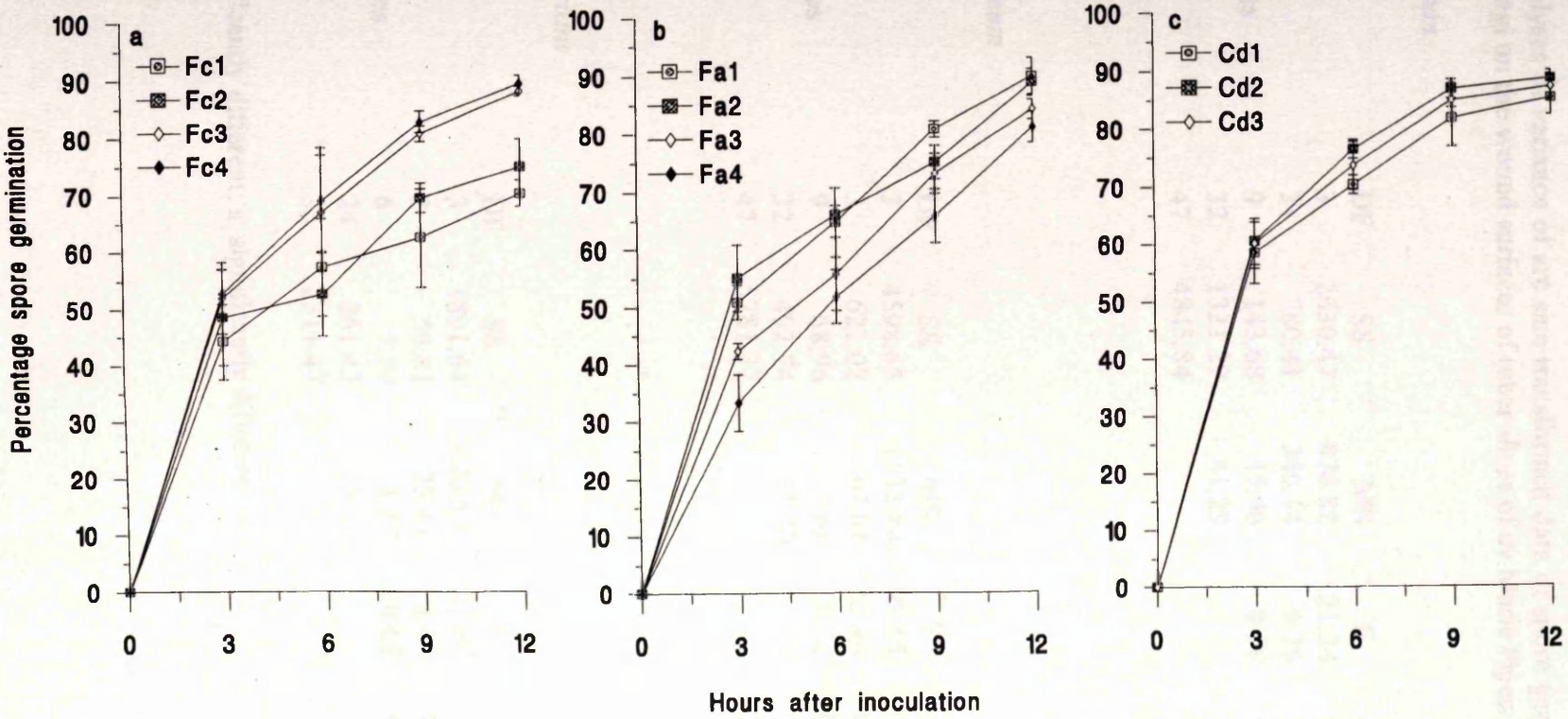

a
Fc1
Fc2
Fc3
Fc4
b
Fa1
Fa2
Fa3
Fa4
c
Cd1
Cd2
Cd3
Percentage spore germination
Hours after inoculation
0
10
20
30
40
50
60
70
80
90
100
0
3
6
9
12

Table 14. Analyses of variance of arc sine transformed data of spore germination of dry rot causing fungi on the wound surfaces of tuber slices of cv Maris Piper.

(a) *F. coeruleum*

Source	DF	SS	MS	F	P	
Time	3	2630.47	876.82	21.24	0.000	s
Isolates	3	780.41	260.14	6.30	0.002	s
Time x Isolates	9	143.68	15.96	0.39	0.933	n.s
Error	32	1321.29	41.29			
Total	47	4845.84				

(b) *F. avenaceum*

Source	DF	SS	MS	F	P	
Time	3	4598.65	1532.88	99.55	0.000	s
Isolates	3	621.02	207.01	13.44	0.000	s
Time x Isolates	9	68.96	7.66	0.50	0.865	n.s
Error	32	492.74	15.40			
Total	47	5781.37				

(c) *C. destructans*

Source	DF	SS	MS	F	P	
Time	3	1891.64	630.55	41.87	0.000	s
Isolates	2	50.81	25.41	1.69	0.206	n.s
Time x Isolates	6	7.59	1.27	0.08	0.997	n.s
Error	24	361.43	15..6			
Total	35	2311.47				

n.s: not significantly different; s: significantly different

4.2.2. *F. avenaceum*

The ANOVA (Table 14b) shows significant differences ($P \leq 0.001$) in percentage spore germination between isolates with isolates Fa1 and Fa2 having slightly higher germination than Fa3 and Fa4.

4.2.3. *C. destructans*

The ANOVA (Table 14c) shows that there were no significant differences between the three isolates in spore germination.

4.3. Germ-tube growth

The germ-tube lengths at different times after inoculation are plotted in Figure (16 a, b, c). Isolates of all three species showed a large variation in germ-tube length at each time of measurement.

Germ-tube growth of all isolates of the three species continued after 12 h but the germ-tubes became so long, and so highly branched that it was not impossible to isolate individual sporelings for measurement. Thus only results obtained during the first 12 h were analysed.

An analysis of variance was carried out for each species to determine if any of the differences between isolates were significant

4.3.1. *F. coeruleum*

The ANOVA (Table 15a) shows significant differences ($P \leq 0.01$) in germ tube length between isolates at each time with isolate Fc1 having the slowest growth and isolate Fc4 the fastest, Isolates Fc2 and Fc3 had growth rates intermediate between Fc1 and Fc4.

4.3.2 *F. avenaceum*

The ANOVA (Table 15b) shows significant differences ($P \leq 0.001$) in germ tube length between isolates. with isolates Fa2 and Fa3, growing significantly faster than isolates Fa1 and Fa4.

4.3.3. *C. destructans*

The ANOVA (Table 15c) shows no significant differences in germ tube length between the three isolates.

Figure 16 (a, b, c). Time course of germ tube growth on the wound surfaces of tuber slices of cv Maris Piper by different isolates of the dry rot causing fungi of (a) *F. coeruleum,* (b) *F. avenaceum* and (c) *C. destructans.*

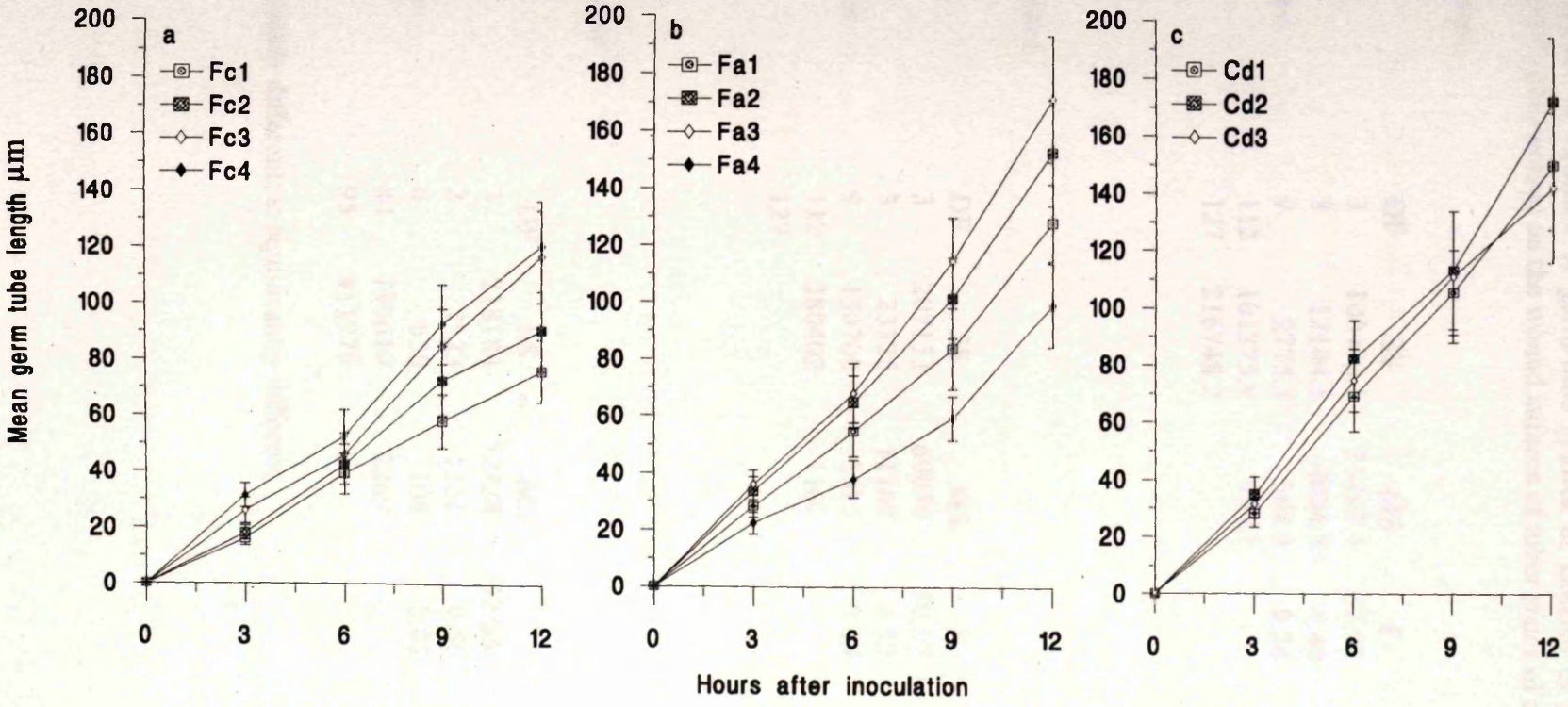

a
Fc1
Fc2
Fc3
Fc4
b
Fa1
Fa2
Fa3
Fa4
c
Cd1
Cd2
Cd3
Mean germ tube length µm
Hours after inoculation
0
20
40
60
80
100
120
140
160
180
200
0
3
6
9
12

Table 15. Analyses of variance of germ-tube growth of isolates of *F. coeruleum*, *F. avenaceum* and *C. destructans* on the wound sufaces of tuber slices of cv Maris Piper.

(a) *F. coeruleum*

Source	DF	SS	MS	F	P	
Times	3	100492.1	33497.4	37.01	0.000	s
Isolates	3	12104.5	4034.8	4.46	0.000	s
Time x Isolates	9	2775.7	308.4	0.34	0.959	n.s
Error	112	101375.9	905.1			
Total	127	216748.2				

(b) *F. avenaceum*

Source	DF	SS	MS	F	P	
Time	3	207151	69050	59.17	0.000	s
Isolates	3	33325	11108	9.52	0.000	s
Time x Isolates	9	130700	1025	0.88	0.547	n.s
Error	112	380402	1167			
Total	127					

(c) *C. destructans*

Source	DF	SS	MS	F	P	
Time	3	218184	72728	32.05	0.000	s
Isolates	2	2523	1261	0.56	0.576	n.s
Time x Isolates	6	650	108	0.05	1.000	n.s
Error	84	190619	2269			
Total	95	411976				

n.s: not significantly different; s: significantly different

4.4. Development of dry rot into the slice

Two days after inoculation considerable hyphal growth of all three species had occurred over the surfaces of the slices of both cultivars.

4.4.1. *F. coeruleum*

Four days after inoculation all isolates had produced a brown to black lesion over the whole slice surface and by 8 days a high density of fungal mycelium was present over the surface as well. The lesions had spread very rapidly and deeply, (up to 6.4-8.8 mm), into the tissue by the 8th day (Plates 3 and 6). The ANOVA (Table 16a) shows that none of the differences between isolates in the depth of lesion produced were significant. The ANOVA also showed that differences between the two cultivars were not significant.

4.4.2. *F. avenaceum*

By 2 days after inoculation the four isolates had produced a brown to black lesion over the whole surface of the slice. Fungal growth was very rapid and by 8 days after inoculation a high density of fungal mycelium was present over the whole of the surface (Plates 4 and 6). Dry rot lesions spread rapidly and deeply into the tissues, (between 7-10mm), by the 8th day. The ANOVA (Table 16b) shows that none of the differences between isolates in the depth of lesions produced by the 8th day were significantly different.

The ANOVA also showed that none of the differences between the two cultivars were significant.

4.4.3. *C. destructans*

One of the isolates of *C. destructans,* isolate Cd2 produced a brown lesion over the whole surface of the slice by 4 days after inoculation and by 8 days a high density of fungal mycelium was present over the whole surface. A brown black depressed lesion was formed, which spread into the tissues. The two other isolates Cd1 and Cd3, had produced slight brown lesions at various sites over the slice surface by 8 days after inoculation and the mycelium produced was much less dense than that of Cd2 (Plates 5 and 6). Penetration of the isolates into the slice tissues was limited (between 2.8-5.9 mm) by 8 days after inoculation The ANOVA (Table 16c) shows that the differences in the depth of lesions produced in the two cultivars by each isolate were significant ($P \leq 0.001$).

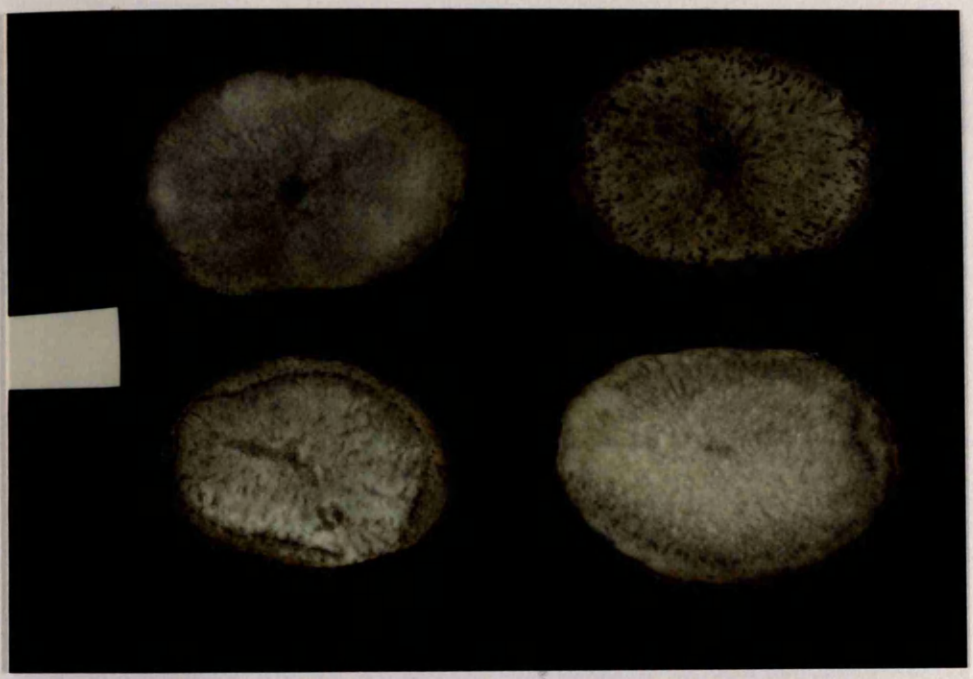


Plate 3. Development of *F. coeruleum* isolates Fc1 (top left) Fc2 (top right) Fc3 (bottom left) Fc4 (bottom right) over the surfaces of tuber slices 8 days after inoculation.

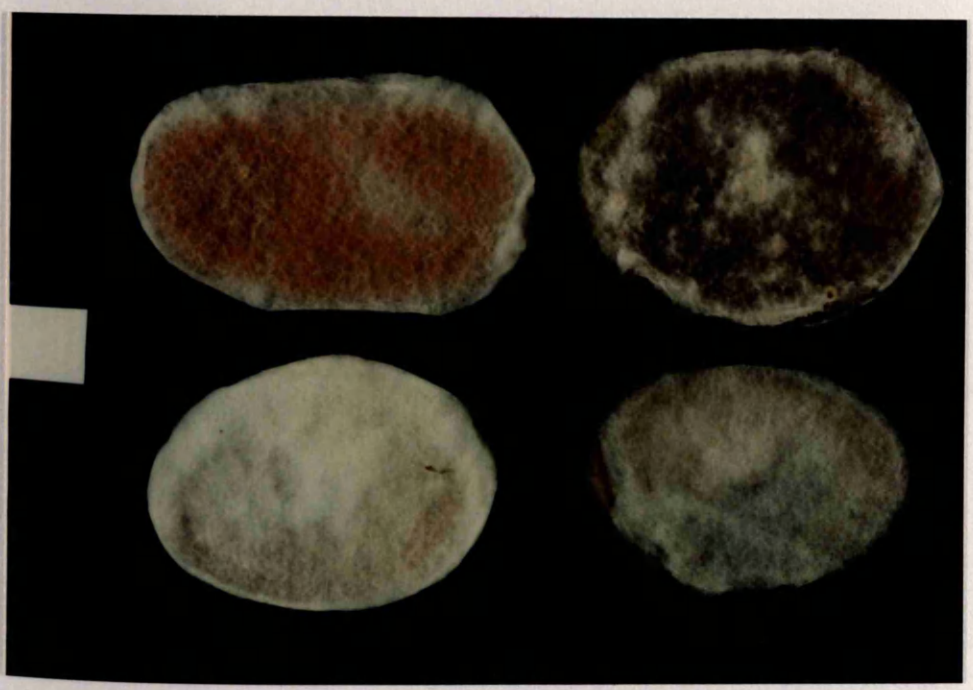


Plate 4. Development of *F. avenaceum* isolates Fa1 (top left) Fa2 (top right) Fa3 (bottom left) Fa4 (bottom right) over the surfaces of tuber slices 8 days after inoculation.

Plate 5. Development of *C. destructans* isolates Cd1 (left) Cd2 (middle) and Cd3 (right) over the surfaces of tuber slices 8 days after inoculation.

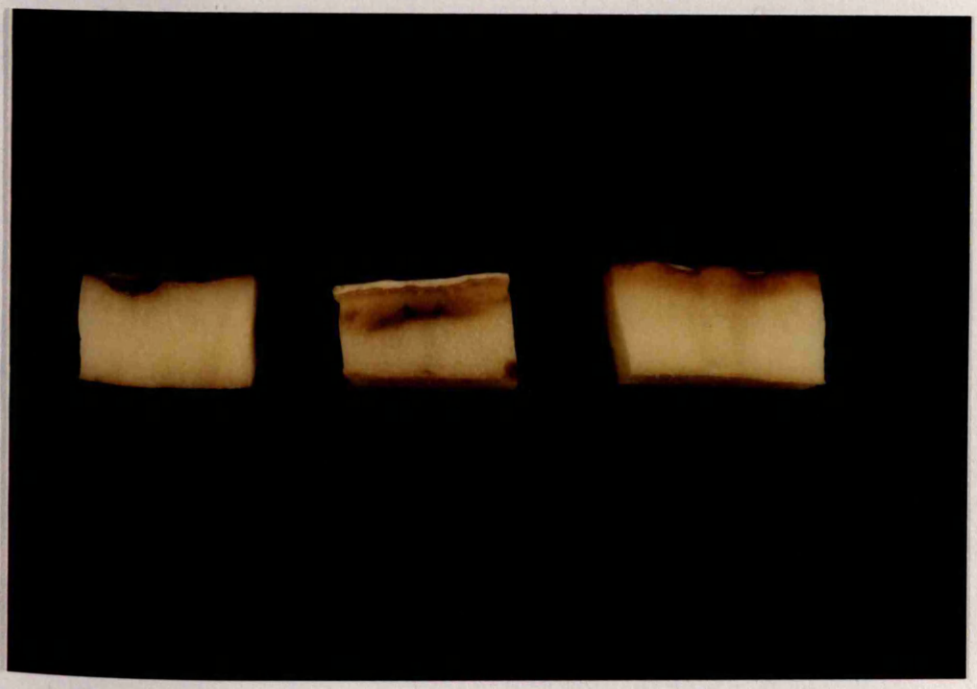


Plate 6. Depth of dry rot lesion produced in slices inoculated with *C. destructans* (left) *F. avenaceum*, (middle) *F. coeruleum* (right) 8 days after inoculation.

Table 16. Analyses of variance of the depth of dry rot lesions developed in tuber slices of cv Maris Piper after inoculation by different isolates of *F. coeruleum*, *F. avenaceum*, and *C. destructans*.

a) *F. coeruleum*

Source	DF	SS	MS	F	P	
Isolates	3	38.600	12.867	2.87	0.052	n.s
Varieties	1	10.000	10.000	2.23	0.145	n.s
Isolates x varieties	3	7.400	2.467	0.55	0.652	n.s
Error	32	143.600	4.488			
Total	39	199.600				

(b) *F. avenaceun*

Source	DF	SS	MS	F	P	
Isolates	3	43.475	14.492	2.74	0.059	n.s
Varieties	1	18.225	18.225	3.45	0.073	n.s
Isolates x varieties	3	0.475	0.158	0.03	0.993	n.s
Error	32	169.200	5.287			
Total	39	231.375				

(c) *C. destructans*

Source	DF	SS	MS	F	P	
Isolates	2	62.067	31.033	34.48	0.000	s
Varieties	1	6.533	6.533	7.26	0.013	s
Isolales x varieties	2	1.267	0.633	0.70	0.505	n.s
Error	24	21.600	0.900			
Total	29	91.467				

n.s: not significantly different; s: significantly different

4.5. Hyphal growth measured by analysis of chitin produced in inoculated slices

The amounts of chitin present on the surfaces and inside the tissue of the potato tuber slices inoculated with one isolate of each of the three different dry rot causing fungi were assayed to get a more exact measure of hyphal growth. Twenty seven slices were inoculated with each species. Three sections (30 mg samples) from the outer infected tissues and three sections for the internal infected tissues (30 mg of rotted tissues) were taken on the day of inoculation and at daily intervals up to 8 days after inoculation. The results of the analyses are plotted in Fig 17. The amounts of glucosamine detected in the external and internal tissues of uninfected, control, slices at each time were small, indicating that tissues contain little glucosamine (Fig 17). The amounts of glucosamine on the external surfaces of slices infected by each pathogen increased rapidly (Fig 17) from the earliest stages of infection, with the increase being most rapid with *F. avenaceum* and least with *C. destructans* (Fig 17).

The ANOVA Table 22 shows that the differences between the amounts of glucosamine produced by each species on the slice surface were significant. Much lower amounts (Fig 17) of glucosamine were present in the internal tissues of the slice than were present on the external surface but the ANOVA Table 17 shows the differences between the three species were significant. Again Fa1 showing the earliest and most rapid increase while Cd2 showed the least.

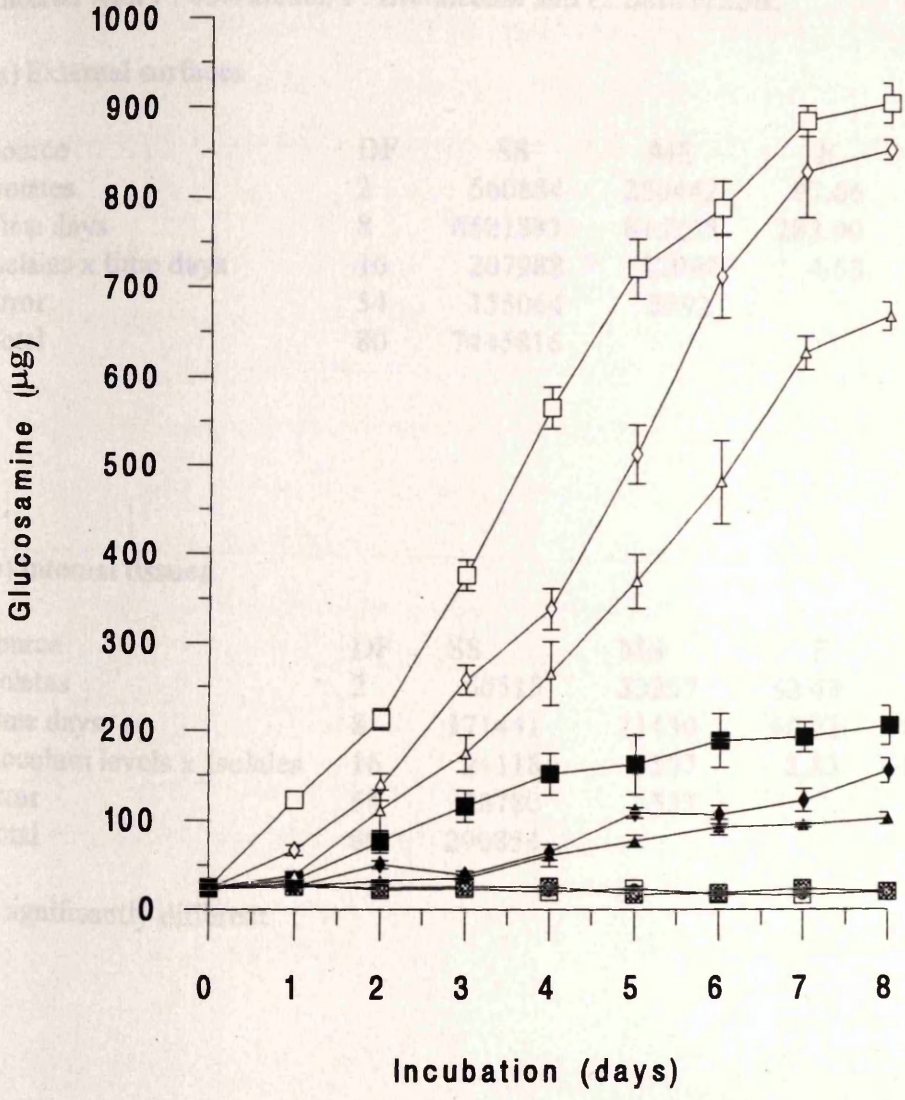


Fig. 17 The course of accumulation of glucosamine in the external and internal tissues of tuber slices of cv Maris Piper after inoculation with F. coeruleum, F. avenaceum and C. destructans.

external control
internal control
external Fc1
internal Fc1
external Fa1
internal Fa1
external Cd2
internal Cd2

Table 17. Analyses of variance of the amounts of chitin detected in potato tuber slices infected with *F. coeruleum*, *F. avenaceum* and *C. destructans*.

(a) External surfaces

Source	DF	SS	MS	F	P	
Isolates	2	560884	280442	97.66	0.000	s
Time days	8	6521881	815235	283.90	0.000	s
Isolales x time days	16	207988	12999	4.53	0.000	s
Error	54	155064	2892			
Total	80	7445816				

(b) Internal tissues

Source	DF	SS	MS	F	P	
Isolates	2	66515	33257	62.40	0.000	s
Time days	8	171441	21430	40.21	0.000	s
Inoculum levels x Isolales	16	24118	1507	2.83	0.000	s
Error	54	28780	533			
Total	80	290854				

s: significantly different

4.6. Discussion

Germination and the early stages of infection of tuber slices by all isolates of the three species *F. coeruleum, F. avenaceum*, and *C. destructans* were compared.

Spores of all isolates started to germinate soon after inoculation and most of the spores of all isolates of three species had germinated within 3 hours. Germination and the early germ tube growth was faster by isolates *C. destructans* than by isolates *F. avenaceum* and *F. coeruleum*. Although the differences in germination between isolates of *C. destructans* were not significant those between isolates of *F. coeruleum* and *F. avenaceum* were significantly different.

Measurement of germ-tube lengths 12 h after inoculation showed that isolates of *F. avenaceum* and *C. destructans* were slightly faster growing than isolates of *F. coeruleum*, but chitin analysis indicated that subsequent growth of *F. coeruleum* and *F. avenaceum* was much higher than that of *C. destructans*.

The reduced growth of *C. destructans*, particularly isolates Cd1 and Cd3 was clearly evident in the extent to which lesions developed over the slice. However, subsequent growth of *C. destructans* particularly of Cd1 and Cd3 was reduced and the lesions did not coalesce over the slice surface. The early none rapid growth of isolates of *C. destructans* on slice surface could have been supported by material released by wound tissue but this species did not penetrate readily into the tissues. In contrast *F. coeruleum* and *F. avenaceum* produced a dense mycelium which spread over the slice surfaces and penetrated deeply into the tissue to colonise the pith and cortex. The tissue clearly has no resistance to those isolates (Moore, 1945; McKee and Boyd, 1952; McKee, 1954).

The amount of glucosamine produced by all 3 species in the external layers of the slices infected by each pathogen increased rapidly with time. The amount of glucosamine produced over the surfaces by isolates *F. avenaceum* and *F. coeruleum* was much larger than that produced by *C. destructans*. Also the amounts of glucosamine produced by *F. avenaceum* in the internal tissues of slices was larger than that produced by *F. coeruleum* and *C. destructans*. Much larger amounts of glucosamine were found over the external surfaces of the slice than that detected in the internal tissues for all pathogens.

CHAPTER V

ELECTRON MICROSCOPY OF DRY ROT LESIONS

CHAPTER V

ELECTRON MICROSCOPY OF DRY ROT LESIONS

5.1. Uninfected tissue

Sections through the cell walls of healthy tuber tissue show a clear stratified structure to the primary wall. In the centre of the wall the middle lamella is present as a thin electron dense region. The outer layers of the primary wall, bordering the cell lumen, are also more electron dense than the inner regions adjacent to the middle lamella. (Plates 7 & 8).

5.2. *F. coeruleum* infected tissue

Sections through tissue infected with *F. coeruleum* show that the hyphae grow within the primary cell wall. The hyphae are surrounded by a thick wall and contain large lipid bodies. Hyphae were not found crossing the middle lamella but they appear to remain in the primary wall on one side of the middle lamella only (Plates 9, 10, 11, 12, 13). Obviously they must grow through the middle lamella at some stage in order to grow from one cell to another, but this was not seen in any section. The middle lamella adjacent to the hyphae remains intact thus appearing relatively resistant to degradation, but the colonised primary wall is highly swollen (Plate 9). The swelling is mainly restricted to the primary wall on the colonised side of the middle lamella and the outer layer of this wall shows a loss of electron dense material. The primary wall on the far side also of the middle lamella to the colonised side appears to be degraded. in pockets or patches, but shows little, if any swelling. Even the outer electron dense region of the primary wall is eroded.

5.3. *F. avenaceum* infected tissue

Sections through tissue infected with *F. avenaceum* (Plate 14 & 15) show that hyphae grow within the primary cell wall as well as within the lumina of dead cells. The hyphae have thick walls and contain lipid bodies and a lot of electron dense material. Walls colonised by the hyphae of *F. avenaceum* appear to split on a line just beneath the electron dense outer region of the primary wall with the hyphae occupying the pocket so formed. Neither the wall external to the hyphae nor the wall internal to the hyphae between it and the middle lamella, appears to undergo significant degradation (Plate 14). The primary wall on the opposite side of the middle lamella is slightly swollen but its

laminar structure is clearly visible and it remains more or less continuous. Walls adjacent to living (Plate 15) or dead (Plate 16) hyphae which are growing within the cell lumina are basically continuous. The middle lamella may not be clearly visible in all cases but the primary wall is still intact (Plate 16). The host wall adjacent to the living hypha shows a loss of electron dense material in pockets. However the wall adjacent to the dead hypha has accumulated a mass of electron dense material. The greater electron density of the host cell wall compared to the walls of healthy tissue could be due to the accumulation of suberin or-lignin-like molecules in the walls after death.

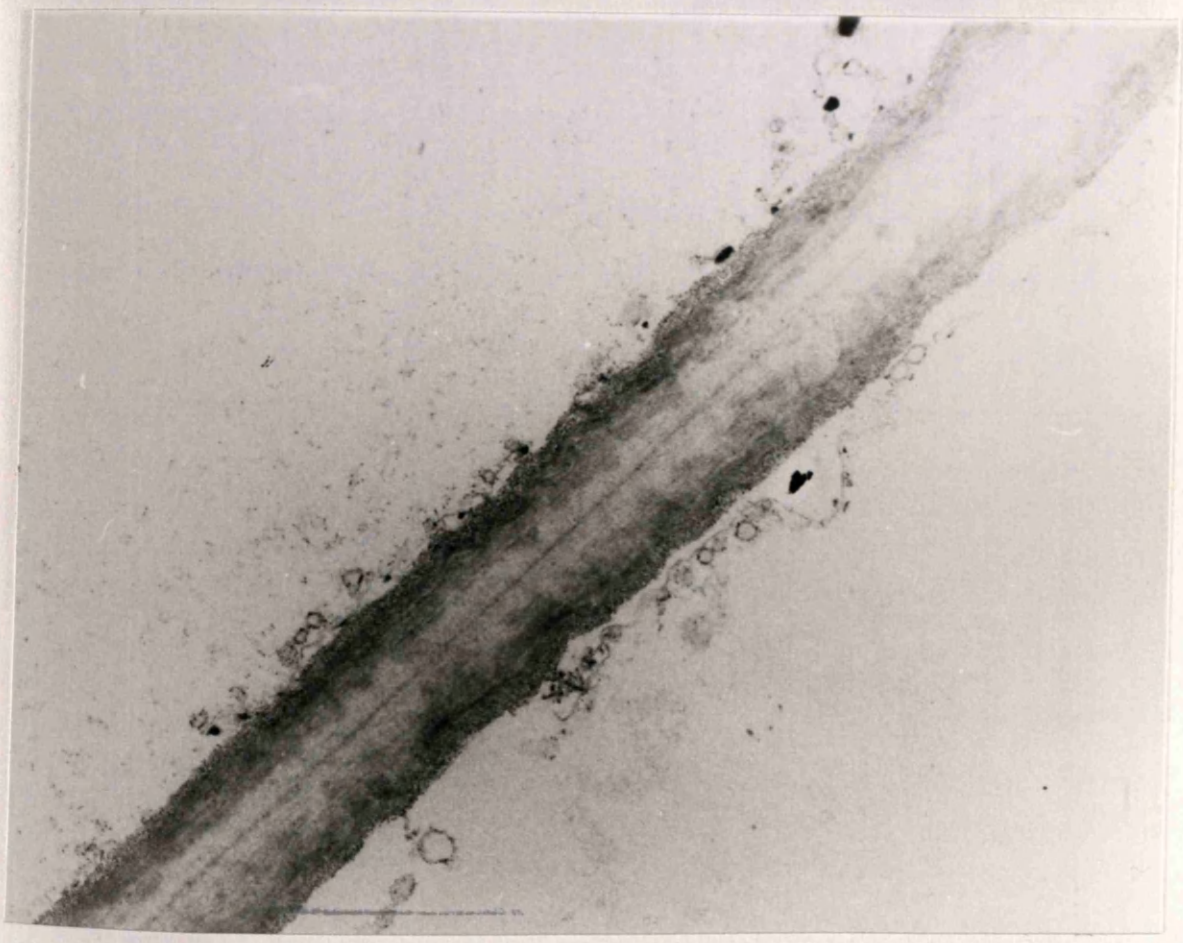


Plate 7. Section through uninfected tissue of a potato tuber cv Maris Piper showing the stratified structure of the cell wall. The electron dense region in the centre marks the position of the middle lamella. The outer layers of the primary wall, adjacent to the cell lumen, are also very electron dense (x 9,000).

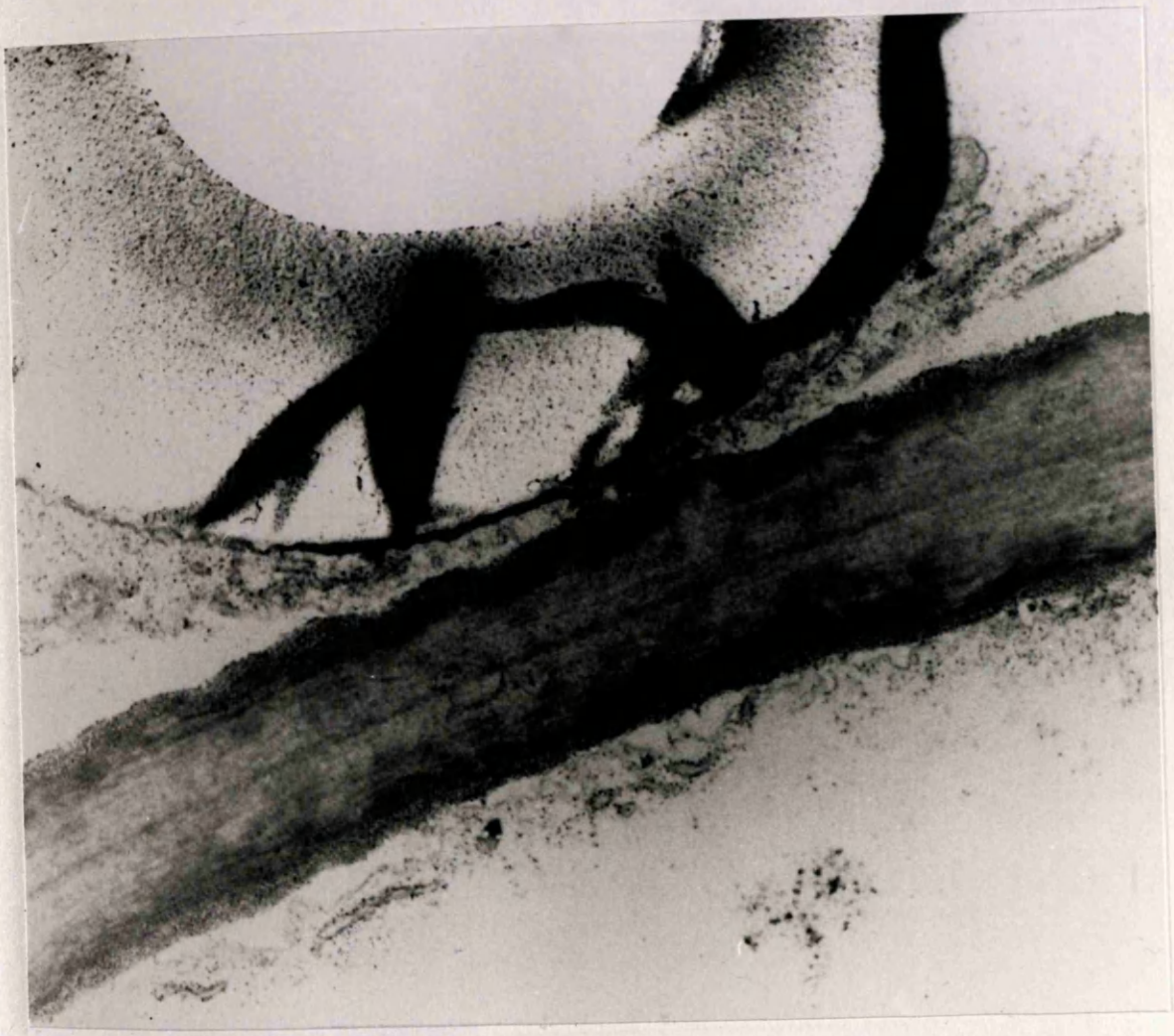


Plate 8. Section at a slightly higher magnification showing the stratified structure of the wall more clearly. The two cells in the section contained cytoplasm. A starch grain is present in the upper cell, adjacent to the wall (x 13,200).

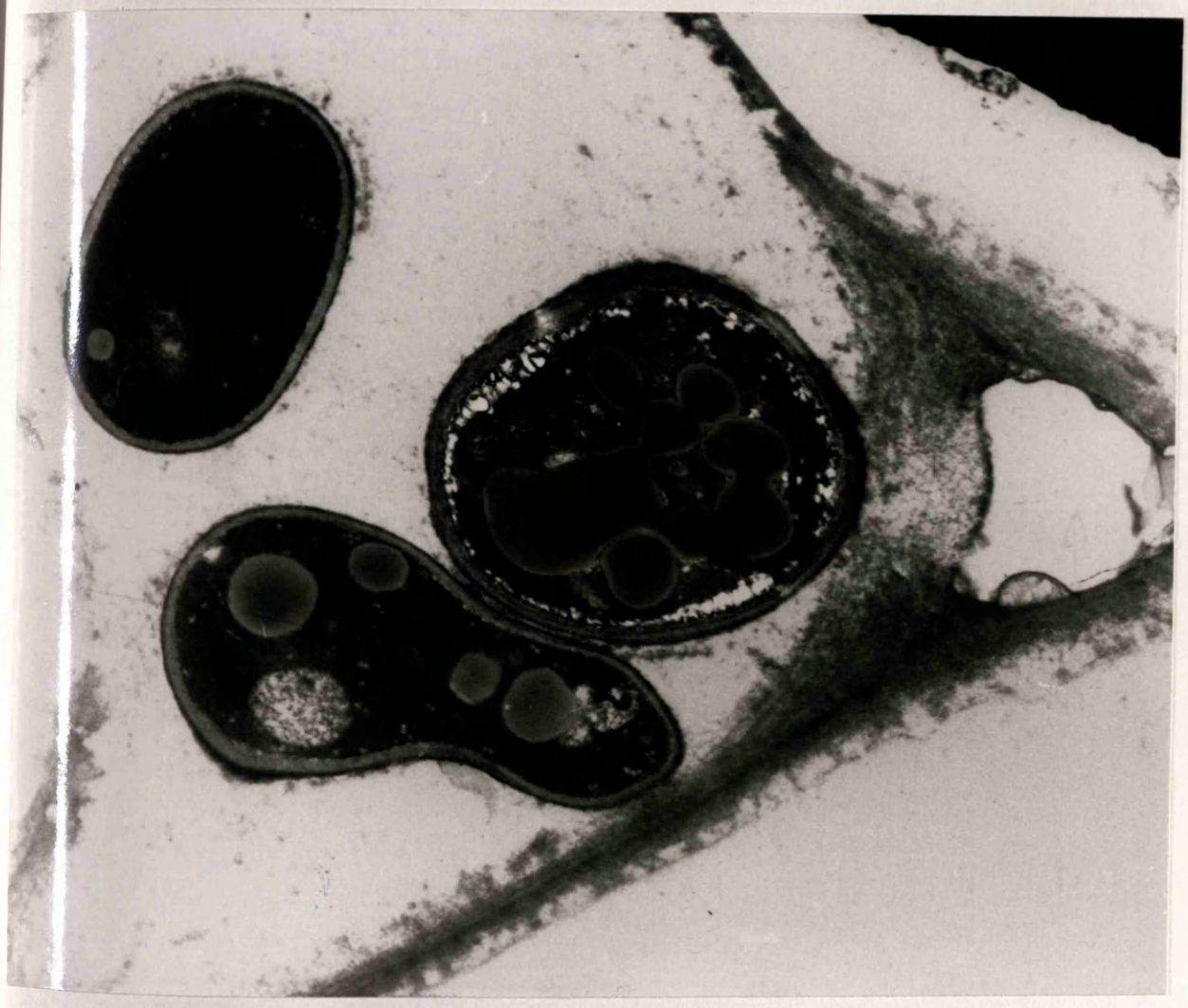


Plate 9. Section showing three fungal hyphae of *F. coeruleum* (Fc1) in cross section growing within a highly swollen section of primary wall near an intercellular space. The middle lamella region on the lower side shows relatively little change but the middle lamella in the wall on the upper right appears to have been degraded in places. The wall between the intercellular space and the hypha on the right shows a light area which probably represents a region of degradation. However, in general, the primary wall between the hyphae and the middle lamella is relatively unchanged. The outer very electron dense layer of the primary wall together with the associated less electron dense material, is highly degraded, particularly towards the upper left of the section. The wall on the lower side of the middle lamella, below the hyphae, shows some erosion in pockets but it is not swollen. The hyphae contain large lipid bodies and are surrounded by thick walls (x 9,000).

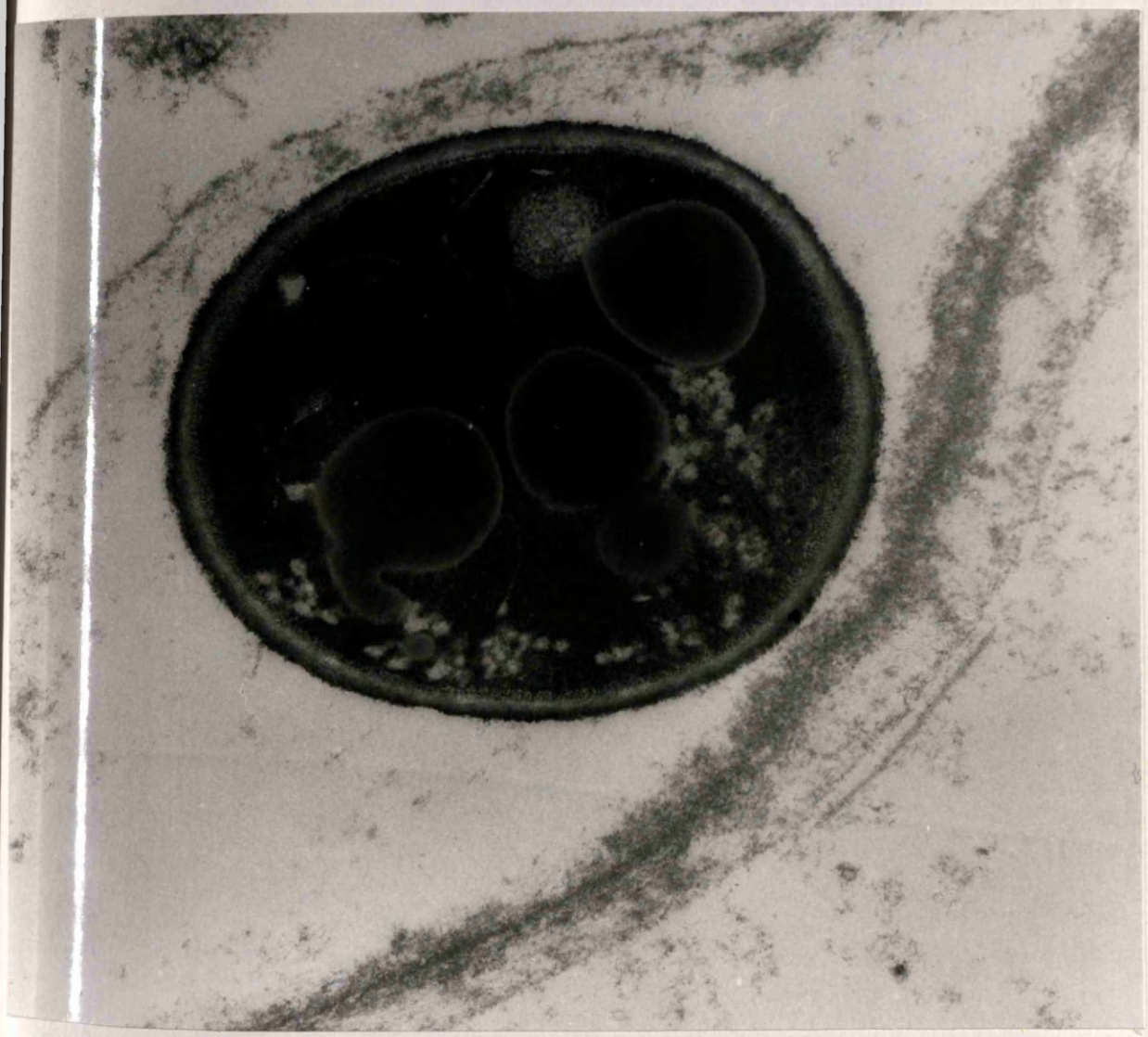


Plate 10. Section of a fungal hypha of *F. coeruleum* (Fc1) growing inside a section of primary wall. The middle lamella is relatively continuous and appears intact. The primary walls on both sides of the middle lamella are highly degraded but swollen only on the side colonised by the hypha. Significant loss of electron dense material from the outer layers of the primary wall has occurred on both sides of the middle lamella (x 30,000).

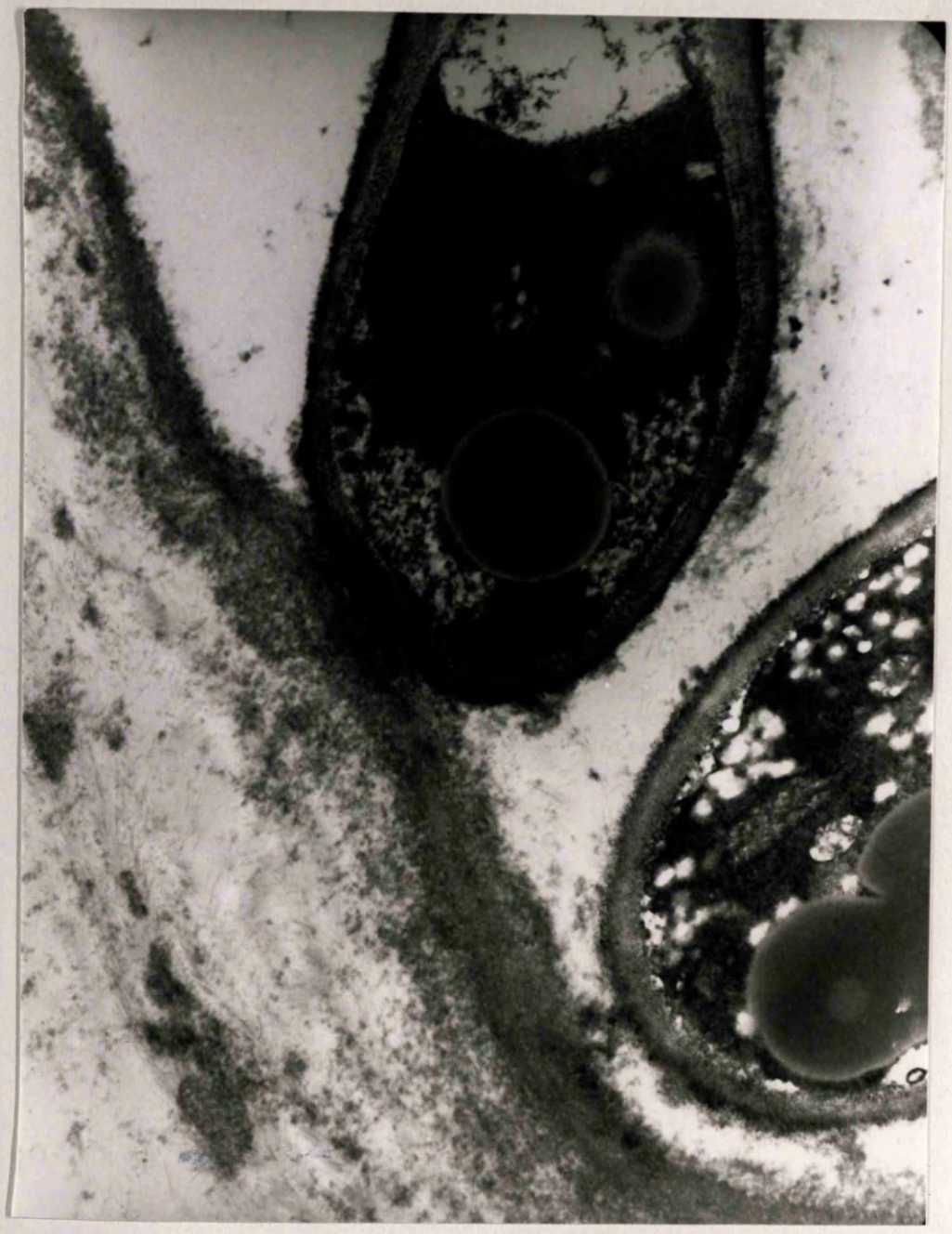


Plate 11. Section showing hyphae of *F. coeruleum* (Fc1) growing within the cell wall adjacent to the middle lamella. The middle lamella is present in the centre as a thin electron dense region. The primary wall colonised by the hyphae appears almost completely degraded although patches of electron dense material from the outer surfaces are still present. The primary wall on the opposite side of the middle lamella shows much less degradation and erosion and contains a lot of fibrous material which is largely absent from the upper wall. The outer boundary of the lower primary wall is very indistinct. The electron dense region adjacent to the middle lamella particularly in the lower primary wall could reflect the accumulation of lignin and suberin like molecules (x 30,000).

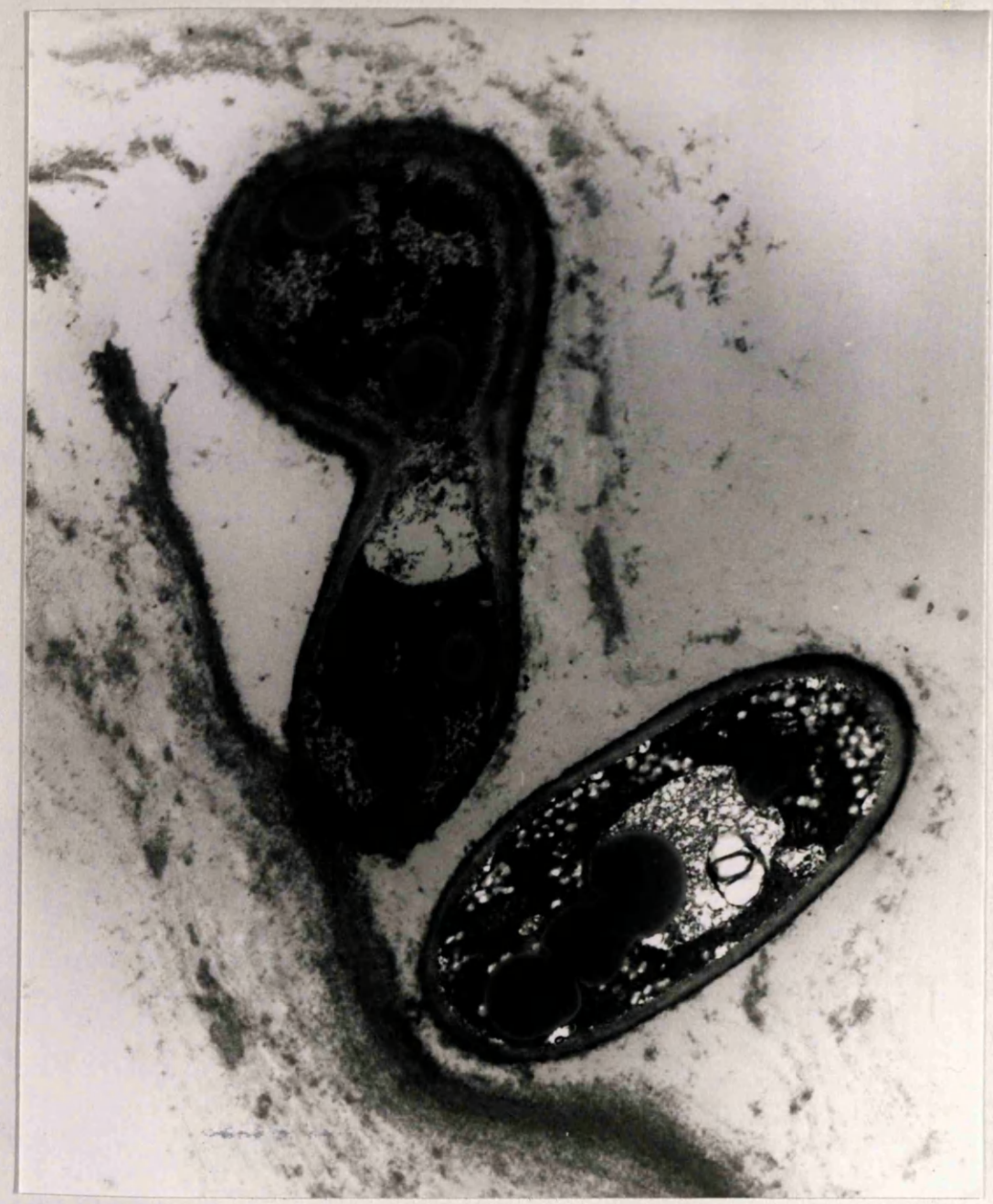


Plate 12. Primary cell wall of tissue infected with *F. coeruleum* (Fc1) showing the fibrillar material in the wall, particularly the lower primary wall (x 11,000).

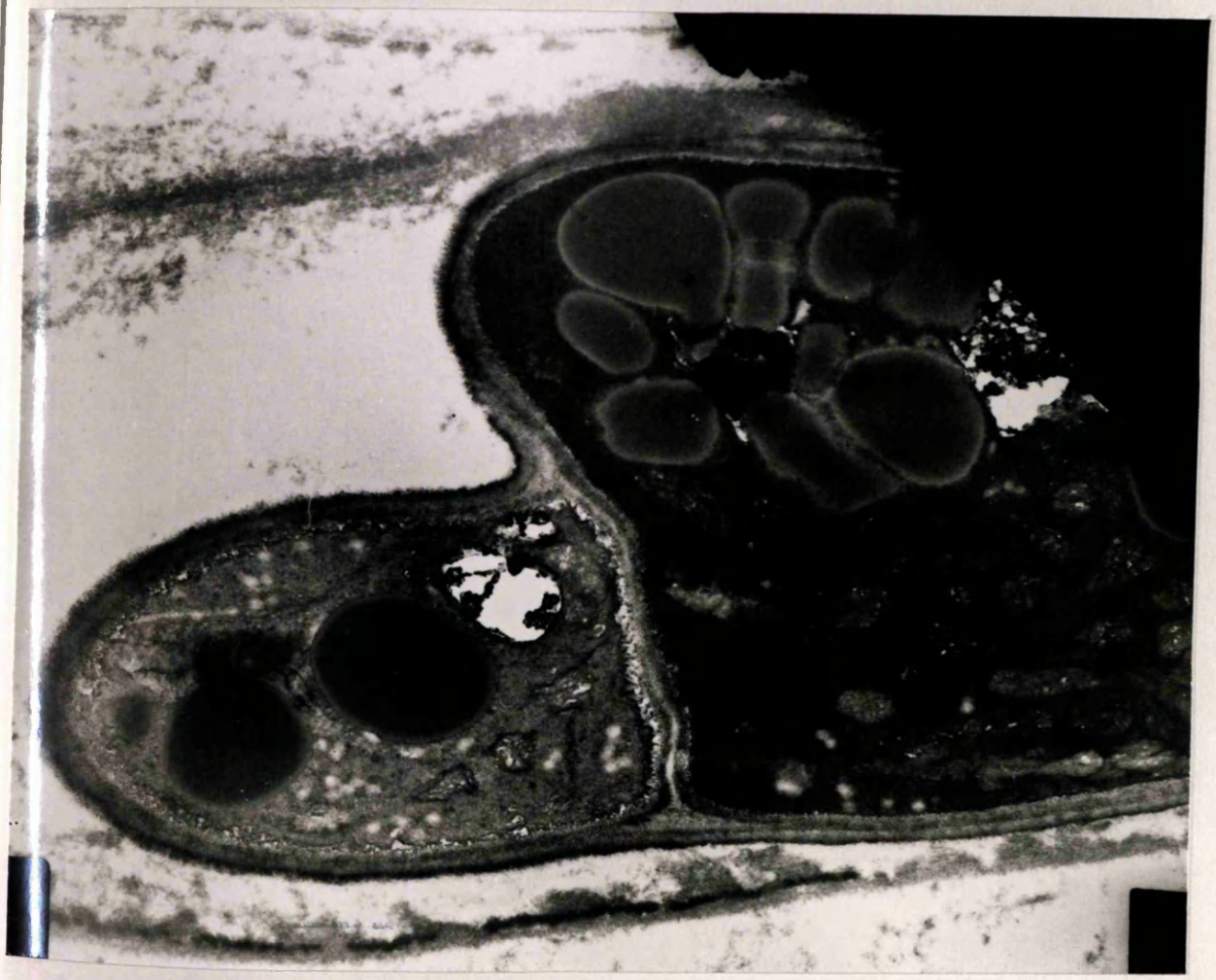


Plate 13. Oblique longitudinal section through a hyphal apex of *F. coeruleum* (Fc1) near a branch apex. The hypha is growing within the primary cell wall. The middle lamella of the wall is electron dense and shows little degradation. The wall in which the hypha is growing between the outer electron dense layer and the middle lamella appears to have largely disappeared. The wall layers on the lower side of the middle lamella also shows significant degradation but less so than the upper side. The outer electron dense regions on both sides show least degradation. These electron dense regions in both primary walls could be due to lignin and suberin deposition. The cell contents have mostly disappeared. The hypha is surrounded by a thick wall and contains large lipid bodies. (x 30,000).

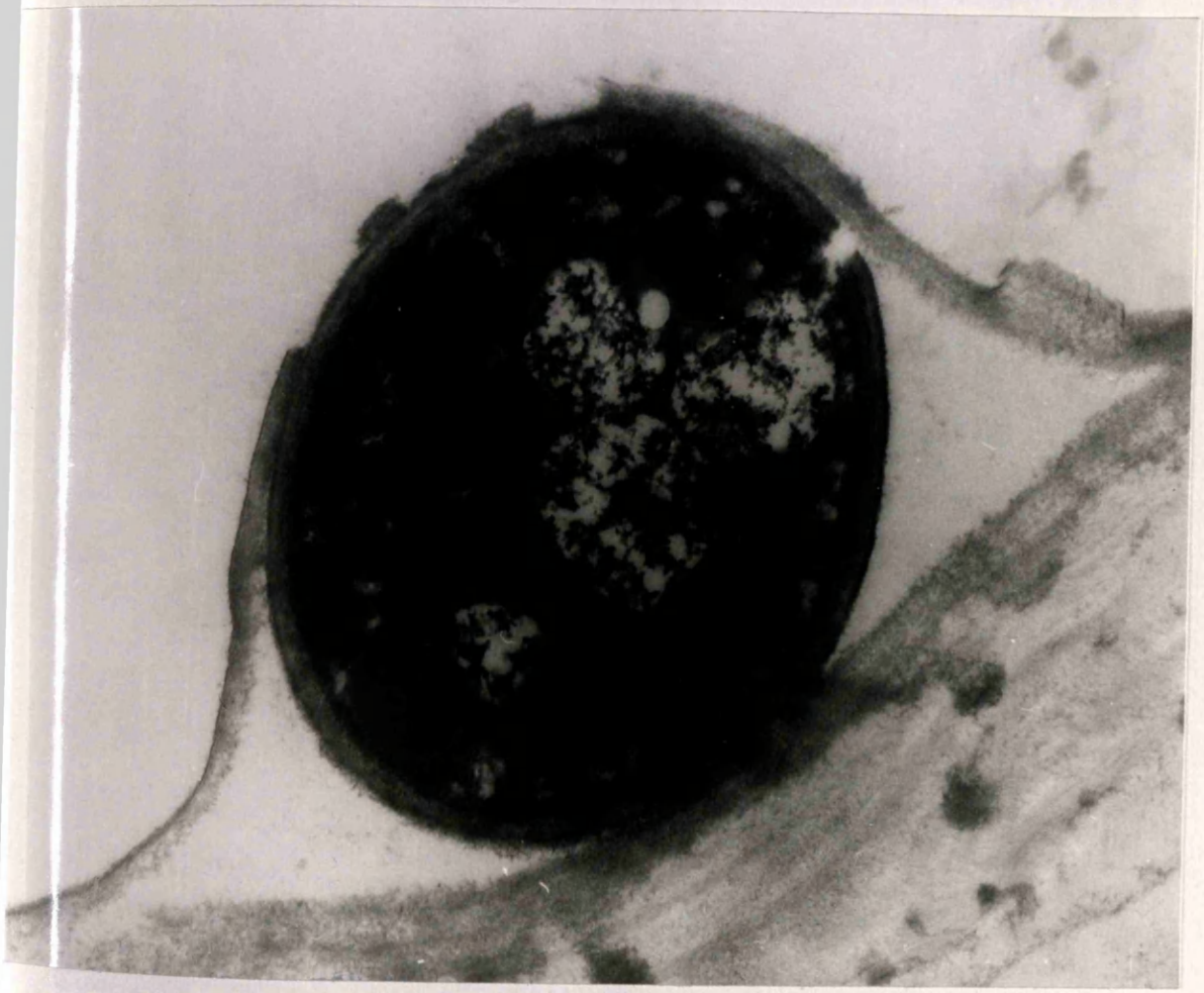


Plate 14. Section through a living hypha of *F. avenaceum* (Fa1) located inside a wall which appears to have separated just beneath the outer electron dense region of the primary wall. The wall on the lower side is swollen but its laminar structure is clearly visible. The middle lamella is not distinct. The wall layer surrounding the hypha is thin and contains electron dense material. This region is only partly degraded (24,000).

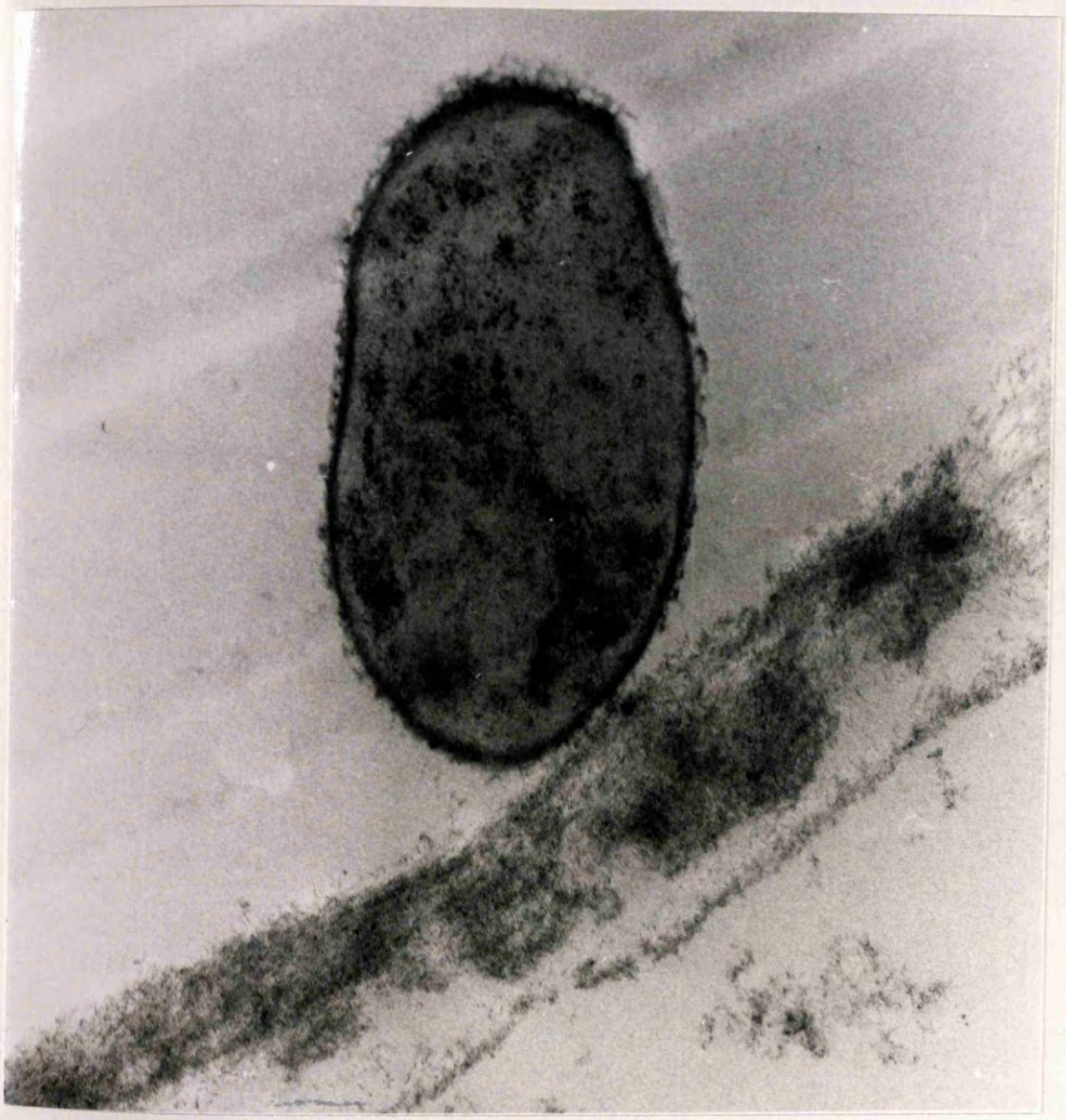


Plate 15. Section through a hypha of *F. avenaceum* (Fa1) growing in the lumen of the cell, adjacent to the primary cell wall. The host wall is basically continuous although the middle lamella is not clearly visible. The host wall near the hypha contains a lot of electron dense material. The hypha has little internal structure and is probably dead. Its wall is thin and coated with fibrillar material indicating that it may be in the process of being degraded (x 30,000).

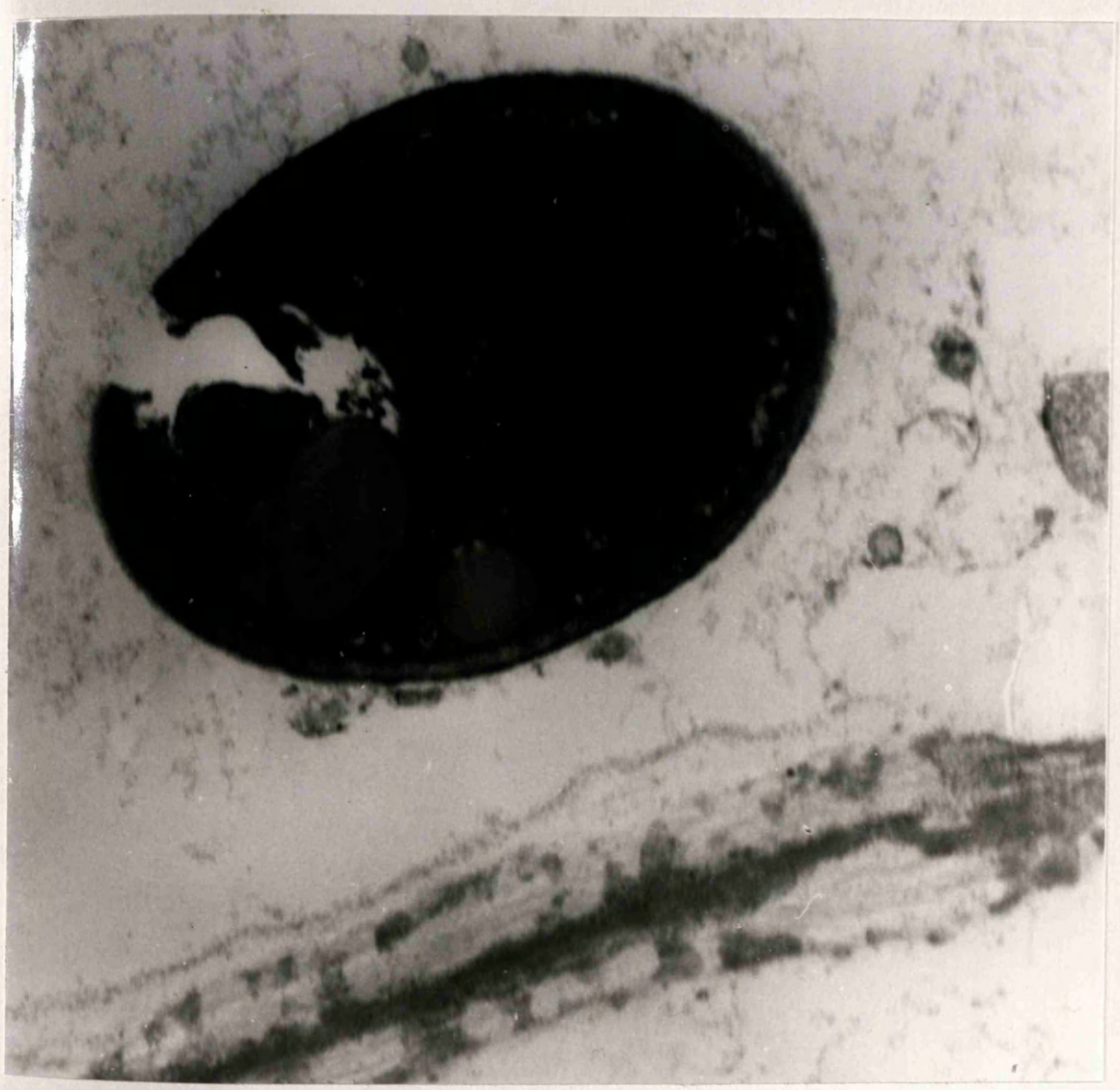


Plate 16. Section of a living hypha of *F. avenaceum* (Fa1) in the lumen of a cell. It has a thick cell wall and contains lipid bodies and a lot of electron dense material. The primary walls are slightly swollen and show significant deposition of electron dense material near the middle lamella. The middle lamella shows some degradation on the right in the section but it is otherwise still complete (x 24,000).

CHAPTER VI

PRODUCTION OF CELL WALL MACERATING ENZYMES, PECTIC ENZYMES AND TOXINS BY *F. coeruleum, F. avenaceum* AND *C. destructans* IN DIFFERENT MEDIA AND POTATO TISSUE

CHAPTER VI

PRODUCTION OF CELL WALL MACERATING ENZYMES, PECTIC ENZYMES AND TOXINS BY *F. coeruleum, F. avenaceum* AND *C. destructans* IN DIFFERENT MEDIA AND POTATO TISSUE

6.1. Introduction

The electron microscopic study described in the last chapter showed that the middle lamellar region of the tuber cell wall was relatively resistant to degradation compared to the primary wall and even in tissue heavily colonised by *F. coeruleum* or *F. avenaceum* it remained intact. However, the primary cell walls underwent considerable degradation, particularly when colonised by *F. coeruleum*. The resistance of the middle lamellar region to degradation explains the dry rot nature of the lesions caused by these fungi since, by remaining intact, the middle lamella effectively keeps the cells attached to each other. In contrast, the development of wet rots in tuber tissue usually involves the early and rapid degradation of the middle lamella, so that the cells become separated.

The middle lamellar region of the cell wall of parenchyma tissue is rich in rhamnogalacturonans (Darvill *et al.*, 1980). These are the substrates of pectic lyases and hydrolases, the enzymes which are produced in abundance by soft rot organisms and which are usually considered to be the enzymes responsible for the maceration of the middle lamella. Galacturonic acid polymers make up about 27% of the cell wall of potato tuber tissue (Burton, 1989) but not all of this is present in the middle lamella. A significant proportion is present in the matrix of the primary cell wall itself.

Several possibilities were considered in this study which might explain the development of dry rot lesions in potato tuber tissue infected by *F. coeruleum*, *F. avenaceum* and *C. destructans*. Firstly, the pathogens do not produce enzymes capable of macerating the middle lamella. Secondly, enzymes are produced but their activity is inhibited by other metabolites produced by or released from the tissues. Thirdly, the middle lamella, at a very early stage of infection, becomes resistant to degradation.

In this chapter are described a series of experiments with the three dry rot causing fungi to determine how active they are at producing cell wall macerating

enzymes in general and certain specific cell wall degrading enzymes in various media in culture, and how this activity might be regulated, in an attempt to differentiate between the various possibilities listed above.

As another significant feature of dry rot development is cell death, the ability of the three species to produce toxic fractions in various media was examined. This part of the work is on essential preliminary the use of toxins to select for resistant germ plasm in subsequent work.

6.2. Production of cell wall macerating enzymes, pectic enzymes and toxins by *F. coeruleum* isolate Fc1 in different media

6.2.1. Czapek-Dox and Pringle & Scheffer's liquid medium with sucrose as the carbon source

6.2.1.1. Fungal growth

F. coeruleum grew well in both Czapek-Dox liquid medium and Pringle & Scheffer's liquid medium. Growth was rapid during the first two days after inoculation, reaching a maximum biomass around 12 to 14 days after inoculation, after which the biomass slowly declined (Fig 18 a, b). The pH of the cultures changed during growth, decreasing during the first 4 days after inoculation to below pH 6.0 then increasing to above pH 8.0 towards the end of growth (Fig 18 a, b).

6.2.1.2. Tissue maceration

At no stage of growth did culture filtrates, when tested at either pH 5.0 or pH 9.0, macerate tuber tissue discs.

6.1.1.3. Pectin methyl esterase activity

Pectin methyl esterase activity was not found in the culture filtrates from any stage of growth

6.2.1.4. Pectic lyase and hydrolase activities

Pectin and pectate lyase activities and pectin and pectate hydrolase activities, whether tested at pH 5.0 or pH 9.0, were very low or negligible in culture filtrates from both media throughout the whole period of growth (Fig 19 a, b and c, d).

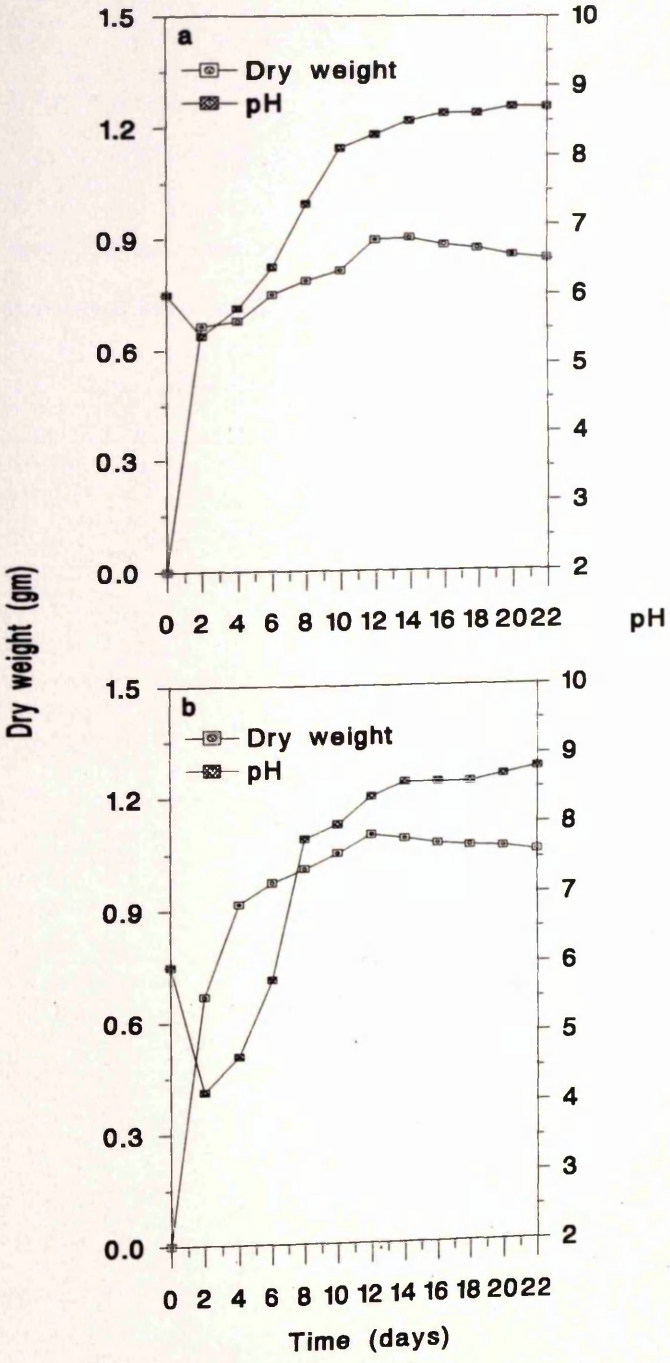
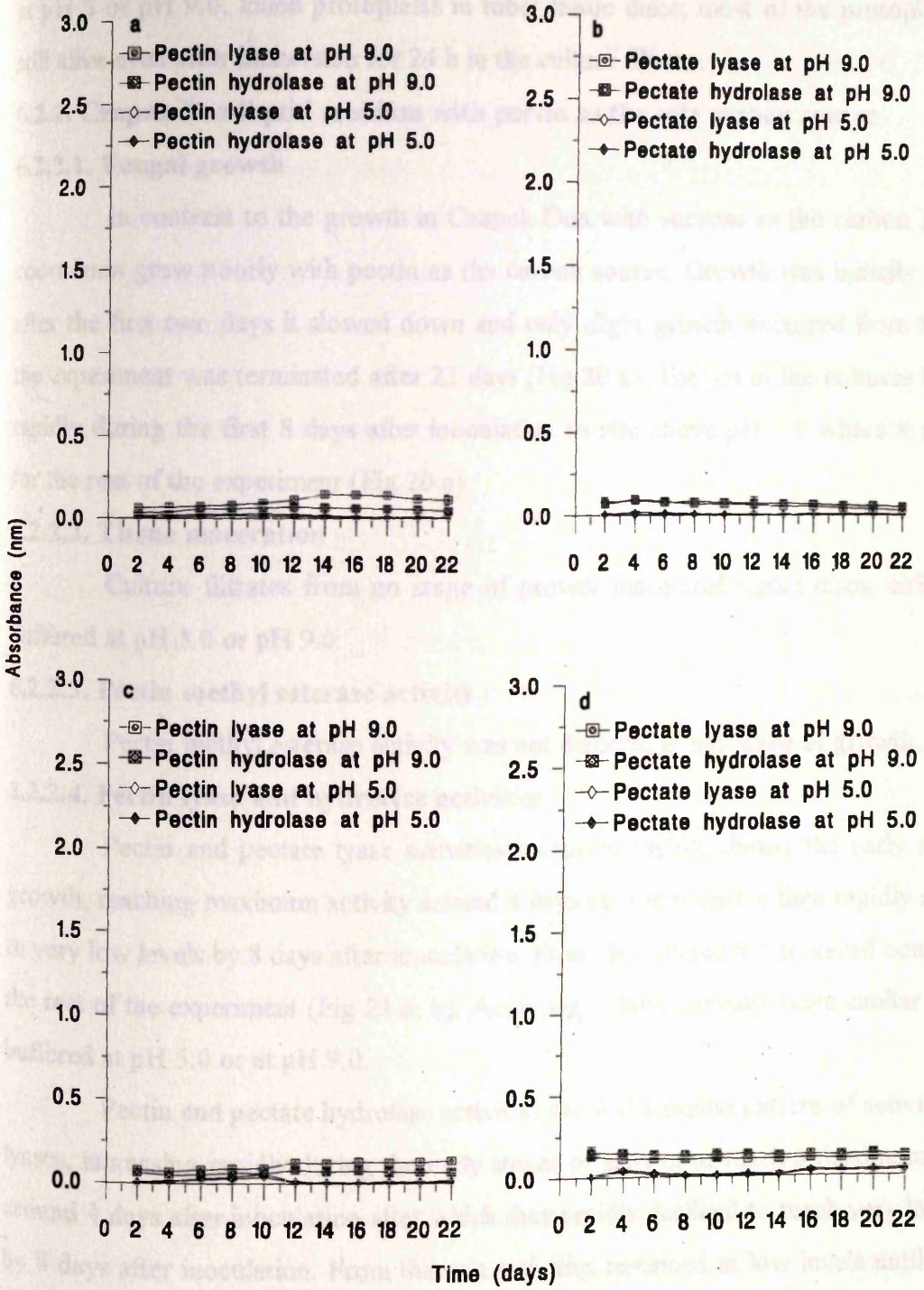


Fig. 18. Growth of F. coeruleum (Fc1), (a) in Czapec-Dox and (b) in (Pringle and Schfer's liquid medium with sucrose as the carbon source.

Figure 19. Pectic enzyme production by *F. coeruleum* (Fc1), (a and b), in Czapek-Dox and (c and d) in Pringle and Scheffer's liquid medium with sucrose as the carbon source.


a
Pectin lyase at pH 9.0
Pectin hydrolase at pH 9.0
Pectin lyase at pH 5.0
Pectin hydrolase at pH 5.0
b
Pectate lyase at pH 9.0
Pectate hydrolase at pH 9.0
Pectate lyase at pH 5.0
Pectate hydrolase at pH 5.0
c
Pectin lyase at pH 9.0
Pectin hydrolase at pH 9.0
Pectin lyase at pH 5.0
Pectin hydrolase at pH 5.0
d
Pectate lyase at pH 9.0
Pectate hydrolase at pH 9.0
Pectate lyase at pH 5.0
Pectate hydrolase at pH 5.0
Absorbance (nm)
Time (days)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0 2 4 6 8 10 12 14 16 18 20 22

6.2.1.5. Toxicity to potato cells

None of the culture filtrates, either before or after boiling and whether buffered at pH 5 or pH 9.0, killed protoplasts in tuber tissue discs; most of the protoplasts were still alive even after immersion for 24 h in the culture filtrate.

6.2.2. Czapek-Dox liquid medium with pectin as the sole carbon source

6.2.2.1. Fungal growth

In contrast to the growth in Czapek Dox with sucrose as the carbon source *F. coeruleum* grew poorly with pectin as the carbon source. Growth was initially rapid but after the first two days it slowed down and only slight growth occurred from then until the experiment was terminated after 22 days (Fig 20 a). The pH of the cultures increased rapidly during the first 8 days after inoculation to rise above pH 9.0 where it remained for the rest of the experiment (Fig 20 a).

6.2.2.2. Tissue maceration

Culture filtrates from no stage of growth macerated tissue discs, either when buffered at pH 5.0 or pH 9.0.

6.2.2.3. Pectin methyl esterase activity

Pectin methyl esterase activity was not detected at any stage of growth.

3.2.2.4. Pectin lyase and hydrolyse activities

Pectin and pectate lyase activities increased rapidly during the early stages of growth, reaching maximum activity around 4 days after inoculation then rapidly declining to very low levels by 8 days after inoculation. From then on the levels stayed constant for the rest of the experiment (Fig 21 a, b). Activities of both enzymes were similar whether buffered at pH 5.0 or at pH 9.0.

Pectin and pectate hydrolase activities showed a similar pattern of activity to the lyases, increasing rapidly during the early stages of growth to reach a maximum activity around 4 days after inoculation after which they rapidly declined to reach very low levels by 8 days after inoculation. From then on activities remained at low levels until the end of the experiment. Pectin hydrolase activities at pH 9.0 were higher than those at pH 5.0 (Fig 21 a) but pectate hydrolase activities at pH 9.0 and pH 5.0 were similar (Fig 21 b).

6.2.2.5. Toxicity to potato cells

None of the culture filtrates tested, either before, or after boiling, or whether buffered at pH 5 or pH 9.0 killed the protoplasts in tissue discs. Most of the protoplasts were still alive at the end of 24 h incubation in the culture filtrate.

6.2.3. Czapek-Dox liquid medium with sodium polypectate as the sole carbon source

6.2.3.1. Fungal growth

F. caeruleum grew poorly in Czapek Dox with sodium polypectate as the sole carbon source. Growth was initially rapid but after the first two days it slowed down and only slight growth occurred from then on until the experiment was terminated after 22 days. The pH of the cultures increased rapidly during the first 8 days after inoculation to rise above pH 9.0 where it remained for the rest of the experiment (Fig 20 b).

6.2.3.2. Tissue maceration

Culture filtrates from no stage of growth, whether buffered at pH 5.0 or 9.0, macerated tissue discs.

6.2.3.3. Pectin methyl esterase activity

Pectin methyl esterase activity was not found at any stage of growth.

6.2.3.4. Pectic lyase and hydrolase activities

Pectin lyase activity, tested at pH 9.0, was not detectable in the culture filtrates during the first 4 days of growth, but then activity increased rapidly reaching maximum levels around 8 days after inoculation. This rapid increase was followed by a rapid decline to very low levels 18 days after inoculation at which activity remained to the end of the experiment (Fig 21 c). Activity at pH 5.0 showed a slight increase around 10 days after inoculation, then rapidly declined to very low levels 12 days after inoculation, staying constant from then on until the end of the experiment.

Pectate lyase activity was not detectable in the culture filtrates during the first 6 days of growth, but activity tested at pH 9.0 then increased rapidly, reaching maximum levels around 10 days after inoculation, followed by an equally rapid decline to very low levels around 14 days after inoculation. Activity remained at low levels from 14 days to the end of the experiment, Activity at pH 5.0 increased slightly around 10 days after inoculation, then rapidly declined to very low levels within 2 days, remaining at those levels for the rest of the experiment (Fig 21 d).

Pectin hydrolase activity at pH 9.0 increased slightly around 8 to 10 days after inoculation, but then fell back to very low levels by 12 days after inoculation at which level it remained to the end of the experiment. Pectin hydrolase activity at pH 5.0 was very low or negligible at all stages of growth (Fig 21 c). Pectate hydrolase activity

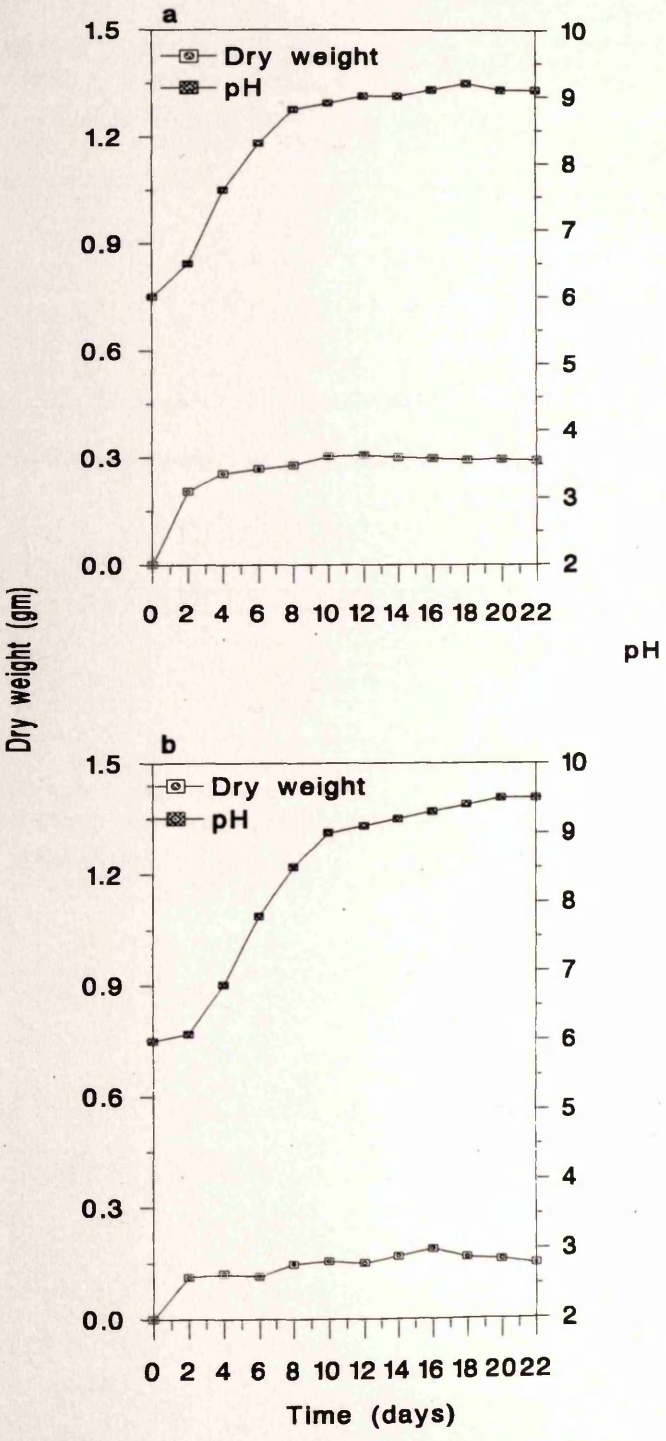
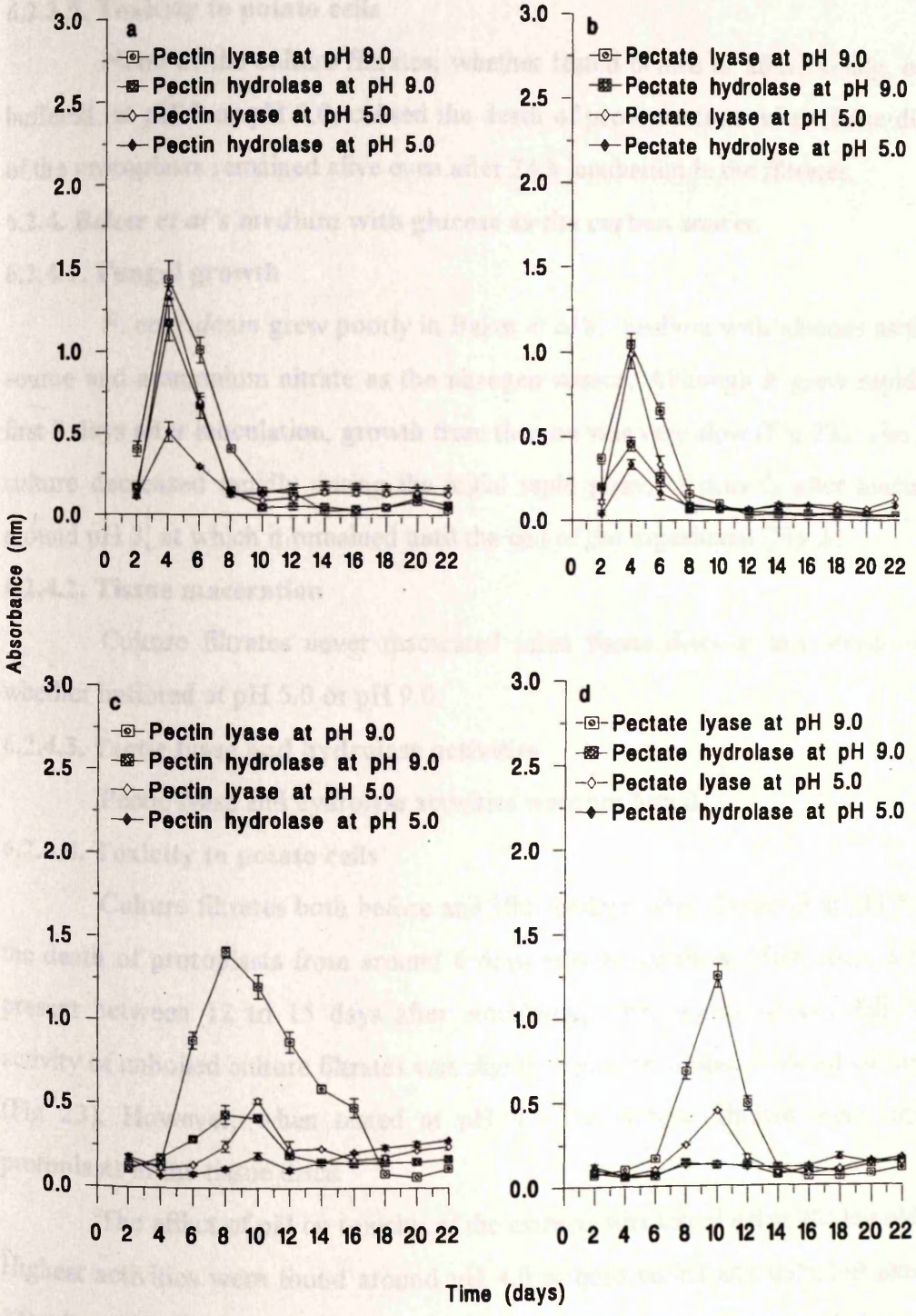


Fig. 20. Growth of F. coeruleum (Fc1) in Czapek-Dox medium, (a) with pectin and (b) with sodium polypectate as carbon source.

Figure 21. Pectic enzyme production by *F. coeruleum* (Fc1), (a and b) in Czapek-Dox liquid medium with pectin and (c and d) with sodium polypectate as the carbon source.


a
Pectin lyase at pH 9.0
Pectin hydrolase at pH 9.0
Pectin lyase at pH 5.0
Pectin hydrolase at pH 5.0
b
Pectate lyase at pH 9.0
Pectate hydrolase at pH 9.0
Pectate lyase at pH 5.0
Pectate hydrolyse at pH 5.0
c
Pectin lyase at pH 9.0
Pectin hydrolase at pH 9.0
Pectin lyase at pH 5.0
Pectin hydrolase at pH 5.0
d
Pectate lyase at pH 9.0
Pectate hydrolase at pH 9.0
Pectate lyase at pH 5.0
Pectate hydrolase at pH 5.0
Absorbance (nm)
Time (days)

whether tested at pH 5.0 or pH 9.0 was very low or negligible throughout the whole period of growth (Fig 21 d).

6.2.3.5. Toxicity to potato cells

None of the culture filtrates, whether tested before or after boiling, or whether buffered, at pH 5 or pH 9.0, caused the death of protoplasts in tuber tissue discs. Most of the protoplasts remained alive even after 24 h incubation in the filtrates.

6.2.4. Baker *et al's* medium with glucose as the carbon source

6.2.4.1. Fungal growth

F. coeruleum grew poorly in Baker *et al's.*, medium with glucose as the carbon source and ammonium nitrate as the nitrogen source. Although it grew rapidly for the first 3 days after inoculation, growth from then on was very slow (Fig 22). The pH of the culture decreased rapidly during the initial rapid phase of growth after inoculation, to around pH 3, at which it remained until the end of the experiment (Fig 22).

6.2.4.2. Tissue maceration

Culture filtrates never macerated tuber tissue discs at any stages of growth whether buffered at pH 5.0 or pH 9.0.

6.2.4.3. Pectic lyase and hydrolase activities

Pectic lyase and hydrolase activities were not tested.

6.2.4.4. Toxicity to potato cells

Culture filtrates both before and after boiling, when buffered at pH 5.0, caused the death of protoplasts from around 6 days after inoculation. Most toxic activity was present between 12 to 15 days after inoculation, after which activity fell. The toxic activity of unboiled culture filtrates was slightly higher than that of boiled culture filtrates (Fig 23). However, when tested at pH 9.0 the culture filtrates were nontoxic to protoplasts in the tissue discs.

The effect of pH on toxicity of the extracts was tested using 12 day old cultures. Highest activities were found around pH 4.0 in both boiled and unboiled extracts (Fig 24) whereas activity at pH 7.0 was very low and no activity was detected above pH 7.0.

6.2.5. Effect of nitrogen source in Baker *et al*'s medium on toxin production

Because the pH of the medium containing ammonium nitrate rapidly dropped to pH 3.0 after inoculation, probably because of the preferential uptake of the ammonium ion, the effect of two other nitrogen sources, ammonium sulphate and sodium nitrate, were tested as well, with all three media containing equivalent nitrogen levels.

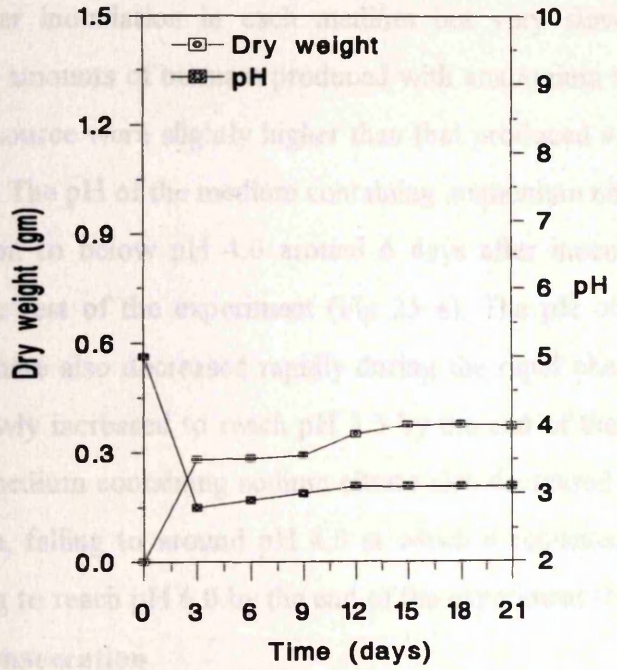


Fig. 22 Growth of F. coeruleum (Fc1) in (Baker et al's medium.

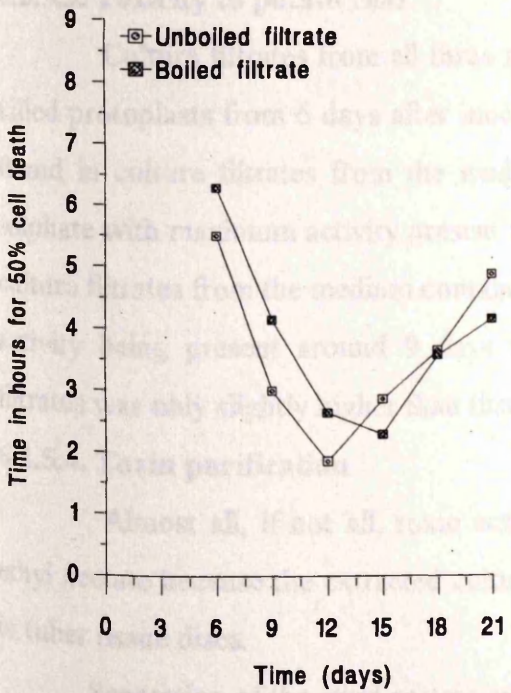


Fig. 23 Toxicity to potato tissue of culture filtrates of F. coeruleum (Fc1) from Baker et al's liquid medium. Unboiled and boiled filtrates were tested at pH 5.0.

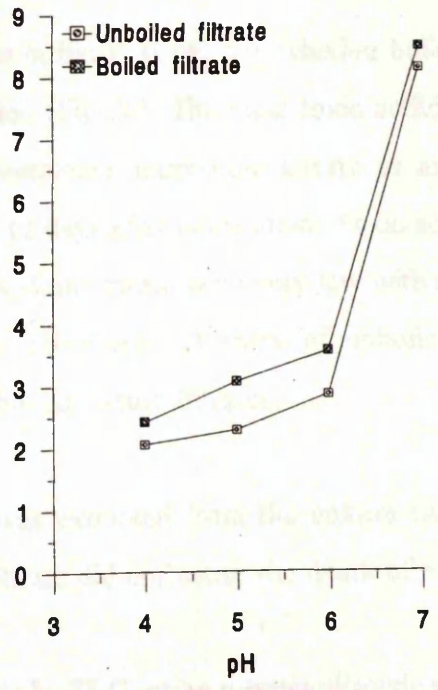


Fig. 24 The effect of pH on the toxicity to potato tissue of boiled and umboiled culture filtrates of F. coeruleum (Fc1) from Baker et al's medium.

6.2.5.1. Fungal growth

The fungus grew poorly with all three nitrogen sources. It grew rapidly for the first 3 days after inoculation in each medium but very slowly for the rest of the experiment. The amounts of biomass produced with ammonium nitrate or sodium nitrate as the nitrogen source were slightly higher than that produced with ammonium sulphate (Figs 25 a, b, c). The pH of the medium containing ammonium nitrate decreased from pH 5.0 at inoculation to below pH 4.0 around 6 days after inoculation at which level it remained for the rest of the experiment (Fig 25 a). The pH of the medium contained ammonium sulphate also decreased rapidly during the rapid phase of growth to around pH 3.0 then slowly increased to reach pH 3.5 by the and of the experiment (Fig 25 b). The pH of the medium containing sodium nitrate also decreased rapidly during the rapid phase of growth, falling to around pH 4.0 at which it remained for about 9 days then slowly increasing to reach pH 6.0 by the end of the experiment (Fig 25 c).

6.2.5.2. Tissue maceration

None of the culture filtrates from the three media macerated tuber tissue discs when tested at either pH 5.0 or pH 9.0.

6.2.5.3. Toxicity to potato cells

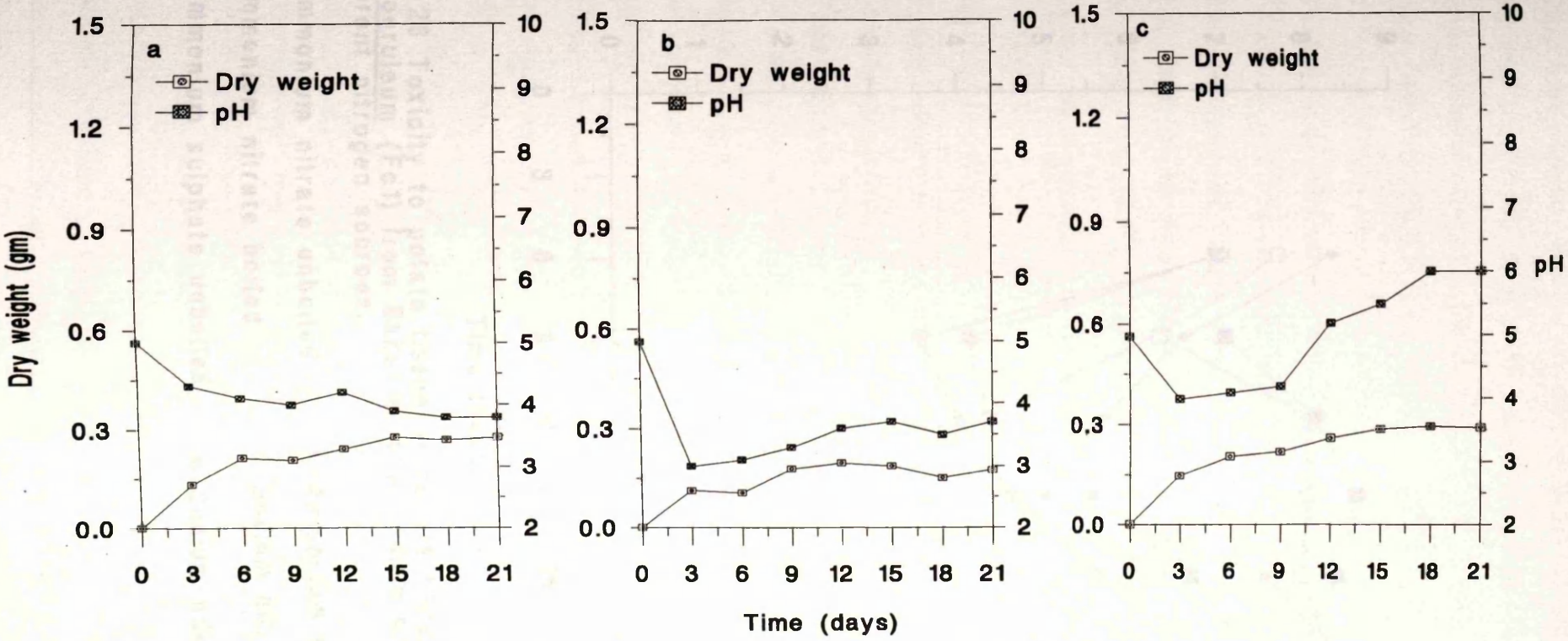
Culture filtrates from all three media buffered at pH 5.0, whether boiled or not killed protoplasts from 6 days after inoculation (Fig 26). The most toxic activities were found in culture filtrates from the media containing ammonium nitrate or ammonium sulphate with maximum activity present 9 to 12 days after inoculation. Toxic activities in culture filtrates from the medium containing sodium nitrate were very low with maximum activity being present around 9 days after inoculation. Activity of unboiled culture filtrates was only slightly higher than that of boiled culture filtrates.

6.2.5.4. Toxin purification

Almost all, if not all, toxic active was extracted from the culture filtrate with ethyl acetate because the extracted culture filtrate did not cause the death of protoplasts in tuber tissue discs.

Separation of the ethyl acetate extracts by TLC, using n-butanol/acetic acid/water (4:1:5,v/v/v) as solvent, gave 4 fractions. The R_f values of the fractions were (1) 0.17, (2) 0.67, (3) 0.89 and (4) 0.94. The fractions were removed from the TLC plates and dissolved in 10 ml water. The pH values of fraction 1 and 2 dissolved in water were both pH 4.5 but that of fraction 3 was pH 4.0 and that of fraction 4 was pH 4.9. The pH value

Figure 25. Growth of *F. coeruleum* (Fc1) in Baker *et al*'s liquid medium with different nitrogen sources (a) ammonium nitrate, (b) ammonium sulphate, (c) sodium nitrate.


a
b
c
Dry weight
pH
Dry weight (gm)
pH
Time (days)
1.5
1.2
0.9
0.6
0.3
0.0
10
9
8
7
6
5
4
3
2
0 3 6 9 12 15 18 21

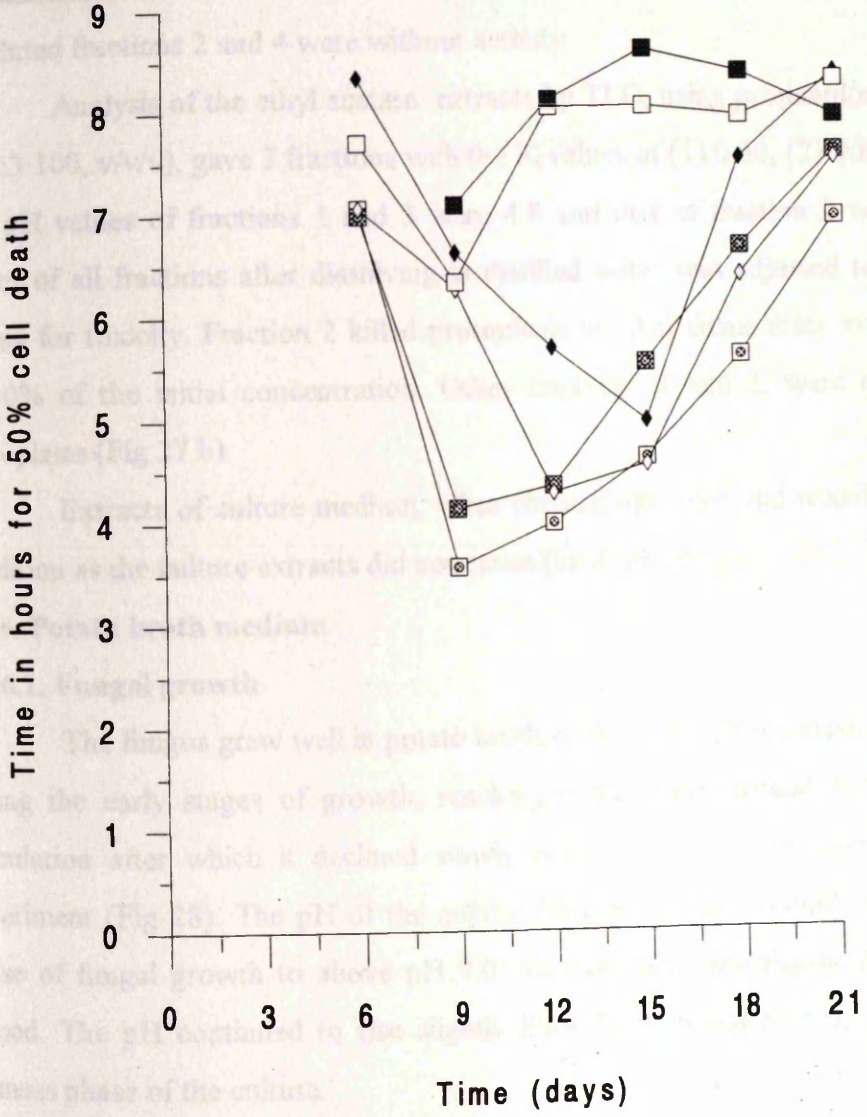


Fig. 26 Toxicity to potato tissue of culture filtrates of F. coeruleum (Fc1) from Baker et al's medium containing different nitrogen sources.

Ammonium nitrate unboiled
Ammonium nitrate boiled
Ammonium sulphate unboiled
Ammonium sulphate boiled
Sodium nitrate unboiled
Sodium nitrate boiled

of all fractions after dissolving in water was adjusted to pH 5.0 before testing. Solutions of both fractions 1 and 3 (Fig 27 a) killed protoplasts in tuber tissue discs with about equal activity. However activity was lost from fraction 1 by diluting to 50% of the initial concentration, and from fraction 3 by diluting to 33% of the initial concentration. Undiluted fractions 2 and 4 were without activity.

Analysis of the ethyl acetate extracts by TLC, using propanol/acetic acid/water (200:3:100, v/v/v), gave 3 fractions with the R_f values of (1) 0.60, (2) 0.85 and (3) 0.96. The pH values of fractions 1 and 3 were 4.8 and that of fraction 2 was 4.1. The pH values of all fractions after dissolving in distilled water was adjusted to pH 5.0 before testing for toxicity. Fraction 2 killed protoplasts in tuber tissue discs even when diluted to 50% of the initial concentration. Other fractions, 1 and 3, were not toxic to the protoplasts (Fig 27 b).

Extracts of culture medium, when chromatographed and tested under the same condition as the culture extracts did not cause the death of tissue discs.

6.2.6. Potato broth medium

6.2.6.1. Fungal growth

The fungus grew well in potato broth medium. Fungal biomass increased rapidly during the early stages of growth, reaching a maximum around 6 to 10 days after inoculation after which it declined slowly over the next 12 days to the end of the experiment (Fig 28). The pH of the culture filtrates increased rapidly during the active phase of fungal growth to above pH 9.0, 12 days after inoculation when growth had ceased. The pH continued to rise slightly from 12 days onwards during the declining biomass phase of the culture.

6.2.6.2. Tissue maceration

The culture filtrates did not contain tissue macerating activity at any stage of growth either when tested at pH 5.0 or at pH 9.0.

6.2.6.3. Pectin methyl esterase activity

Pectin methyl esterase activity was not found at any stage of growth.

6.2.6.4. Pectic lyase and hydrolase activities

Pectin lyase activity (Fig 29 a) at pH 9.0 increased slowly during the first 8 days after inoculation but increased rapidly from 8 days reaching maximum activity around 12 days after inoculation. The activity then rapidly declined so that by 14 days it was present at very low levels and activity continued to decrease slowly to the end of the experiment.

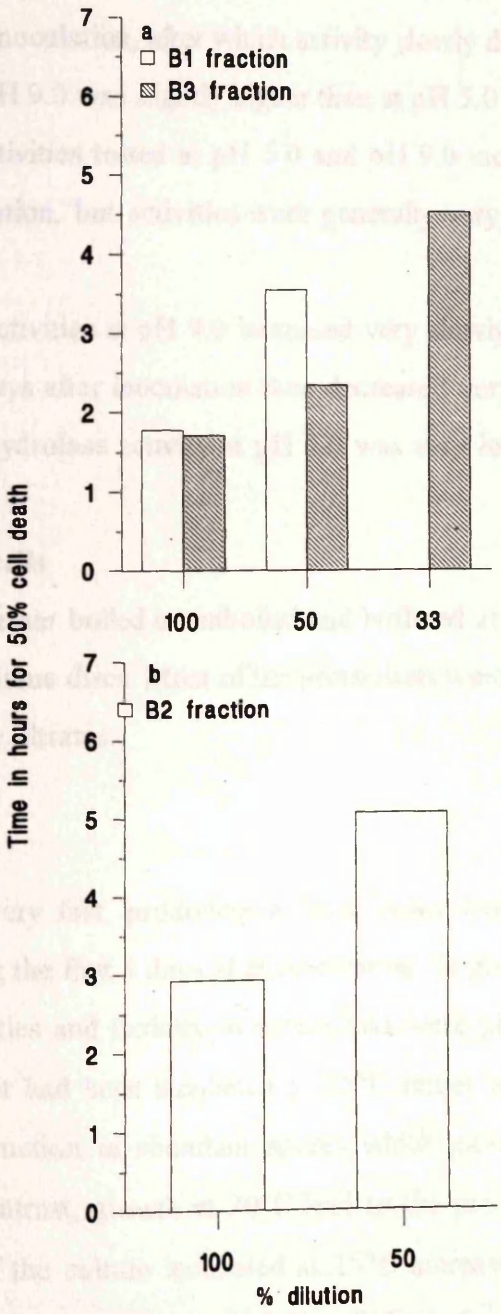


Fig. 27 Toxic activity of fractions obtained by TLC of ethyl acetate extracts from cultures of F. coeruleum (Fc1) grown in Baker et al's medium using TLC (a) n-butanol/acetic acid/water or (b) propanol/acetic acid/water as developing solvents.

Pectin lyase activity at pH 5.0 was not detectable in the culture filtrates during the first 10 days after inoculation but it showed a small increase from then on to reach maximum activity around 12 days after inoculation. Activity then slowly declined during the rest of the experiment. Activity at pH 9.0 was slightly higher than at pH 5.0 (Fig 29 a).

Pectate lyase activities at pH 5.0 and 9.0 increased slowly, with maximum activities being reached 14 days after inoculation, after which activity slowly declined to the end of the experiment. Activity at pH 9.0 was slightly higher than at pH 5.0 (Fig 29 b).

Pectin hydrolase activities tested at pH 5.0 and pH 9.0 increased slightly from around 12 days after inoculation, but activities were generally very low at all stages of growth (Fig 29 a).

Pectate hydrolase activities at pH 9.0 increased very slowly reaching maximum activities around 12 to 14 days after inoculation then decreased very slowly for the rest of the experiment. Pectate hydrolase activity at pH 5.0 was very low or negligible (Fig 29 b).

6.2.6.5. Toxicity to potato cells

Culture filtrates whether boiled or unboiled and buffered at pH 5 or pH 9.0 did not kill protoplasts in tuber tissue discs. Most of the protoplasts were still alive even after 24 h incubation in the culture filtrates.

6.2.7. Tuber tissue medium

6.2.7.1. Fungal growth

The fungus grew very fast, producing a thick brown-black growth over the surface of the medium during the first 4 days after inoculation. In preliminary tests it was found that macerating activities and toxicity to protoplasts were present at the highest levels in the medium when it had been incubated at 15°C rather than 20°C. At 15°C, growth resulted in the production of abundant spores which gave a deep blue-green colour to the medium. In contrast, growth at 20°C lead to the production of mycelium with few spores. The pH of the culture incubated at 15°C increased during the first 2 days after inoculation rising rapidly to above pH 8.0. and then stayed relatively constant at that level for the rest of the experiment (Fig 30).

6.2.7.2. Tissue maceration

Extracts obtained from the early stages of growth macerated potato tissue discs when tested at either pH 5.0 or 9.0 (Fig 31 a, b). Maximum activity was greatest at around 8 days after inoculation but it remained high for the rest of the experiment.

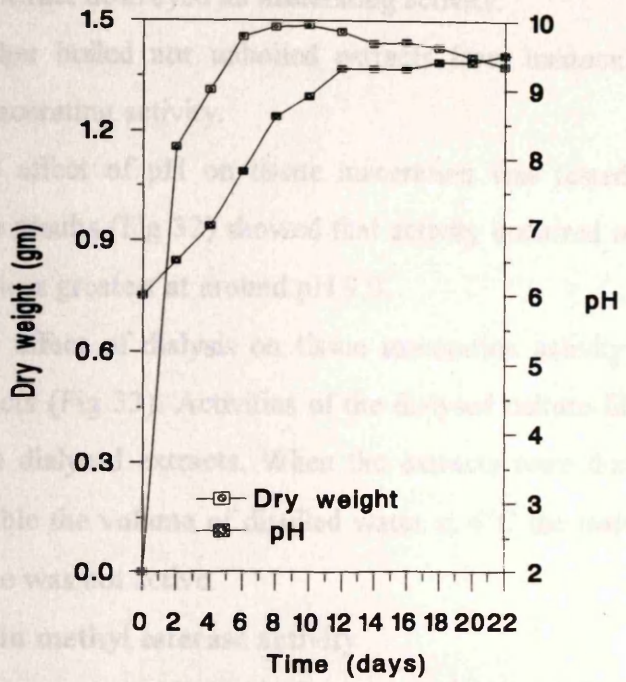


Fig. 28. Growth of F. coeruleum in potato broth medium.

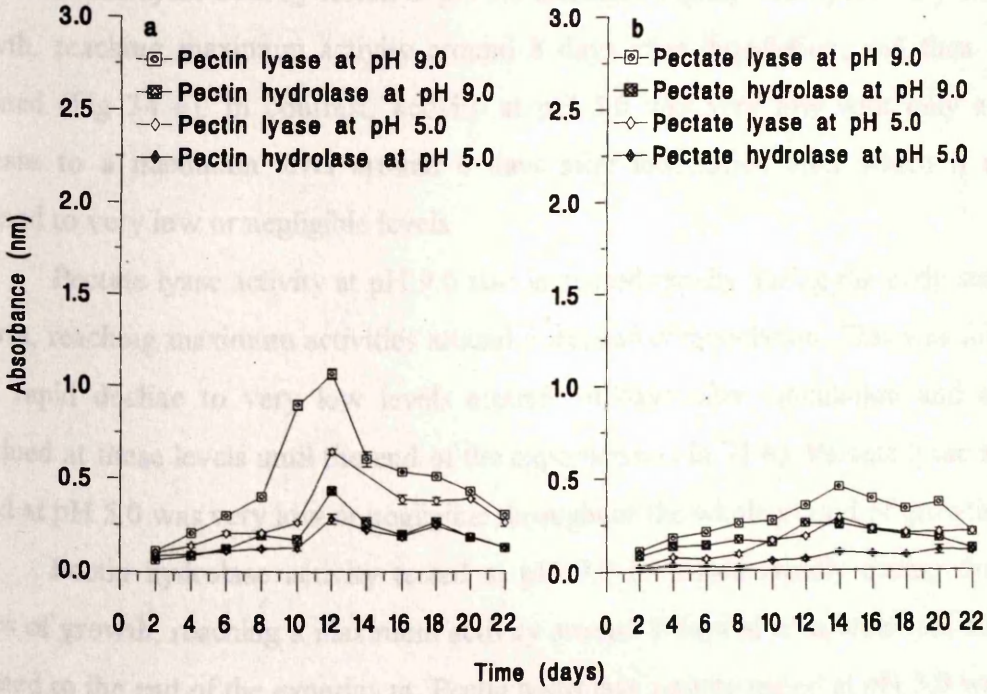


Fig. 29 Pectic enzyme production by F. coeruleum (Fc1) in potato broth mediun.

Macerating activity at pH 9.0 was slightly higher than that at pH 5.0 (Fig 31 a, b). Boiling the extract destroyed all macerating activity.

Nether boiled nor unboiled extracts from uninoculated tuber tissue medium possessed macerating activity.

The effect of pH on tissue maceration was tested using 12-day-old culture extracts. The results (Fig 32) showed that activity occurred over the whole range of pH tested but it was greatest at around pH 9.0.

The effect of dialysis on tissue maceration activity was tested in 12-day-old culture extracts (Fig 33). Activities of the dialysed culture filtrates were very similar to those of non dialysed extracts. When the extracts were dialysed overnight against an equal or double the volume of distilled water at 4°C the material which passed through the membrane was not active.

6.2.7.3. Pectin methyl esterase activity

Pectin methyl esterase activity was not found in the extracts of any of the isolates at any stage of growth.

6.2.7.4. Pectic lyase and hydrolase activities

Pectin lyase activity tested at pH 9.0 increased rapidly during the early stages of growth, reaching maximum activity around 8 days after inoculation, and then slowly declined (Fig 34 a). In contrast, activity at pH 5.0 was very low with only a slight increase to a maximum level around 6 days after inoculation after which it rapidly declined to very low or negligible levels.

Pectate lyase activity at pH 9.0 also increased rapidly during the early stages of growth, reaching maximum activities around 6 days after inoculation. This was followed by a rapid decline to very low levels around 10 days after inoculation and activity remained at these levels until the end of the experiment (Fig 34 b). Pectate lyase activity tested at pH 5.0 was very low or negligible throughout the whole period of growth.

Pectin hydrolase activity tested at pH 9.0 increased rapidly during the early stages of growth, reaching a maximum activity around 8 days after inoculation, and then declined to the end of the experiment. Pectin hydrolase activity tested at pH 5.0 was very low at all stages of growth. Pectate hydrolase activities tested at pH 5.0 and 9.0 were very low or negligible throughout the whole period of growth.

The effect of pH on enzymes activities was tested using extracts from 12 days old cultures (Fig 35). Both pectin lyase and pectate lyase activities had rather sharp optima,

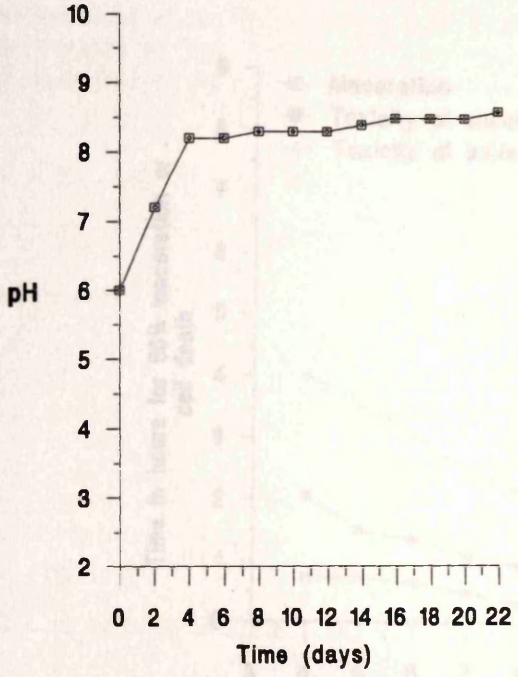


Fig. 30 Change in pH in autoclaved tissue medium during growth of F. coeruleum (Fc1).

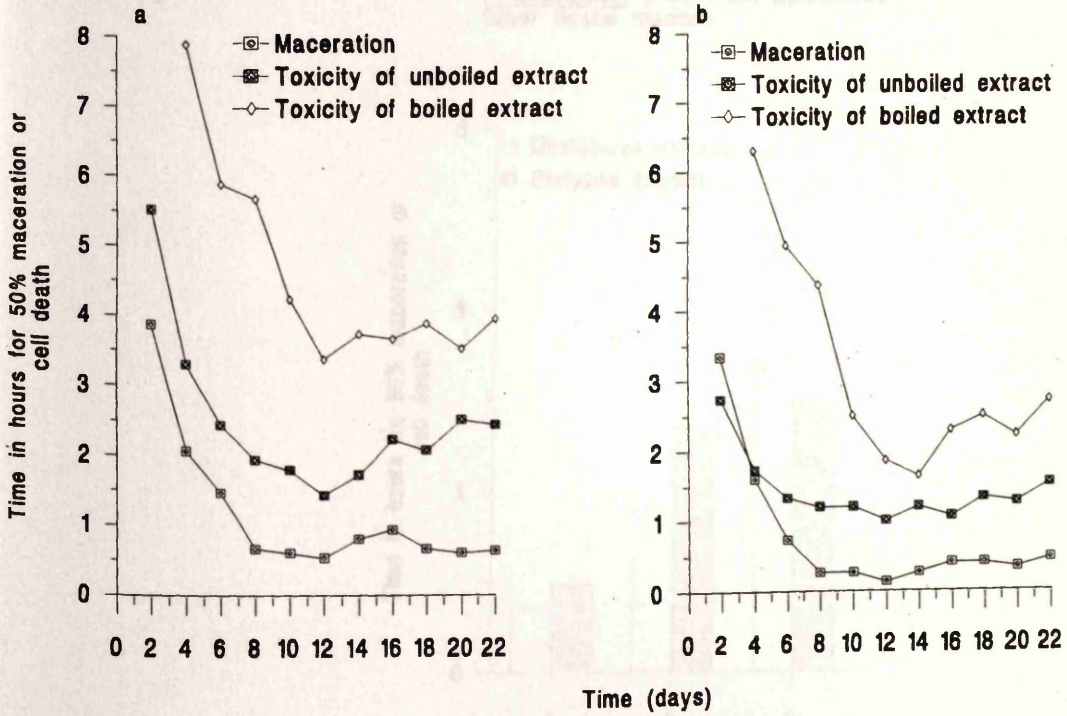


Fig. 31 Production of tissue macerating activity and toxic activity by F. coeruleum (Fc1) in autoclaved tuber tissue medium. (a) tested at pH 5.0, (b) tested at pH 9.0.

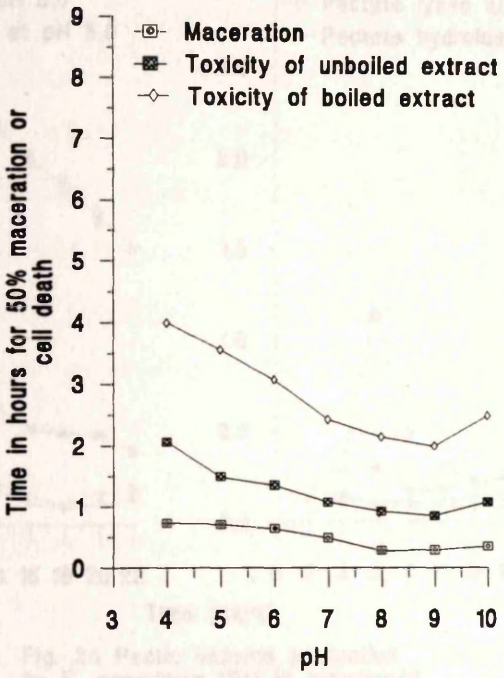


Fig. 32 Effect of pH on macerating activity and toxicity of extracts of F. coeruleum (Fc1) from autoclaved tuber tissue medium.

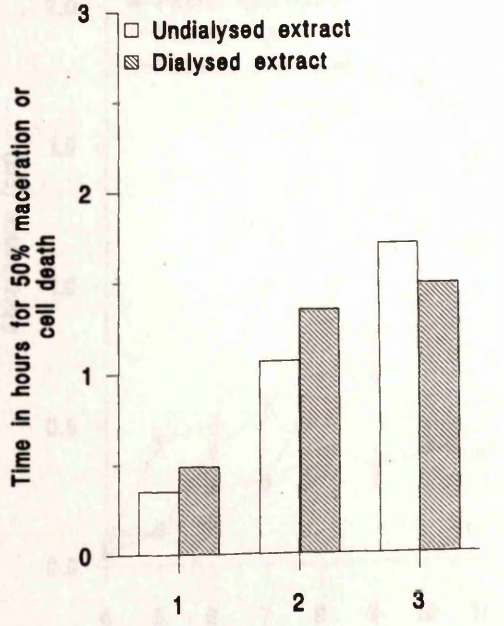


Fig. 33 Effect of dialysis on macerating activity of extracts of F. coeruleum (Fc1) grown on autoclaved tuber tissue medium.

1-Maceration

2-Toxicity of unboiled extract

3-Toxicity of boiled extract

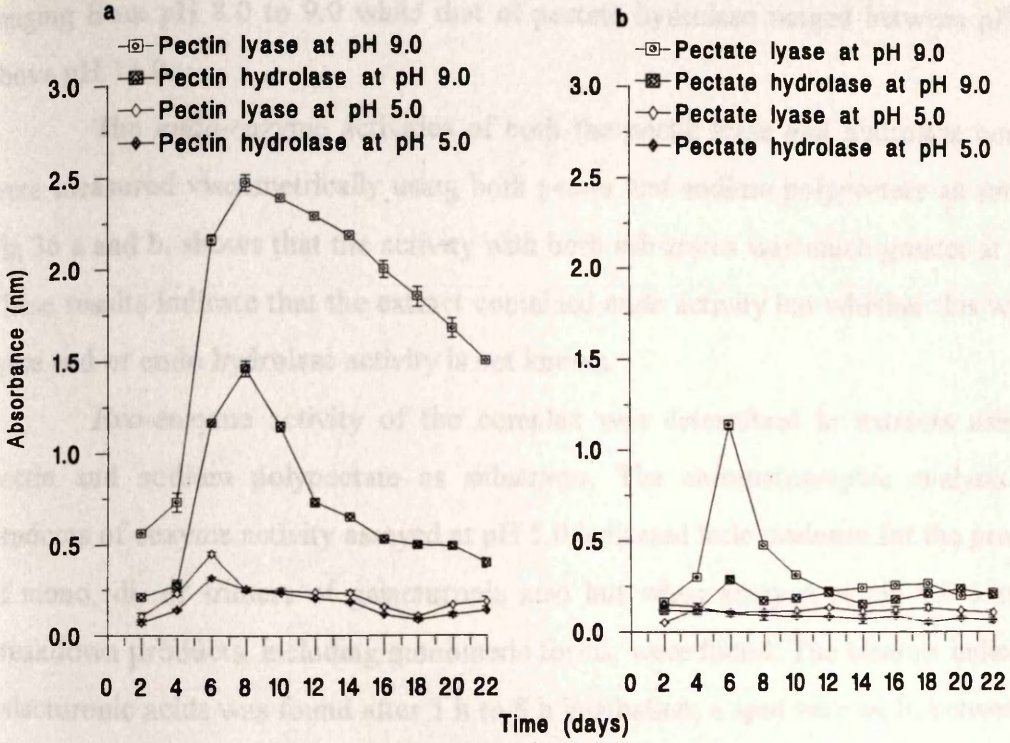


Fig. 34 Pectic enzyme production by F. coeruleum (Fc) in autoclaved tuber tissue medium.

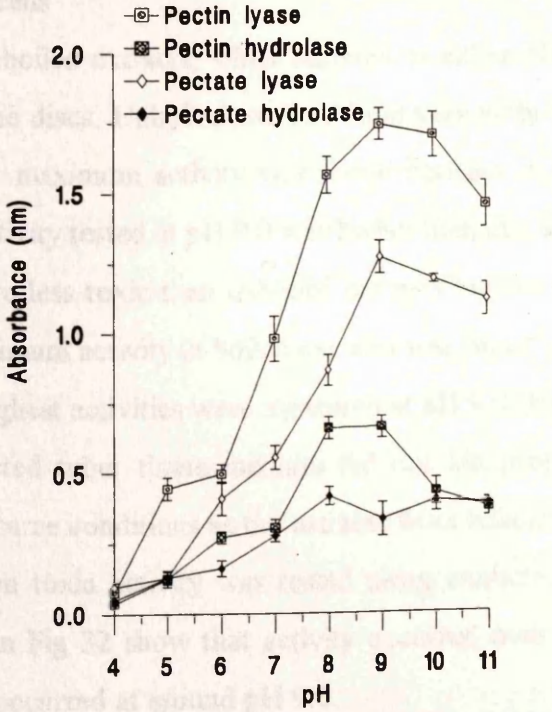


Fig. 35 Effect of pH on pectic enzyme activities of F. coeruleum (Fc1) in extracts from autoclaved tuber tissue medium 12 days after inoculation.

around pH 9.0, whereas hydrolase activities had broader optima, that of pectin hydrolase ranging from pH 8.0 to 9.0 while that of pectate hydrolase ranged between pH 8.0 to above pH 11.0.

The *endo*-enzyme activities of both the pectic lyase and hydrolase complexes were measured viscometrically using both pectin and sodium polypectate as substrates. Fig 36 a and b, shows that the activity with both substrates was much greater at pH 9.0. These results indicate that the extract contained *endo* activity but whether this was endo lyase and or endo hydrolase activity is not known.

Exo-enzyme activity of the complex was determined in extracts using both pectin and sodium polypectate as substrates. The chromatographic analysis of the products of enzyme activity assayed at pH 5.0 indicated little evidence for the production of mono, di, or trimers of galacturonic acid but when assayed at pH 9.0 a range of breakdown products, including monomeric forms, were found. The clearest indication of galacturonic acids was found after 1 h to 8 h incubation; a spot with an R_f between 0.46-0.57 was present, the R_f of galacturonic acid being between 0.50-0.55. Chromatographic analysis of the products of prolonged incubation was difficult because large amounts of material were produced which streaked on the chromatography sheets.

6.2.7.5. Toxicity to potato cells

Both boiled and unboiled extracts, when buffered at either pH 5.0 or pH 9.0, killed the protoplasts in tissue discs. Unboiled extracts from very early stages of growth killed some protoplasts, but maximum activity was found between 4 to 22 days after inoculation (Fig 31 a, b). Activity tested at pH 9.0 was higher then that at pH 5.0.

Boiled extracts were less toxic than unboiled extracts but the activity was still very high (Fig 31 a, b). Maximum activity in boiled extracts was found between 10 to 22 days after inoculation and highest activities were measured at pH 9.0 (Fig 31 b).

Extracts of uninfected tuber tissue medium did not kill protoplasts of tissue discs when tested under the same conditions as the extracts from infected tissues.

The effect of pH on toxin activity was tested using extracts from 12-day-old cultures. The results given in Fig 32 show that activity occurred over the whole range tested but optimum activity occurred at around pH 9.0.

The effect of dialysis on toxic activity was tested with 12-day-old culture extracts (Fig 33). Activities of dialysed culture filtrates were very similar to those of undialysed extracts. To test the activity of materials which passed through the membrane the

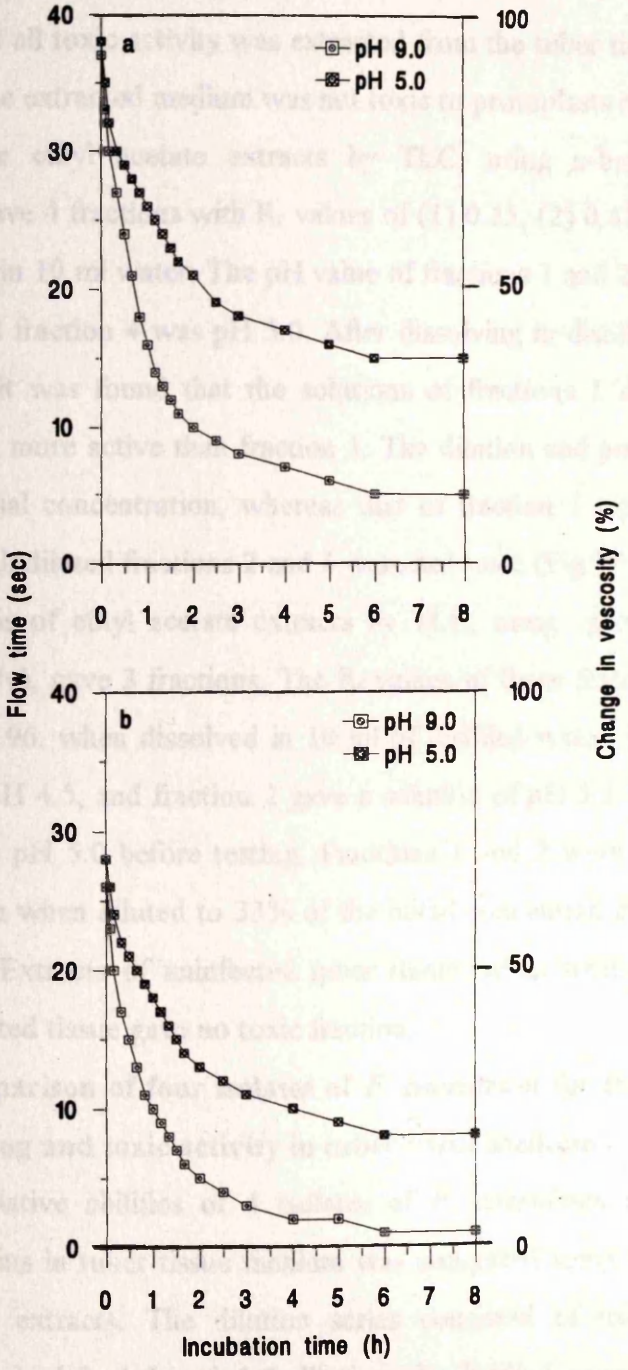


Fig. 36 Endo-enzyme activity of pectic enzyme complex produced by F. coeruleum (Fc1) in autoclaved tuber tissue medium (a) pectin lyases and hydrolases, (b) pectate lyases and hydrolases.

extracts were dialysed overnight against twice the volume of distilled water at 4°C; the material which passed through the membrane was not active.

6.2.7.6. Toxin purification

Almost all toxic activity was extracted from the tuber tissue medium with ethyl acetate, since the extracted medium was not toxic to protoplasts in tuber tissue discs. Analysis of the ethyl acetate extracts by TLC, using n-butanol/acetic acid/water (4:1:5,v/v/v), gave 4 fractions with R_f values of (1) 0.25, (2) 0.41, (3) 0.83 and (4) 0.91 when dissolved in 10 ml water. The pH value of fractions 1 and 2 was pH 4.6, fraction 3 was pH 4.4 and fraction 4 was pH 5.0. After dissolving in distilled water and adjusting the pH to 5.0 it was found that the solutions of fractions 1 and 3 were toxic, with fraction 3 being more active than fraction 1. The dilution end point of fraction 1 was at 50% of the initial concentration, whereas that of fraction 3 was at 33% of the initial concentration. Undiluted fractions 2 and 4 were not toxic (Fig 37 a).

Analysis of ethyl acetate extracts by TLC, using propanol/acetic acid/water (200:3:100, v/v/v), gave 3 fractions. The R_f values of these fractions were (1) 0.44, (2) 0.88 and (3) 0.96. when dissolved in 10 ml of distilled water. Fractions 1 and 3 gave solutions with pH 4.5, and fraction 2 gave a solution of pH 5.1. The pH of all fractions was adjusted to pH 5.0 before testing. Fractions 1 and 2 were toxic to protoplasts in tissue discs even when diluted to 33% of the initial concentration (Fig 37 b). Fraction 3 was not toxic. Extracts of uninfected tuber tissue, when treated in the same way as extracts of infected tissue gave no toxic fraction.

6.2.7.7. A comparison of four isolates of *F. coeruleum* for their ability to produce tissue macerating and toxic activity in tuber tissue medium

The relative abilities of 4 isolates of *F. caeruleum* to produce macerating activity and toxins in tuber tissue medium was compared using a dilution series of 12-day-old culture extracts. The dilution series consisted of undiluted culture extract together with 1:1, 1:3, 1:5 and 1:7 dilutions in distilled water. Before testing, each solution was buffered to pH 9.0, The time for 50% maceration or protoplast death to occur in each dilution was determined.

6.2.7.7.1. Tissue maceration

The undiluted extracts and extracts diluted 50% showed rather similar levels of activity although in each case isolate Fc2 was slightly less active than the others. Clear

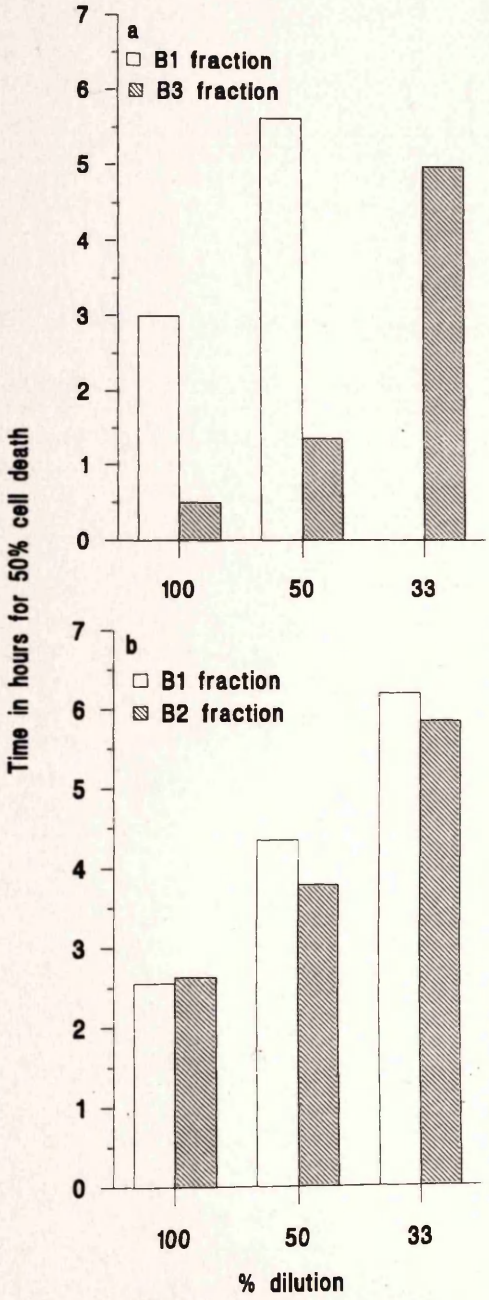
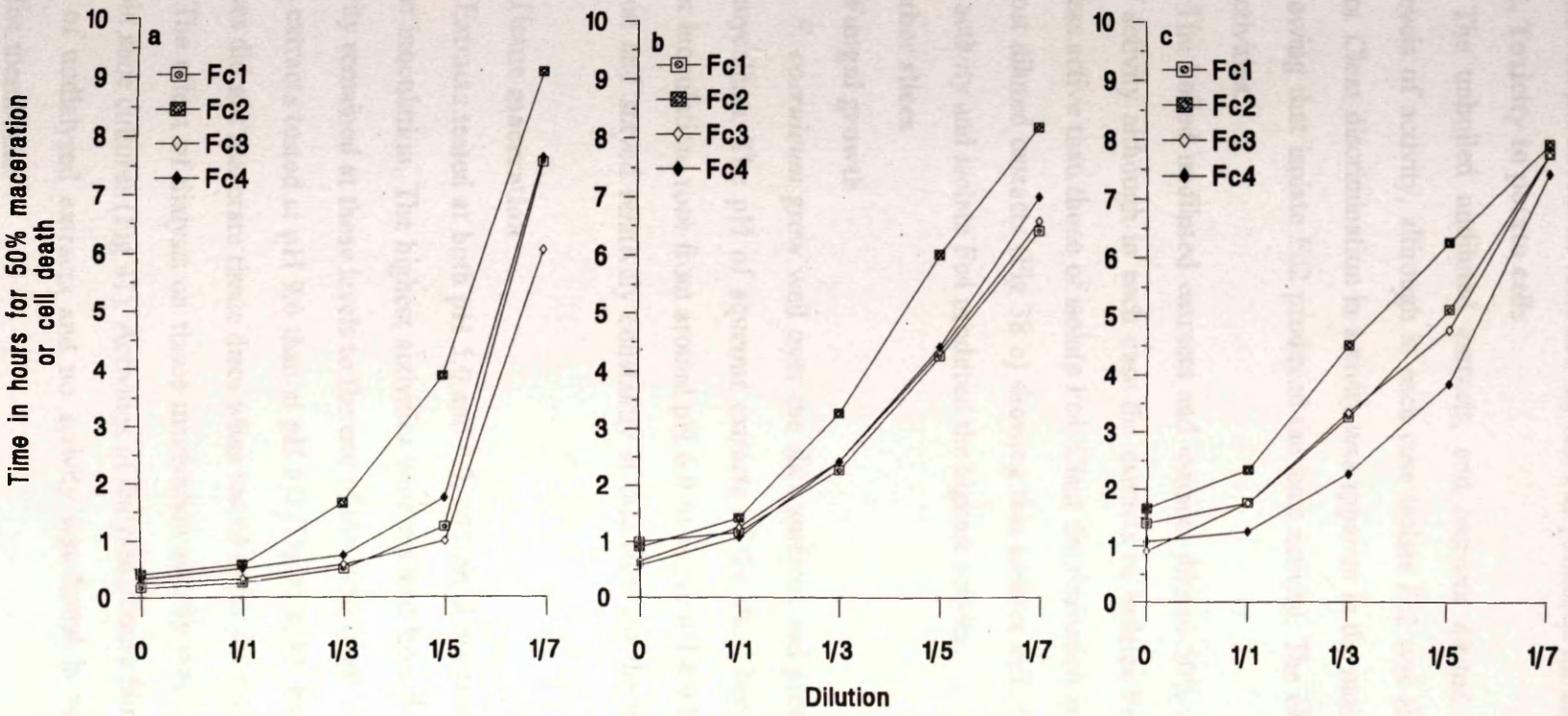


Fig. 37 Toxic activity of fractions obtained by TLC of ethyl acetate extracts from cultures of F. coeruleum grown in autoclaved tuber tissue medium using TLC (a) n-butanol/acetic acid/water or (b) propanol/acetic acid/water as developing solvents

Figure 38. Comparison of tissue macerating activity and toxic activity produced by four isolates (Fc1, Fc2, Fc3, Fc4) of *F. coeruleum* in autoclaved tuber tissue medium (a) macerating activity, (b) toxicity of unboiled cultur, (c) toxicity of boiled culture.


Time in hours for 50% maceration or cell death
a
b
c
Fc1
Fc2
Fc3
Fc4
0
1
2
3
4
5
6
7
8
9
10
0
1/1
1/3
1/5
1/7
Dilution

discrimination in activity was apparent in the most diluted extracts (Fig 38 a), showing that isolate Fc2 produced the least activity and Fc3 produced the highest activity.

6.2.7.7.2. Toxicity to potato cells

The unboiled undiluted extracts, and extracts diluted 50%, showed rather similar levels of activity, although in each case isolate Fc2 was slightly less active than the others. Clear discrimination in activity was apparent in the most diluted extracts (Fig 38 b) showing that isolate Fc2 produced the least activity. The other isolates produced similar activities.

The boiled undiluted extracts and extracts diluted 50% showed rather similar levels of activity although in each case the extracts of isolates Fc1, Fc3 and Fc2 were slightly less active than those of isolate Fc4. Clear discrimination in activity was apparent in the most diluted extracts (Fig 38 c) showing that isolates Fc1, Fc2 and Fc3 produced the least activity and isolate Fc4 produced the highest activity.

6.2.8. Tuber slices

6.2.8.1. Fungal growth

F. coeruleum grew well over the slice surfaces and produced a thick layer of sporing mycelium. The pH of aqueous extracts of the slice, beginning from around 2 days after inoculation, rose from around pH 6.0 to above pH 8.0 by 12 to 14 days after inoculation and stayed relatively constantly at that level for the rest of the experiment (Fig 39).

6.2.8.2. Tissue maceration

Extracts tested at both pH 5.0 and 9.0, contained macerating activity from very early after inoculation. The highest activities were reached from 8 days after inoculation and activity remained at these levels to the end of the experiment. Activities were slightly higher in extracts tested at pH 9.0 than at pH 5.0 (Fig 40 a, b). Extracts of uninoculated tuber slices did not macerate tissue discs when tested under the same conditions.

The effect of dialysis on tissue maceration activity was tested in extracts from 12-day-old slice cultures (Fig 41). Activities of dialysed culture filtrates were very similar to those of undialysed extracts and no activity was found in materials which passed through the membrane.

6.2.8.3. Pectin methyl esterase activity

Pectin methyl esterase activity was not found in extracts from any stage of colonisation.

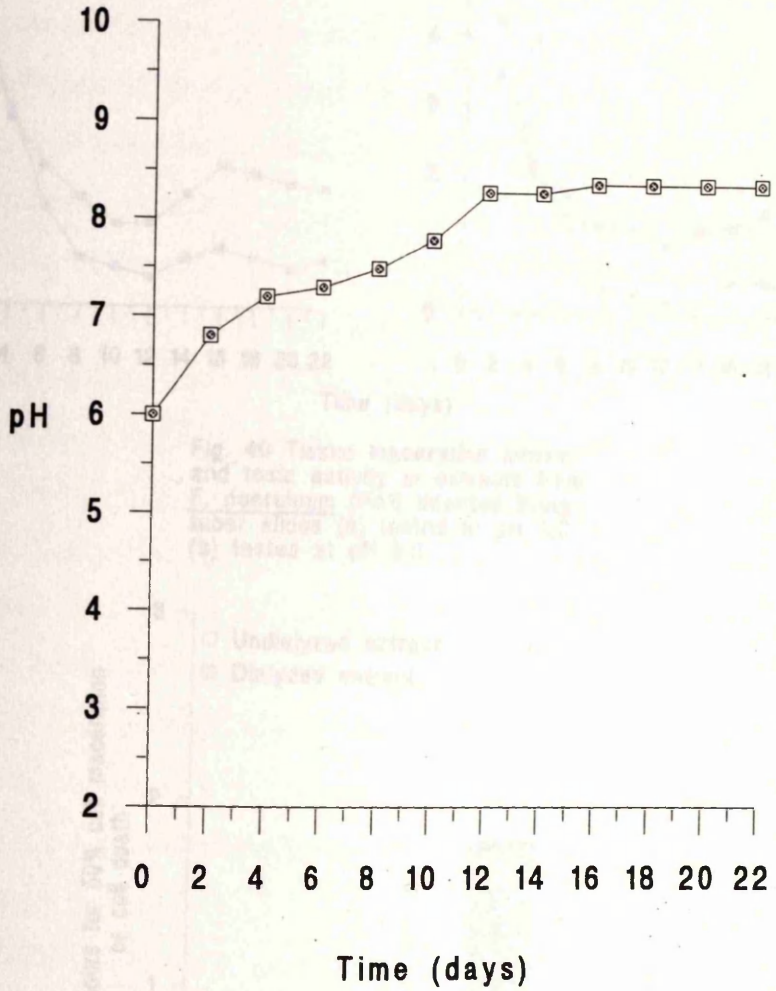


Fig. 39 Change in pH during culture of F. coeruleum (Fc1) in tuber slices.

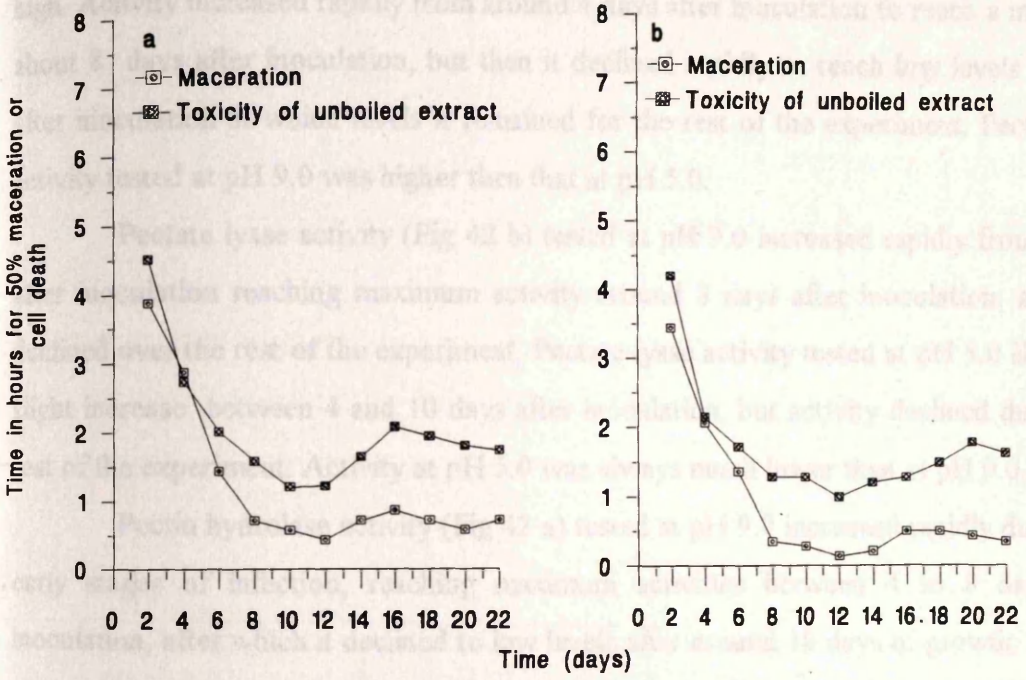


Fig. 40 Tissue macerating activity and toxic activity in extracts from F. coeruleum (Fc1) infected living tuber slices (a) tested at pH 5.0 (b) tested at pH 9.0.

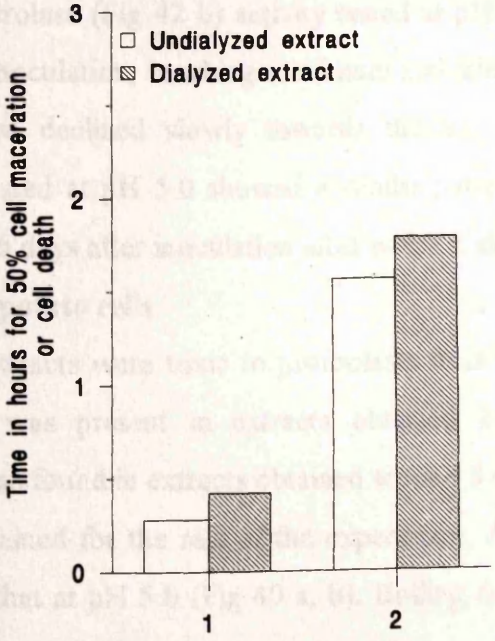


Fig. 41 Effect of dialysis on macerating activity and toxicity in extracts from F. coeruleum (Fc1) infected living tuber slices.

1-Maceration

2-Toxicity of unboiled extract

6.2.8.4. Pectic lyase and hydrolase activities

Pectin lyase (Fig 42 a) activities tested at both pH 5.0 and pH 9.0 were very high. Activity increased rapidly from around 4 days after inoculation to reach a maximum about 8 days after inoculation, but then it declined rapidly to reach low levels 10 days after inoculation at which levels it remained for the rest of the experiment. Pectin lyase activity tested at pH 9.0 was higher then that at pH 5.0.

Pectate lyase activity (Fig 42 b) tested at pH 9.0 increased rapidly from 6 days after inoculation reaching maximum activity around 8 days after inoculation, and then declined over the rest of the experiment. Pectate lyase activity tested at pH 5.0 showed a slight increase between 4 and 10 days after inoculation, but activity declined during the rest of the experiment. Activity at pH 5.0 was always much lower than at pH 9.0.

Pectin hydrolase activity (Fig 42 a) tested at pH 9.0 increased rapidly during the early stages of infection, reaching maximum activities between 4 to 8 days after inoculation, after which it declined to low levels after around 10 days of growth. Activity then remained at these low levels for the rest of the experiment. Activity tested at pH 5.0 increased to a maximum around 8 days after inoculation then declined during the rest of the experiment. Activity tested at pH 9.0 was always higher than that at pH 5.0.

Pectate hydrolase (Fig 42 b) activity tested at pH 9.0 increased slightly during the early stages of inoculation, reaching maximum activities after between 4 to 12 days of growth, and then declined slowly towards the end of the experiment. Pectate hydrolase activity tested at pH 5.0 showed a similar pattern of activity with maximum activity around 4 to 6 days after inoculation after which it slowly declined.

6.2.8.5. Toxicity to potato cells

Unboiled extracts were toxic to protoplasts from the early stages of infection. Significant activity was present in extracts obtained 2 days after inoculation but maximum toxicity was found in extracts obtained around 8 days after inoculation and this level of activity remained for the rest of the experiment. Activity tested at pH 9.0 was slightly higher than that at pH 5.0 (Fig 40 a, b). Boiling destroyed toxic activity almost completely.

Extracts of uninfected tuber tissue, when tested under the same conditions were not toxic.

Toxic activity of extracts obtained 12 days after inoculation was not dialyzable since the activities were very similar both before and after dialysis (Fig 41). The materials

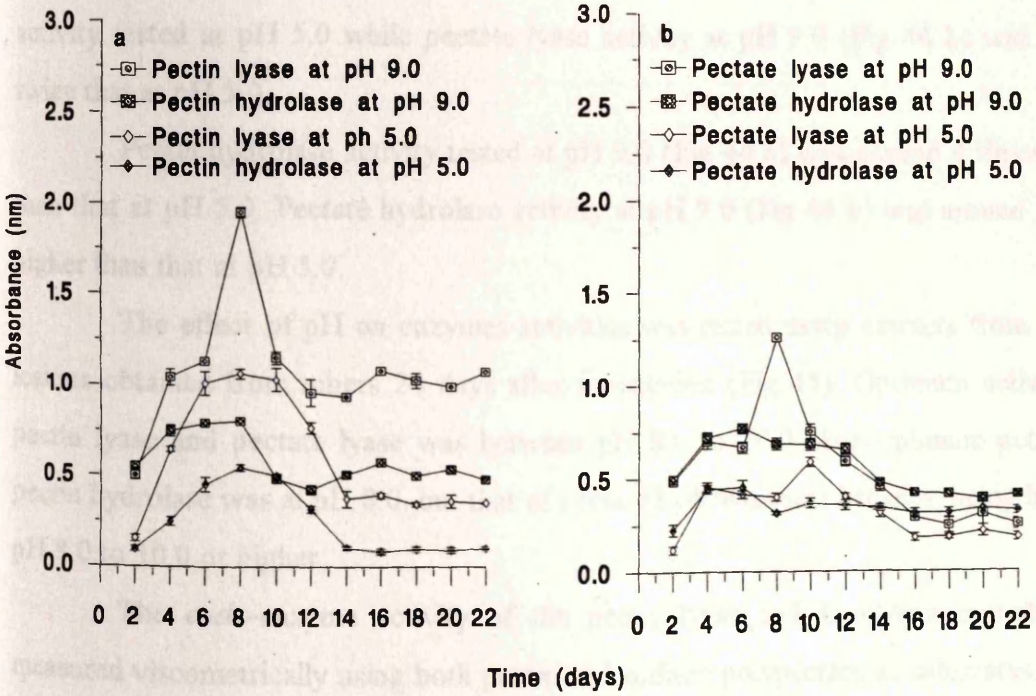


Fig. 42 Pectic enzyme production by F. coeruleum (Fc1) in living tuber slices.

which passed through the membrane were not toxic.

6.2.9. Extracts from dry rot lesions in whole tubers

6.2.9.1. Fungal growth

The pH of the extracts obtained from dry rotted tissues 24 day after inoculation was around pH 8.5.

6.2.9.2. Tissue maceration

Extracts from dry rot lesions prepared 24 days after inoculation, when tested at either pH 5.0 or 9.0, macerated tissue discs. Activities were slightly higher in extracts tested at pH 9.0, than at pH 5.0 (Fig 43). Extracts of uninfected tuber tissue medium did not macerate tissue discs when tested under the same conditions.

6.2.9.3. Pectic lyase and hydrolase activities

Pectin lyase activity tested at pH 9.0 (Fig 44 a) was around 3 times higher than activity tested at pH 5.0 while pectate lyase activity at pH 9.0 (Fig 44 b) was around twice that at pH 5.0.

Pectin hydrolase activity tested at pH 9.0 (Fig 44 a) was around 4 times higher than that at pH 5.0. Pectate hydrolase activity at pH 9.0 (Fig 44 b) was around 5 times higher than that at pH 5.0.

The effect of pH on enzymes activities was tested using extracts from dry rot lesions obtained from tubers 24 days after inoculation (Fig 45). Optimum activities of pectin lyase and pectate lyase was between pH 8.0 to 10.0. The optimum activity of pectin hydrolase was at pH 8.0, but that of pectate hydrolase was broader, being between pH 8.0 to 10.0 or higher.

The *endo*-enzyme activity of the pectic lyase and hydrolase complex was measured viscometrically using both pectin and sodium polypectate as substrates. Figure 46 a, b shows that *endo*-activity of extracts was present in extracts at pH 9.0 and 5.0 on both pectin and sodium polypectate but that activity on both was much higher at pH 9.0. These results indicate that the extract contains *endo*-enzyme activity but whether this is *endo*-lyase and or *endo*-hydrolyse activity is not known.

Exo-enzyme activity of the enzyme complex was determined in extracts using both pectin and sodium polypectate as substrates. The chromatographic analysis of the products of enzyme activity assayed at pH 5.0 indicated little evidence for the production of mono, di, or trimers of galacturonic acid but when assayed at pH 9.0 a range of

breakdown products, including monomeric forms, were found. The clearest indication of galacturonic acid was found after 1 to 8 h of incubation.

6.2.9.4. Toxicity to potato cells

Unboiled extracts when tested at either pH 5 or 9.0 caused cell death. Activity was slightly higher in extracts tested at pH 9.0 than pH 5.0 (Fig 43). Boiling destroyed toxic activity almost completely.

Extracts of uninfected tubers did not kill protoplasts when prepared and tested under the same conditions.

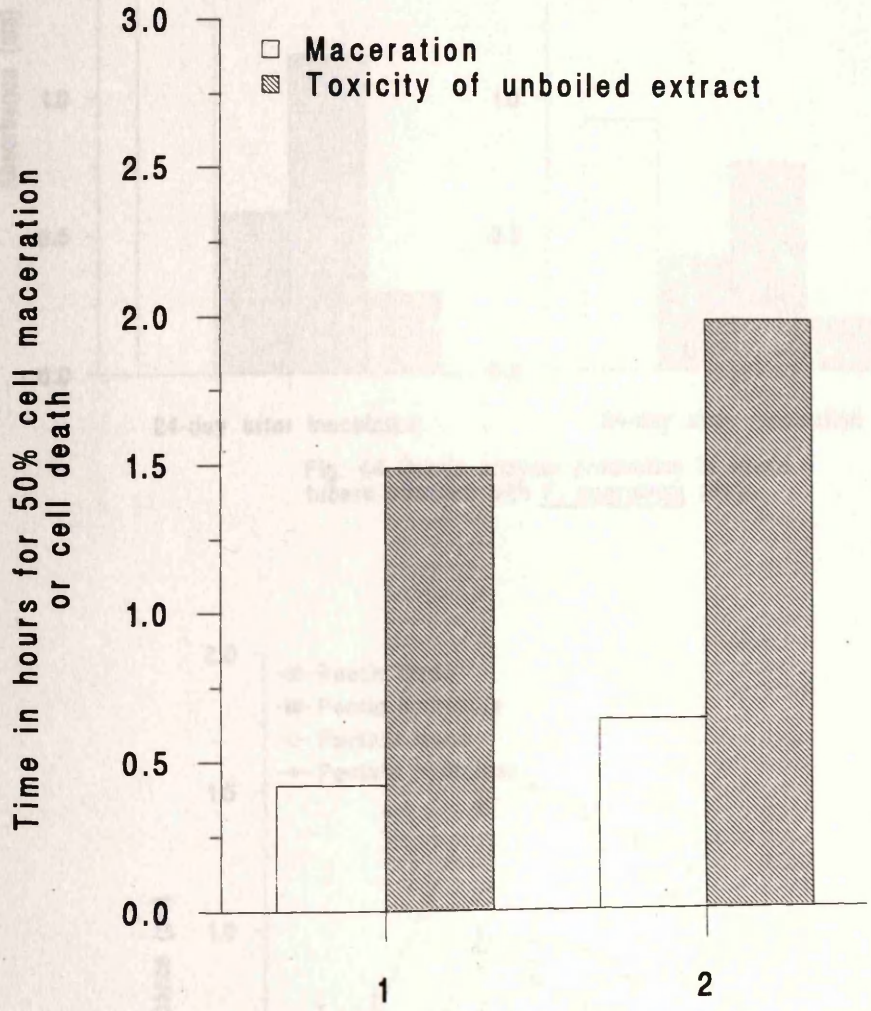


Fig. 43 Production of macerating activity and toxicity of extracts from dry rot lesions produced by F. coeruleum (Fc1) in whole tubers

1-Tested at pH 9.0

2-Tested at pH 5.0

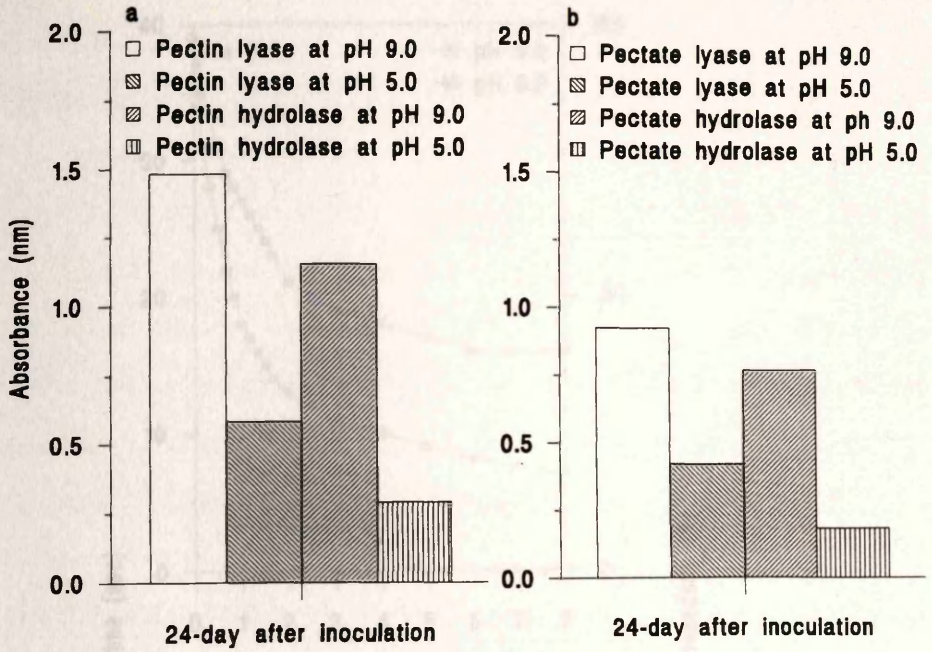


Fig. 44 Pectic enzyme production in whole tubers infected with F. coeruleum (Fc1).

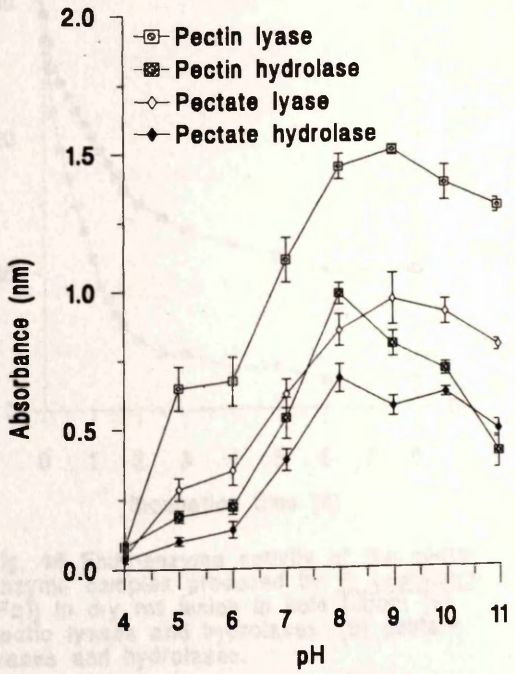


Fig. 45 Effect of pH on pectic enzyme activities in extracts from dry rot lesions produced by F. coeruleum in whole tubers.

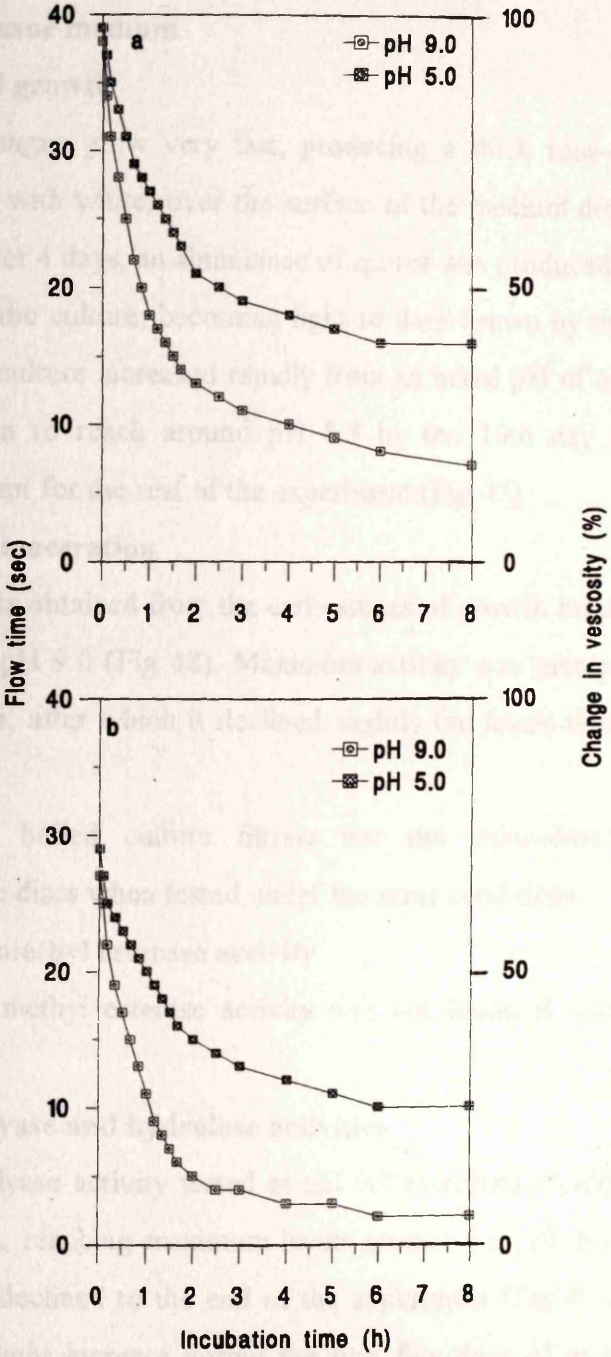


Fig. 46 Endo-enzyme activity of the pectic enzyme complex produced by F. coeruleum (Fc1) in dry rot lesion in hole tubers (a) pectic lyases and hydrolases, (b) pectate lyases and hydrolases.

6.3. Production of cell wall macerating enzymes, pectic enzymes and toxins by *F. avenaceum* isolate Fa1 in different media

6.3.1. Tuber tissue medium

6.3.1.1. Fungal growth

The fungus grew very fast, producing a thick rose-pink or brown-yellowish colony, fringed with white, over the surface of the medium during the first 4 days after inoculation. After 4 days, an abundance of spores was produced, which gave a yellowish-pink colour to the culture, becoming light to dark-brown by the end of the experiment. The pH of the culture increased rapidly from an initial pH of 6.0 during the first 6 days after inoculation to reach around pH 8.5 by the 10th day at which level it stayed relatively constant for the rest of the experiment (Fig 47).

6.3.1.2. Tissue maceration

Extracts obtained from the early stages of growth macerated potato tissue discs when tested at pH 9.0 (Fig 48). Maximum activity was greatest between 4 to 12 days after inoculation, after which it declined slightly but levels remained high to the end of the experiment.

Nether boiled culture filtrate nor the unincubated tuber tissue medium macerated tissue discs when tested under the same conditions.

6.3.1.3. Pectin methyl esterase activity

Pectin methyl esterase activity was not found in extracts from any stage of growth.

6.3.1.4. Pectic lyase and hydrolase activities

Pectin lyase activity tested at pH 9.0 increased rapidly during the first 2 days after inoculation, reaching maximum levels around 4 to 10 days after inoculation, after which it slowly declined to the end of the experiment (Fig 49 a). Activity tested at pH 5.0 showed a slight increase during the first few days of growth, reaching maximum activity around 6 to 10 days after inoculation and then declined to low levels during the rest of the experiment. Pectate lyase activity tested at pH 9.0 also increased during the early stages of growth, reaching maximum activities around 4 to 10 days after inoculation after which it declined to low levels around 12 days after inoculation and this level of activity remained to the end of the experiment (Fig 49 b). Pectate lyase activity tested at pH 5.0 reached maximum levels after around 4 to 6 days of growth after which it declined to very low levels by 12 days after inoculation and remained at these levels for

the rest of the experiment. Pectin and pectate lyase activities were higher in extracts buffered at pH 9.0 than that at pH 5.0.

Pectin hydrolase activity tested at pH 9.0 increased to maximum levels within 2 days of inoculation and remained at these levels for around 8 days and then declined (Fig 49 a). Pectin hydrolase activity tested at pH 5.0 showed a slow increase to maximum levels around 10 days after inoculation and then activity declined to low levels during the rest of growth. Pectate hydrolase activities tested at both pH 5.0 and 9.0 (Fig 49 b) increased to maximum levels within 2 days of inoculation. Activity at pH 9.0 only showed a slight decline at these levels for the rest of the experiment but activity at pH 5.0 fell quite sharply from the 4th day after inoculation.

6.3.1.5. Toxicity to potato cells

Unboiled extracts from 4 day old cultures when tested at pH 9.0, were much more active than boiled extracts suggesting that toxic components produced during the first few days of culture were heat labile. However from the 6th day of culture the activity of boiled and unboiled extracts was essentially the same and maximum levels were maintained from the 8th day until the end of the experiment (Fig 48). Extracts of uninfected tuber tissue medium did not kill protoplasts in tissue discs when tested under the same conditions.

6.3.2. Extracts from dry rot lesions in whole tubers

6.3.2.1. Fungal growth

The pH of the extracts obtained from dry rotted lesion in whole tubers 24 days after inoculation was around pH 8.3.

6.3.2.2. Tissue maceration

Extracts from dry rot lesions prepared 24 days after inoculation, when tested at pH 9.0, macerated tissue discs and the activity was very high (Fig 50). Extracts of uninfected tuber tissue medium did not macerate tissue discs when tested under the same conditions.

6.3.2.3. Pectic lyase and hydrolase activities

Pectin lyase activity tested at pH 9.0 (Fig 51 a) was around 6 times greater than activity tested at pH 5.0, while pectate lyase activity at pH 9.0 (Fig 51 b) was around 3 times higher than activity at pH 5.0. Both pectin and pectate hydrolase activities (Fig 51 a, b), tested at pH 9.0, were around 4 times higher than at pH 5.0.

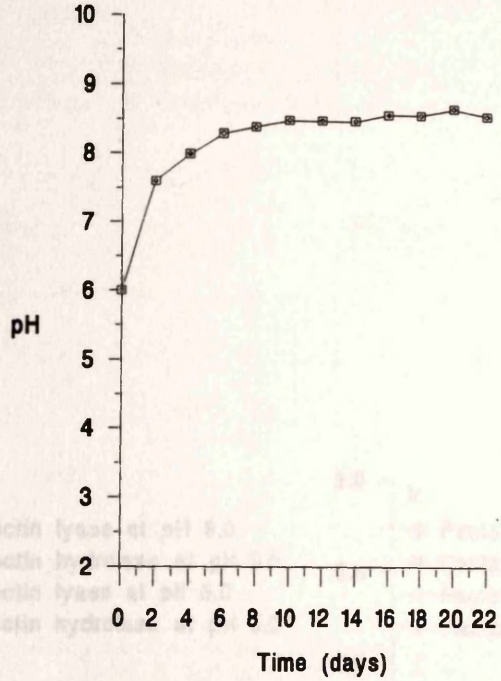


Fig. 47 Change in pH in autoclaved tuber tissue medium during growth by F. avenaceum (Fa1).

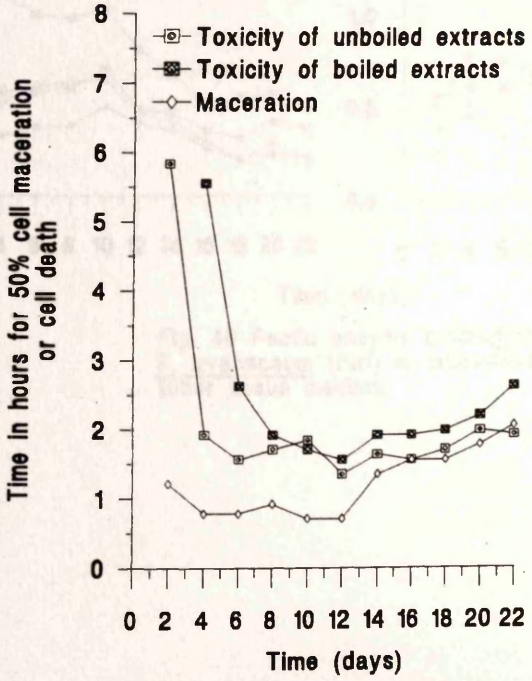


Fig. 48 Tissue macerating activity and toxicity in culture extracts of F. avenaceum (Fa1) from autoclaved tuber tissue medium.

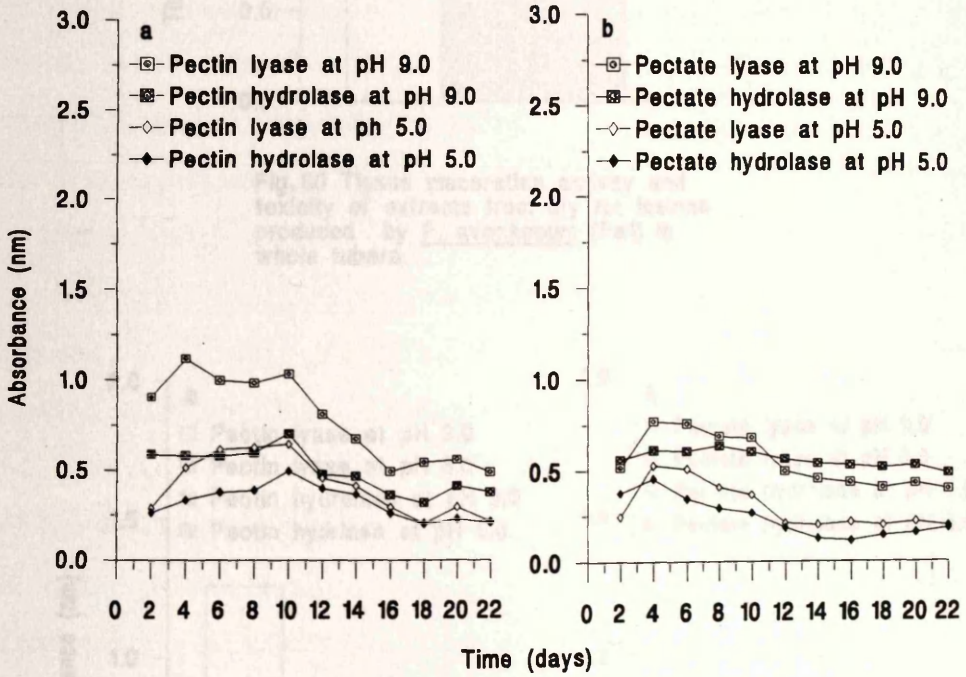


Fig. 49 Pectic enzyme production by F. avenaceum (Fa1) in autoclaved tuber tissue medium.

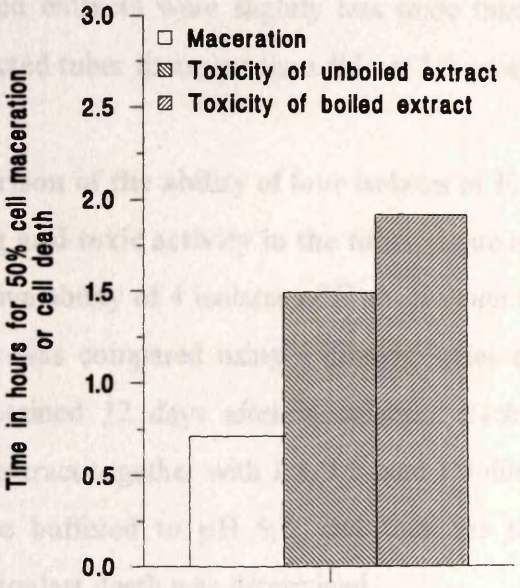


Fig. 50 Tissue macerating activity and toxicity of extracts from dry rot lesions produced by F. avenaceum (Fa1) in whole tubers.

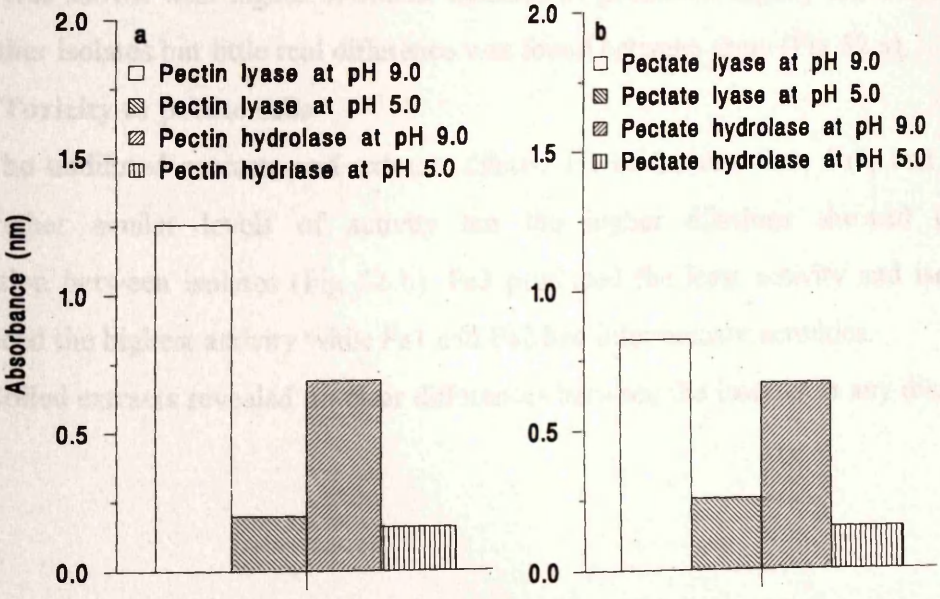


Fig. 51 Pectic enzyme production in whole tubers infected with F. avenaceum (Fa1) 24 days after inoculation.

6.3.2.4. Toxicity to potato cells

Both boiled and unboiled extracts, when tested at pH 9.0, caused cell death although the boiled extracts were slightly less toxic than unboiled extracts (Fig 50). Extracts of uninfected tuber tissue medium did not kill protoplasts when tested under the same conditions.

6.3.2.5. A comparison of the ability of four isolates of *F. avenaceum* to produce tissue macerating and toxic activity in the tuber tissue medium

The relative ability of 4 isolates of *F. avenaceum* to macerate potato tissue and to kill protoplasts was compared using a dilution series of culture filtrates from tuber tissue medium obtained 12 days after inoculation. Each dilution series consisted of undiluted culture extract together with 1:1, 1:3, and 1:5 dilutions with distilled water. All the dilutions were buffered to pH 9.0, and then the time for each to cause 50% maceration or protoplast death was determined.

6.3..2.5.1. Tissue maceration

The undiluted extracts for all isolates showed high levels of activity with dilution reducing activity. The reduction was slight with the 1:1 dilution but a linear reduction was shown with higher dilutions. Isolate Fa4 produced slightly more activity then the other isolates but little real difference was found between them (Fig 52 a).

6.3.2.5.2. Toxicity to potato cells

The undiluted extracts and extracts diluted 1:1 of isolates Fa1, Fa2, and Fa4 showed rather similar levels of activity but the higher dilutions showed clear discrimination between isolates (Fig 52 b). Fa3 produced the least activity and isolate Fa4 produced the highest activity while Fa1 and Fa2 had intermediate activities.

Boiled extracts revealed no clear differences between the isolates at any dilution (Fig 52 c).

Figure 52. Comparison of tissue macerating activity and toxic activity produced by four isolates (Fa1, Fa2, Fa3, Fa4) of *F. avenaceum* in autoclaved tuber tissue medium (a) macerating activity, (b) toxicity of unboiled culture, (c) toxicity of boiled culture.

Time in hours for 50% cell maceration
or cell death
a
b
d
Fa1
Fa2
Fa3
Fa4
0
1
2
3
4
5
6
7
8
9
1
1/1
1/3
1/5
Dilution

6.4. Production of cell wall macerating enzymes pectic enzymes and toxins by *Cylindrocarpon destructans* isolate Cd2 in different media

6.4.1. Tuber tissue medium

6.4.1.1. Fungal growth

The fungus grew very fast, producing a thick brown-yellowish mycelium over the surface of the medium during the first 4 days after inoculation. From 4 days the culture began to produce abundant conidia which imparted a brown-yellowish to dark-brown colour to the culture. The pH of the culture increased during the first 2 days after inoculation rising rapidly to above pH 8.0. and then stayed relatively constant at this level for the rest of the experiment (Fig 53).

6.4.1.2. Tissue maceration

Extracts obtained from the early stages of growth, tested at pH 9.0, macerated potato tissue (Fig 54). Activity was high even 2 days after inoculation and it remained high throughout the course of the experiment with maximum activity being found around 10 days after inoculation. Neither boiled nor unboiled extracts from uninoculated tuber tissue medium macerated tissue discs when tested under the same conditions.

6.4.1.3. Pectin methyl esterase activity

Pectin methyl esterase activity was not found in extracts from any stage of growth.

6.4.1.4. Pectic lyase and hydrolase activities

Pectin lyase activity tested at pH 9.0 increased slightly during the first few days after inoculation, reaching maximum activity around 4 days after inoculation, This increase was followed by a sharp decline to low levels from 10 days after inoculation at which it remained to the end of the experiment (Fig 55 a). Activity tested at pH 5.0 was very low or negligible throughout the whole period of growth. Pectate lyase activity tested at pH 9.0 were very low throughout the whole period of growth (Fig 55 b).

Pectin hydrolase activity tested at pH 9.0 showed a similar pattern of activity to lyase activity increasing slightly during the early stages of growth to reach maximum activity around 4 days after inoculation, after which it declined to low levels for the rest of the experiment (Fig 55 a). Pectin hydrolase activity tested at pH 5.0 was very low or negligible throughout the whole period of growth. Pectate hydrolase activity at both pH 5.0 and 9.0 was very low or negligible throughout the whole period of growth (Fig 55b).

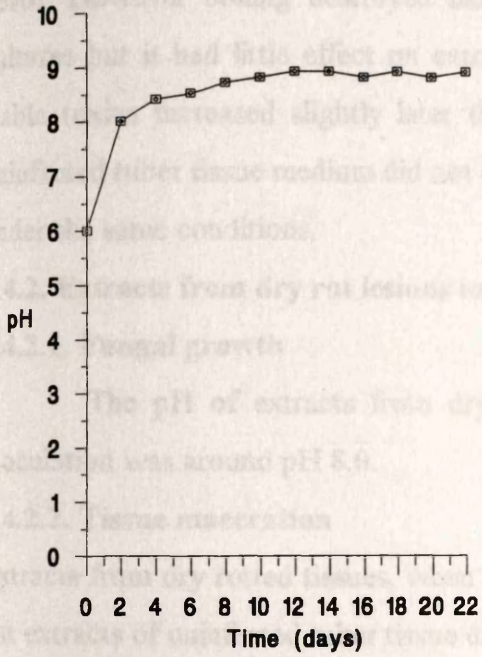


Fig. 53 Change in pH in autoclaved tissue medium during growth by C. destructans.

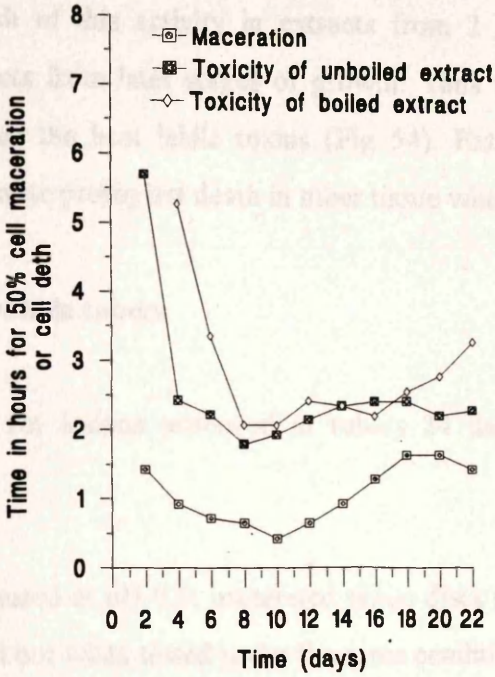


Fig. 54 Production of tissue macerating activity and toxicity of extracts from C. destructans (Cd2) in autoclaved tuber tissue medium.

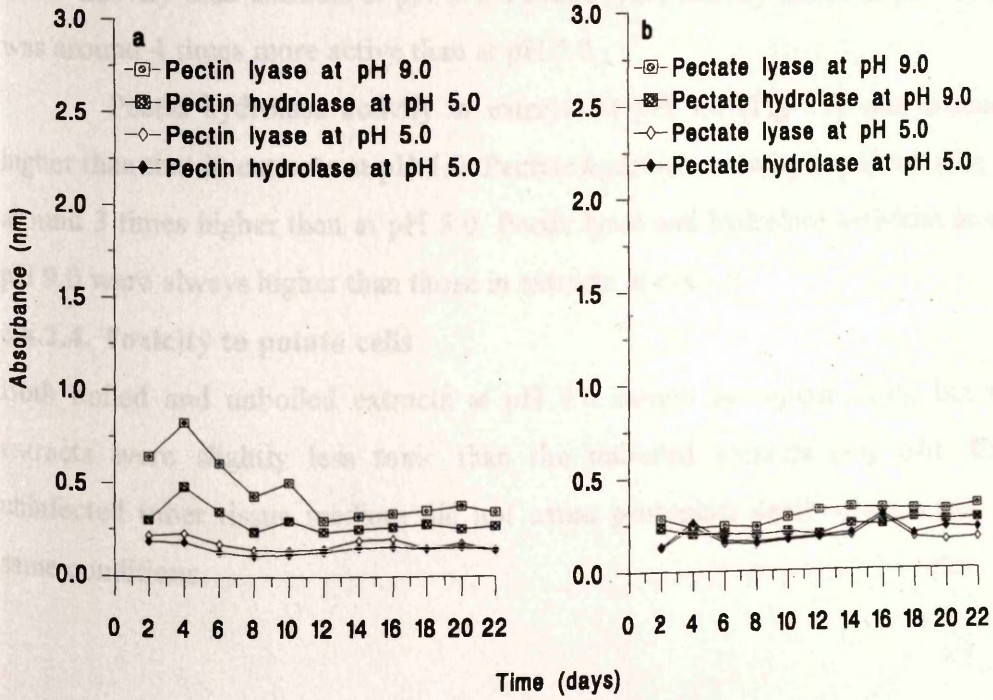


Fig. 55 Pectic enzyme production by C. destructans (Cd2) in autoclaved tuber tissue medium.

6.4.1.5. Toxicity to potato cells

Both boiled and unboiled extracts, when tested at pH 9.0 caused protoplast death. However boiling destroyed much of this activity in extracts from 2 day old cultures but it had little effect on extracts from later stages of growth. Thus the heat stable toxins increased slightly later than the heat labile toxins (Fig 54). Extracts of uninfected tuber tissue medium did not cause protoplast death in tuber tissue when tested under the same conditions.

6.4.2. Extracts from dry rot lesions in whole tubers

6.4.2.1. Fungal growth

The pH of extracts from dry rot lesions produced in tubers 24 days after inoculation was around pH 8.0.

6.4.2.2. Tissue maceration

Extracts from dry rotted tissues, when tested at pH 9.0, macerated tissue discs (Fig 56) but extracts of uninfected tuber tissue did not when tested under the same conditions.

6.4.2.3. Pectic lyase and hydrolase activities

Pectin lyase activity in extracts tested at pH 9.0 (Fig 57 a) had about 3 times more activity than extracts at pH 5.0. Pectate lyase activity tested at pH 9.0 (Fig 57 b) was around 4 times more active than at pH 5.0.

Pectin hydrolase activity in extracts at pH 9.0 (Fig 57) was around 3 times higher than that in extracts at pH 5.0. Pectate hydrolase activity at pH 9.0 (Fig 57 b) was around 3 times higher than at pH 5.0. Pectic lyase and hydrolase activities in extracts at pH 9.0 were always higher than those in extracts at pH 5.0.

6.4.2.4. Toxicity to potato cells

Both boiled and unboiled extracts at pH 9.0 caused protoplast death but the boiled extracts were slightly less toxic than the unboiled extracts (Fig 56). Extracts of uninfected tuber tissue medium did not cause protoplast death when tested under the same conditions.

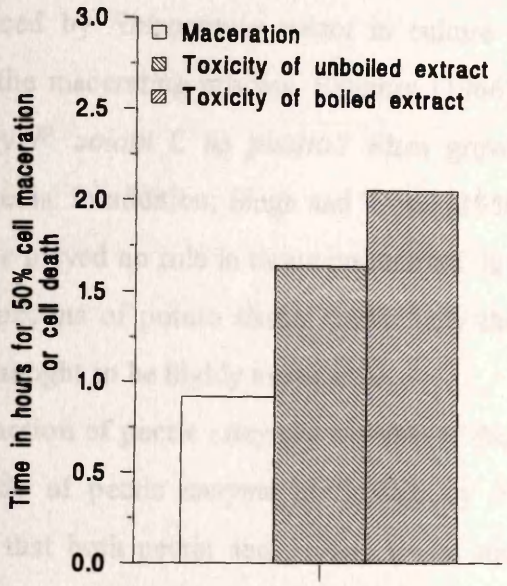


Fig. 56 Production of tissue macerating activity and toxicity of extracts from dry rot lesions produced by C. destrucans (Cd2) in whole tubers 24 days after inoculation.

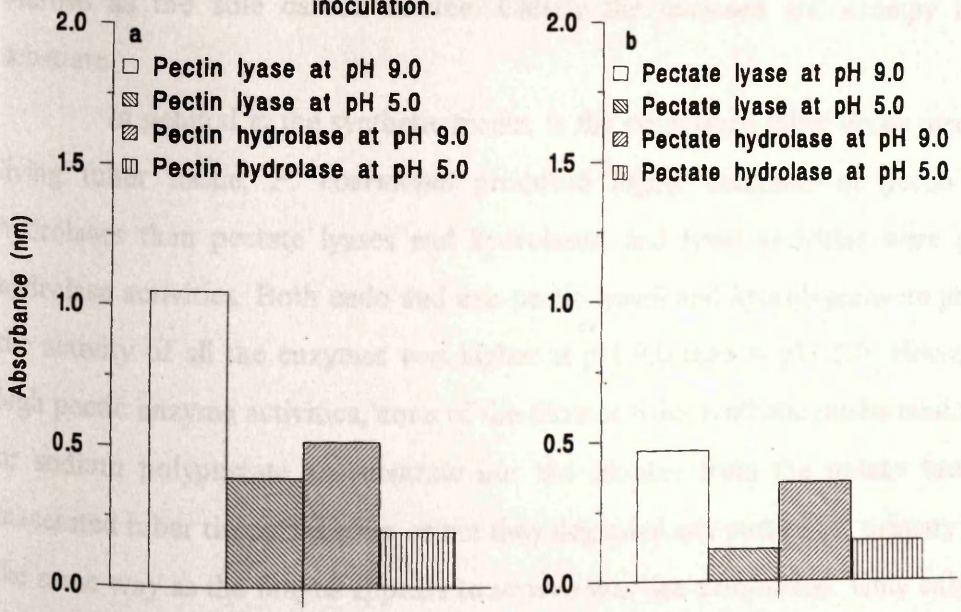


Fig. 57 Pectic enzyme production in whole tubers infected with C. destructans (Cd2) 24 days after inoculation.

6.5. Discussion

6.5.1. The role of pectin methylesterase in pathogenicity

None of the three species produced detectable PME in any of the culture media at any stage of growth. However, Bateman (1963b), found no evidence to suggest that the PME produced by *Rhizoctonia solani* in culture or in infected tissue plays an essential role in the macerating process. Bateman (1966) further reported that no PME was produced by *F. solani* f. sp *phasioli* when grown on potato-pectin or potato-glucose-pectin media. In addition, Singh and Wood (1956) reported that PME produced by *F. moniliforme* played no role in tissue maceration. In any case the largest proportion of the cell wall pectins of potato tissue, particularly those associated with the middle lamella, are not thought to be highly methylated.

6.5.2. The production of pectic enzymes capable of degrading the cell wall

The study of pectic enzyme production by *F. coeruleum* described in this chapter, showed that both pectin and pectate lyases and hydrolases were produced in culture in a variety of media but mainly only those containing pectic substrates as carbon sources. Only negligible amounts were produced in synthetic media with glucose or sucrose as the sole carbon source. Clearly the enzymes are strongly inducible by substrate.

In general in the synthetic media, in the autoclaved tuber tissue medium, and in living tuber tissue, *F. coeruleum* produced higher activities of pectin lyases and hydrolases than pectate lyases and hydrolases, and lyase activities were greater than hydrolase activities. Both endo and exo-pectic lyases and hydrolases were produced and the activity of all the enzymes was higher at pH 9.0 than at pH 5.0. However, despite high pectic enzyme activities, none of the filtrates from synthetic media containing pectin or sodium polypectate as substrate nor the filtrates from the potato broth medium, macerated tuber tissue; whether or not they degraded any part of the primary cell walls in the same way as the fungus appears to *in vivo* was not determined. Only culture extracts from autoclaved tuber tissue medium and from living tuber tissue caused cell wall maceration of tuber tissue discs. When it occurred, maceration occurred over the whole range of pH tested, but high rates of maceration were only evident between pH 7.0 and pH 10.

Potato broth medium was prepared from tuber tissue which had been macerated and then filtered, so that a large part of the cell wall material was probably removed.

Thus the main difference between potato broth medium and autoclaved potato tuber tissue medium was that the former probable contained little cell wall material whereas the latter contained it all. It is thus likely that the cell wall materials which were removed in the preparation of potato broth medium were responsible for the induction of those specific cell wall macerating enzymes which were produced in autoclaved tuber tissue and in living tuber tissue.

F. avenaceum, and *C. destructans,* the two other tuber dry rot fungi used in this investigation, also produced pectic lyases and hydrolases when grown on living and dead (autoclaved) tuber tissue. The culture extracts of these two fungi were also capable of macerating tuber tissue *in vitro* although *in vivo* they both caused dry rots. However, the production of enzymes by these two species in synthetic media with pectic materials as carbon sources, or in potato broth was not investigated.

The inducibility of pectic enzymes by substrate and their inhibition by the end products of action has been reported by many workers. Singh and Wood (1956) reported that *F. moniliforme* secreted macerating enzymes in liquid media but only when these contained certain natural extracts, such as pectic substances or polygalacturonic acid. Mullen & Bateman 1971 and 1975 reported that a potato dry rot causing *Fusarium, F. roseum 'Avenaceum'* (syn *F. avenaceum*) was induced to produce a number of polysaccharide degrading enzymes when cultured on a mineral salts medium supplemented with certain polymeric carbon sources or when grown on potato broth supplemented with isolated potato cell walls. No polysaccharide degrading enzymes were detected in culture filtrates when the fungus was grown in the mineral salts medium supplemented with glucose, starch, mannan, or carboxymethyl cellulose, or in potato broth medium without the addition of pectate or potato cell walls. This latter result is at variance with the results reported in this thesis for *F. coeruleum*, because *F. coeruleum* did produce pectic enzymes in potato broth medium. The isolate of *F. avenaceum* used in this study was not tested in potato broth medium.

Cooper *et al.* (1981) examined the role of plant cell walls in infection by determining their influence on the production and activity of polygalacturonases and pectic lyases by 6 different fungal pathogens able to cause significant wall degradation *in vivo.* These six fungi were, *Botrytis fabae, B. cinerea, Sclerotinia fructigena, S. trifoliorum, F. oxysporum* f. sp. *lycopersici* and *F. oxysporum* f. sp. *pisi.* They were grown in shake cultures containing as sole carbon source, cell walls extracted from host

and non-host plants (broad bean, tomato, apple, clover, pea and dwarf bean). The walls from the various species had a differential effect on the rate and extent of synthesis of some enzymes, but no consistent relationship was found between their production and susceptibility or resistance of the host parasite interaction. The highest levels of polygalacturonase production by *B. fabae, B. cineria* and *F. oxysporum* f. sp. *lycopersici* occurred on host cell walls, whereas the walls from host plants supported the lowest accumulation of pectic lyases by *F. oxysporum* f. sp. *lycopersici,* and *F. oxysporum* f. sp. *pisi* and of polygalacturonase by *S. trifoliorum.* Occasionally, enzyme levels were very low during growth on non-host walls, e.g. *B. fabae* polygalacturonase with apple cell walls, and *F. oxysporum* f. sp. *pisi* polygalacturonase with clover cell walls. Growth of the two *F. oxysporum* ff. sp. varied on cell walls from different species but it was not related to enzyme production nor host-parasite compatibility.

As indicated earlier the pectic enzymes were not produced by *F. coeruleum* in synthetic media containing sucrose or glucose as carbon sources. Both these compounds are known to repress pectic enzyme synthesis in a number of plant pathogens. Horton & Keen (1966) found that pectic enzyme synthesis by *Pyrenochaeta terrestris* was strongly repressed by glucose while Patil and Dimond (1968) also reported that polygalacturonase synthesis by *F. oxysporum* f. sp *lycopersici* was repressed by sugar and sugar derivatives. Many other workers (Mussel and Green, 1970; Cooper and Wood, 1975; Cooper *et al.*, 1981) have found that cell wall polysaccharide degrading enzymes produced by pathogenic micro-organisms are subject to catabolite repression and Mullen & Bateman (1971, 1975) found that the extent of repression of enzyme synthesis varied between enzymes and depended upon the particular catabolite.

In the present study a variety of pectic enzymes were found to be produced by *F. coeruleum* in the synthetic media and in potato broth which were not capable of macerating tuber tissue. The failure of these enzymes to macerate tuber tissue could have several explanations. For example the enzymes produced in synthetic media and in potato broth might be inactive on potato cell wall pectins, particularly those in the middle lamella.

However, all three fungi produced cell wall macerating enzymes when grown on autoclaved potato tissue and such enzymes were extracted from infected living tissue. So why do these fungi not macerate potato tissue *in vivo.* Many workers have reported that cell wall macerating enzymes can be inactivated by oxidised tissue phenolics. (Cole &

Wood, 1961; Deverall & Wood, 1961; Patil & Dimond, 1968; and Mullen & Bateman, 1971, 1975). However enzyme inactivation is unlikely to be the explanation for the failure of the cell wall macerating enzymes to macerate tuber tissue because aqueous extracts could be obtained from infected tissue which contained active tissue macerating enzyme activity.

The most likely explanation for the failure of the macerating enzymes to degrade the middle lamella is that the middle lamella, at a very early stage of infection, becomes impregnated with tuber metabolites, possibly phenolic or lipid substances which make it resistant to degradation. The electron microscope sections of infected tissue indicates the accumulation of electron dense materials in the middle lamella.

6.5.3. Toxin production

All three fungi, *F. coeruleum, F. avenaceum,* and *C. destructans* produced toxic substances when grown on living and dead (autoclaved) tuber tissue which killed potato tuber cells. Culture, or tissue extracts, contained toxic activity from 2-4 days after inoculation and retained activity up to the end of the experiments.

No toxins were produced by *F. coeruleum* growing in Czapek-Dox medium with sucrose, pectin or sodium polypectate as the sole carbon source and neither when grown on potato broth medium. On the other hand it produced toxic substances when grown in Baker's synthetic medium. None of the other two fungi were grown on the synthetic media including Baker's medium and so whether or not they produced toxins on synthetic media is not known.

The activity of the toxic substances produced by *F. avenaceum* and *C. destructans* in living potato slices or in autoclaved potato tissue was only slightly reduced by boiling but those produced by *F. coeruleum* in living tuber tissue were largely destroyed. However the toxicity of substances produced by *F. coeruleum* in autoclaved tuber tissue or in Baker's medium was more stable to heat. Clearly the toxins of *F. avenaceum* and *C. destructans* produced in living tuber tissue were different from those produced by *F. coeruleum* in this substrate. Furthermore, the toxins produced in living tissue by *F. coeruleum* may be different from those produced in culture media.

All of the active extracts of the three fungi retained activity after dialysis indicating that the toxins had molecular masses of 12.000 or greater, the cut off point of the membrane used, Since boiling extracts destroyed macerating activity, but not

toxicity, unlike toxicity with many soft rot organisms, it was not due to the activities of cell wall macerating or other enzyme activities.

The toxic fractions produced by *F. coeruleum* in both synthetic Baker's medium and in autoclaved tuber tissue contained fractions with similar R_f values when run on silica by TLC using butanol/acetic acid/water or propanol/acetic acid/water as the solvent systems. This suggests that the same or very similar compounds were produced in each medium.

The toxicity of extracts from cultures of *F. coeruleum* was affected by pH but those from different media were affected in different ways. The extracts from tuber tissue medium were toxic over the whole pH range tested, from pH 4.0-10.0, but optimum activity was found between pH 7.0 and pH 10.0. In contrast, culture filtrate from Baker's medium, although active over the pH range from pH 4.0-7.0 had optimum activity between pH 4.0-5.0. Thus, in contrast to the results from TLC this suggests that different molecules were involved.

Extracts of *F. coeruleum* still produced rapid maceration when diluted to such a level that toxin activity was negligible. This observation provides further evidence that cell death is not due to any enzymes involved in the macerating activity.

In contrast, dilutions of extracts of *F. avenaceum* showed similar losses of macerating activity and toxic activity. However the toxic activity was relatively unaffected by boiling, whereas macerating activity was totally destroyed clearly indicating that toxicity was not due to the activity of macerating enzymes.

A number of phytotoxins have been reported to be produced by species of *Fusarium* and the subject was reviewed in the Introduction. Among the toxins produced by the Martiella group, the group in which *F. coeruleum* is classified are the naphthazarins Kern and Neaf-Roth (1965); Kern *et al.* (1972); Kern (1978) and the trichothecenes. Elbenna *et al.* (1984) reported that *F. coeruleum* produces trichothecenes including deoxynivalenol and acetyldeoxynivalenol and HT-2 toxin in potato tubers and in liquid media. Trichothecenes were found in dry rotted tubers stored at 4°C or at 15°C for up 71 days and in culture at 23°C for up 21 days. Trichothecenes were also detected in potato tubers rotted by *F. sambucinum*. Thus the production of trichothecene toxins can be a common trait of *Fusarium* species causing dry rot in potato tubers and that the toxins may be produced in potatoes naturally infected with these fungi in the field or in storage. However, whether or not they are involved in pathogenecity

depends upon when they are produced during the growth cycle. To be important in the mechanisms of infection they must be produced at or soon after germination. All the toxin groups are in fact produced early in growth by *Fusarium* spp in culture and thus could be pathogenic factors. Ueno *et al.* (1973) reported that *F. solani* produced the largest amounts when grown in a nutrient medium consisting of glucose, sucrose, peptone and yeast extract either in shake culture or by jar fermentation at 24-27°C. They also reported (Ueno *et al.*, 1975) that the growth of *F. solani* was maximum on day 3 of incubation when the glucose in the medium had been completely consumed. The production of trichothecenes began soon after inoculation and the maximum amounts of toxin were obtained after 2 days of growth after which the levels declined. The trichothecenes are also produced by nembers of the Arthrosporella (Wyllie & Morehouse, 1977) group to which *F. avenaceum* is classified and by *Cylindrocarpon* spp. (Durbin, 1981).

Some of these toxins could be part of the toxic activity produced by *F. coeruleum*, *F. avenaceum* and *C. destructans* in these studies. However further work would be required clarify this point.

CHAPTER VII

GENERAL CONCLUSION

Table 18. Comparison of the sizes of dry rot lesions developed internally or visible externally on tubers of cvs Maris Piper and Record 24 days after inoculation with different isolates of *F. coeruleum* (Fc), *F. avenaceum* (Fa) and *C. destructans* (Cd).

Isolates	Size of lesion developed internally in tuber by 24 days after inoculation				Size of lesion developed externally in tuber by 24 days after inoculation			
	First experiment		Second experiment		First experiment		Second experiment	
	Maris Piper Mean SE	Record Mean SE	Maris Piper Mean SE	Record Mean SE	Maris Piper Mean SE	Record Mean SE	Maris Piper Mean SE	Record Mean SE
Fc1	40.3 ± 1.3	38.4 ± 1.3	30.1 ± 1.3	17.8 ± 1.4	34.7 ± 1.6	30.7 ± 1.6	26.4 ± 1.3	13.1 ± 0.9
Fc2	35.1 ± 1.5	33.4 ± 1.8	29.6 ± 1.3	18.0 ± 1.1	28.5 ± 1.7	29.9 ± 1.8	24.8 ± 1.3	19.1 ± 1.0
Fc3	34.8 ± 1.3	23.3 ± 1.2	30.5 ± 1.5	19.4 ± 1.1	29.4 ± 1.3	15.9 ± 1.0	24.5 ± 1.4	17.1 ± 1.0
Fc4	28.9 ± 1.3	23.3 ± 1.4	26.3 ± 1.2	18.5 ± 1.2	25.8 ± 1.1	19.7 ± 1.1	22.9 ± 1.3	12.9 ± 0.7
Mean	34.8 ± 1.4	29.6 ± 1.4	29.1 ± 1.3	18.4 ± 1.2	29.6 ± 1.4	24.1 ± 1.4	24.7 ± 1.3	15.6 ± 0.9
Fa1	43.7 ± 1.1	35.8 ± 1.2	35.5 ± 1.1	26.7 ± 1.4	43.2 ± 1.7	28.2 ± 1.2	40.7 ± 1.1	23.5 ± 1.2
Fa2	41.3 ± 1.0	35.9 ± 1.2	40.2 ± 1.0	28.6 ± 1.2	39.7 ± 1.3	34.6 ± 1.3	41.5 ± 1.1	26.9 ± 1.3
Fa3	36.5 ± 1.0	32.3 ± 0.8	31.3 ± 1.1	25.4 ± 1.1	35.5 ± 1.0	28.7 ± 1.0	32.5 ± 1.1	21.7 ± 1.3
Fa4	33.7 ± 0.8	30.3 ± 1.1	30.2 ± 1.0	26.1 ± 1.4	30.4 ± 1.0	25.5 ± 0.9	27.7 ± 1.1	18.9 ± 0.8
Mean	38.8 ± 1.0	33.6 ± 1.1	34.3 ± 1.1	26.7 ± 1.3	37.2 ± 1.3	29.3 ± 1.1	35.6 ± 1.1	22.8 ± 1.2
Cd1	09.9 ± 0.7	11.5 ± 0.8	10.0 ± 0.6	10.0 ± 0.5	09.4 ± 0.8	09.3 ± 0.8	09.9 ± 0.7	10.6 ± 0.8
Cd2	12.8 ± 1.0	15.9 ± 1.3	11.7 ± 0.8	13.6 ± 1.1	10.7 ± 1.0	12.7 ± 1.0	11.0 ± 0.8	10.6 ± 0.9
Cd3	09.1 ± 0.5	10.9 ± 0.7	09.6 ± 0.6	10.5 ± 0.8	08.4 ± 0.8	08.4 ± 0.6	09.7 ± 0.7	10.0 ± 0.7
Mean	10.6 ± 0.7	12.8 ± 0.9	10.4 ± 0.7	08.5 ± 0.8	09.5 ± 0.9	10.1 ± 0.6	10.2 ± 0.7	10.4 ± 0.8

Standard Error = SE

For Fc isolates; first experiment was carried out in 1991 and second experiment in 1992

For Fa isolates; first experiment was carried out in 1991 and second experiment in 1992

For Cd isolates; first experiment was carried out in 1990 and second experiment in 1991

GENERAL CONCLUSION

The work presented in this thesis was aimed at investigating the mechanisms of pathogenicity of three different fungi. *F. coeruleum, F. avenaceum* and *C. destructans* which causing dry rot in potato tubers to determine if they caused dry rot lesions by similar or different mechanisms.

All the isolates of *F. caeruleum* and *F. avenaceum* caused infection from an inoculum level of around 60 spores per tuber, but the isolates of *C. destructans* required an inoculum of about 600 spores or above for an infection to develop. Thus the isolates of the two species of *F. coeruleum* and *F. avenaceum* were more pathogenic than any of the isolates of *C. destructans*. However, for all three species the higher the number of spores inoculated the faster the lesions established and the faster the lesions expanded within the tuber. A summary of the relative pathogenicities of all isolates of the three fungi is given in Table 18.

In general, the isolates of *F. avenaceum* established growth faster than those of *F. coeruleum*, but surprisingly, *C. destructans* established infection much faster than either of them but its lesions then expanded at much lower rates. This was confirmed by studies of germination on tuber tissue slices when germination and early germ tube growth of isolates of *C. destructans* occurred faster than those of all isolates of *F. avenaceum* and *F. coeruleum*. On the other hand, just as in whole tubers, subsequent growth of *C. destructans* over the surface of the slice was very limited and lesions did not spread to coalesce nor did they penetrate readily into the inner tissues. Thus early growth may be supported by the nutrients released from the damaged cells at the surface of the slice or in the inoculum hole while resistance of the undamaged tissues beneath the wound may be due to the mechanical and chemical composition of the cell walls. In contrast, *F. coeruleum* and *F. avenaceum* produced dense mycelium over the tuber slice surface and penetrated deeply into the tissue to colonise the pith and cortex and so produce large dry rot lesions.

Chitin analysis showed that much larger amounts of glucosamine were produced by all three species over the external surfaces of the slice than in the internal tissues indicating that all grew much better on damaged tissue than in intact tissue. All isolates of *F. avenaceum* and *F. coeruleum* clearly grew much better than *C. destructans* both on and in tuber tissue. Growth within the tissues by all three species was restricted

much more than on a wound surface. This restriction is unlikely to be related to a shortage of nutrients since all three fungi were necrotrophs and all cell contents should be available to the fungi within the inner tissues once the protoplasts were killed. Thus resistance within intact tissue is probably due to the presence of intact cell walls. However, resistance in the intact tissues to *F. coeruleum* and *F. avenaceum* was gradually lost during storage since lesions spread faster in tubers later in the storage season than early in the storage season. Boyd (1952b) and McKee (1952, 1954) have also reported that resistance to infection decreases as the storage season advanced. In contrast, the level of resistance to *C. destructans* did not change during storage and so the resistance factors which restricted this fungus must be different from those which restrict *F. coeruleum* and *F. avenaceum.*

The electron microscopic studies of tuber tissues colonised by *F. coeruleum* and *F. avenaceum* showed that the middle lamellar region of the cell wall was relatively resistant to degradation compared to the primary wall and remained intact for a considerable time after the tissue was infected. The resistance of the middle lamellar region to degradation explains the dry rot nature of the lesions caused by these fungi and this resistance may be a factor in the resistance of intact tuber tissue to the spread of the two dry rot causing fungi since hyphae must penetrate it in order to advance through the tissue. In contrast to the middle lamella the primary cell walls were considerable degraded, particularly when colonised by *F. coeruleum.* The structure of the primary cell walls of potato tuber tissue is fairly complex, consisting of hemicelluloses and cellulose embedded in a pectin matrix. This matrix also includes some proteinaceous and phenolic compounds. The primary wall is laid down on each side of a layer of pectin substances which are deposited on the cell plate. The cell plate is the structure which forms between adjacent cells at the time of cell division and together with the pectin which is incorporated onto the cell plate at this time forms the middle lamella. The middle lamellar forms the as a cementing component between the primary walls of adjacent cells. The pectin also merges into the primary cell wall on each side of the middle lamella (Burton, 1989). Thus the response of the cell wall to cell wall degrading enzymes and particularly pectic enzymes may be a key to understanding the resistance of the middle lamella to degradation.

All three species, *F. coeruleum, F. avenaceum* and *C. destructans* produced both pectin and pectate degrading enzymes during the early stages of growth on

autoclaved extracts of tuber tissue and living tuber tissue. Enzymes reached maximum activities around 6-12 days after inoculation in both dead and living tissues.

All three fungi produced higher activities of pectin lyases and hydrolases than pectate lyases and hydrolases, and lyase activities were greater than hydrolase activities. Both *endo* and *exo*-pectic lyases and hydrolases were also produced. The production of pectic enzymes by *F. coeruleum* was strongly inducible by substrate and inhibited by simple sugars such as glucose or sucrose. Although the pectic enzymes produced in synthetic media containing pectic substrates and in potato broth medium did not macerate potato tuber tissue those produced in dead (autoclaved) and living slices did thus the middle lamella is not resistant to degradation by some enzymes produced by these fungi growing in living tissue. Thus the failure of the middle lamella to be degraded in infected tissue suggests that it is modified in some ways during infection. Workers have reported that cell wall macerating enzymes can be inactivated by tissue phenolics (Cole & Wood, 1961; Deverall & Wood, 1961; Patil & Dimond, 1967; and Mullen & Bateman, 1971; Mullen & Bateman, 1975) and it may be that the electron density of the middle lamella in infected tissue is due to the accumulation of inhibitory phenolic materials within the structure.

All three species of *F. coeruleum*, *F. avenaceum* and *C. destructans* produced toxic substances in autoclaved tuber tissue medium and in living tuber tissue. The activity of the toxic substances produced by *F. avenaceum* and *C. destructans* in living tuber tissue or in autoclaved tuber tissue medium was only slightly reduced by boiling but the activity of that produced by *F. coeruleum* in living tuber tissue was largely destroyed. Clearly the toxins produced in living tuber tissue by *F. avenaceum* and *C. destructans* are different from those produced by *F. coeruleum.* The toxic fractions produced by all three species were non dialysable through membranes with a cut of point of around 12,000 molecular weight or greater indicating that they are relatively large molecules.

Diluted tissue extracts of *F. coeruleum* still produced rapid maceration at levels where toxic activity was negligible indicating that cell death was not due to macerating enzyme activity. In contrast, dilutions of extracts of *F. avenaceum* showed similar losses of macerating activity and toxic activity but since toxic activity was relatively unaffected by boiling, whereas macerating activity was totally destroyed the evidence again suggests that cell death is not due to macerating enzyme activity.

No toxins were produced by *F. coeruleum* growing in Czapek-Dox medium

Table 19. Summary of pectic enzyme activity, cell wall macerating activity and toxic activity produced by *F. coeruleum*, *F. avenaceum* and *C. destructans* in different culture media and in living potato tissue.

	Pectin and pectate enzyme activity				Maceration		Toxicity			
	Pactin lyase	Pectin hydrolase	Pectate lyase	Pectate hydrolase	Tissue maceration		Unboiled extracts		Boiled extracts	
F. coeruleum	pH 9	pH 9	pH 9	pH 9	pH 5	pH 9	pH 5	pH 9	pH 5	pH 9
Culture media										
Czapek-Dox + Sucrose	+	+	+	+	–	–	–	–	–	–
Pringle's-medium + sucrose	+	+	+	+	–	–	–	–	–	–
Czapek-Dox + Pectin	++++	++++	+++	++	–	–	–	–	–	–
Czapek-Dox + Pectate	++++	++	++++	+	–	–	–	–	–	–
Baker medium + glucose	NT	NT	NT	NT	NT	NT	++++	NT	+++	NT
Potato broth	+++	++	++	+	–	–	–	–	–	–
Culture medium										
Autoclaved tuber tissue	+++++	++++	+++	+	+++++	+++++	++++	++++	++	++++
Living tissue										
Tuber slices	+++++	+++	+++	++	+++++	+++++	++++	++++	–	–
Whole tubers	++++	+++	+++	+++	+++++	+++++	++++	++++	–	–

Table 19. Continued

F. avenaceum										
Culture medium										
Autoclaved tuber tissue	+++	++	++	++	NT	+++++	NT	++++	NT	++++
Living tissue										
Whole tubers	++++	+++	+++	+++	NT	+++++	NT	++++	NT	++++
C. destructant										
Culture medium										
Autoclaved tuber tissue	+++	++	++	+	NT	+++++	NT	++++	NT	++++
Living tissues										
Whole tubers	+++	++	++	++	NT	+++++	NT	++++	NT	++++

Pectic enzyme activity graded on a scale from, + = negligible activity to +++++ = high activity
Maceration and toxicity graded on a scale from, – = no activity to +++++ = high activity. NT = not tested

with sucrose, pectin or sodium polypectate as the sole carbon source and neither when grown on potato broth medium. The toxic fractions produced by *F. coeruleum* in synthetic Baker's medium had similar R_f values when run on silica TLC. to those produced in autoclaved tuber tissue medium and this suggests that the same or very similar compounds were produced in each medium. A summary of the cell wall macerating, pectic enzyme and toxic activities produced by all three fungi when grown in different media and potato tissue is given in Table 19.

In conclusion, the results of this study suggest that, the abilities of isolates of *F. coeruleum, F. avenaceun* and *C. destructans* to cause dry rot in potato tuber tissue is due to a complex interaction of various factors including the ability to produce multiple forms of different types of cell wall degrading enzymes and toxins, but that the mechanisms of pathogenicity of each fungus are not the same.

REFERENCES

REFERENCES

Adams, M. J. and Lapwood, D. H. (1983). Transmission of *Fusarium solani* var. *coeruleum* and *F. sulphureum* from seed potatoes to progeny tubers in the field. *Annals of Applied Biology 103*, 411-417.

Albersheim, P., Jones, T. M. and English, P. D. (1969). Biochemistry of the cell wall in relation to infection processes. *Annual Review of Phytopathology 7*, 171-194.

Allen, E. H. (1970). The nature of antifungal substances in the peel of Irish potato tubers. *Phytopathologische Zeistschrift 69*, 151-159.

Allen, E. H. and Kuc, J. (1968). α-solanine and α-chaconine as fungitoxic compounds in extracts of Irish potato tubers. *Phytopathology 58*, 776-781.

Appel, O. and Fuchs, J. (1912). Zur Kenntnis der Fusariumfäule der Kartoffeln. *Mitteilungen biologische Anst.* (*Reichsanst*), Berl., 14, 16.

Archer, S. A., Byrde, R. J. and Fielding, A. H. (1972). Some effects on higher plant tissues of pectolytic enzymes secreted by *Sclerotinia fructigena*. *Journal of General Microbiology 73*, XVIII.

Arneson, P. A. and Durbin, R. D. (1968). Studies on the mode of action of tomatine as a fungitoxic agent. *Plant Physiology 43*, 683-686.

Ayers, W. A., Papavizas, G. C. and Diem, A. F. (1966). Polygalacturonate trans eliminase and polygalacturonase production by *Rhizoctonia solani*. *Phytopathology 56*, 1006-1011.

Baker, R. A., Tatum, J. H. and Nemec, S. (1981). Toxin production by *Fusarium solani* from fibrous roots of blight-diseased citrus. *Phytopathology 71*, 951-954.

Bamburg, J. R., and Strong, F. M. (1971). 12,13-epoxytrichothecenes. In Microbial Toxins vol Vll, Algal and Fungal Toxins S. Kadis, A. Ciegler, and S. J. Ajl, eds, pp. 207-292. Academic Press, New York.

Barron, G. L. (1972). The genera of Hyphomycetes from Soil. Robert E. Krieger Publshing Company.

Basham, H. G. and Bateman, D. F. (1975a). Killing of plant cells by pectic enzymes: the lack of direct injurious interaction between pectic enzymes or their soluble reaction products and plant cells. *Phytopathology 65*, 141-153.

Basham, H. G. and Bateman, D. F. (1975b). Relationship of cell death in plant tissue treated with a homogeneous endopectate lyase to cell wall degradation. *Physiological Plant Pathology 5*, 249-262.

Bateman, D. F. (1963a). Pectolytic activities of culture filtrates of *Rhizoctonia solani* and extracts of *Rhizoctonia*-infected tissues of bean. *Phytopatology 53*, 197-204.

Bateman, D. F. (1963b). The "macerating enzyme" of *Rhizoctonia solani*. *Phytopathology 53*, 1178-1186.

Bateman, D. F. (1966). A hydrolytic and trans-eliminative degradation of pectic substances by extracellular enzymes of *Fusarium solani* f. sp. *phasioli*. *Phytopathology 56*, 238-244.

Bateman, D. F. and Basham, H. G. (1976). Degradation of plant cell walls and membranes by microbial enzymes. In Encyclopaedia of plant physiology *New series, Physiological Plant Pathology*, ed. R. Heitefuss, P. H. Williams, 4, 16-55. New York: Springer-Verlag.

Bateman, D. F. and Millar R. L. (1966). Pectic enzymes in tissue degradation. *Annual Review of Phytopathology 4*, 119-149.

Bateman, D. F., Van Etten, H. D., English, P. D., Nevins, D. J. and Albesheim, P. (1969). Susceptibility to enzymatic degradation of cell walls from bean plants resistant and susceptible to *Rhizoctonia solani* Khuhn. *Plant Physiology 44*, 641-648.

Behnke, M. (1979). Selection of potato callus for resistance to culture filtrates of *Phytophthora infestans* and regeneration of resistance plants *Theoretical and Applied Genetics 55*, 69-71.

Booth, C. (1971). The genus *Fusarium*. Commonwealth Institute, Kew, Surrey, England, p. 237.

Bossi, R. (1959). Über die wirkug der Fusarinsäure auf die polyphenoloxydase. *Phytopathologische Zeistschrift 37*, 273-316.

Boyd, A. E. W. (1952a). Dry rot disease of the potato. IV. Laboratory methods used in assessing variations in tuber susceptibility. *Annals of Applied Biology 39*, 322-329.

Boyd, A. E. W. (1952b). Dry rot disease of the potato. V. Seasonal and local variations in tuber susceptibility. *Annals of Applied Biology 39*, 330-338.

Boyd, A. E. W. (1952c). Dry rot disease of the potato. VI. Varietal differences in tuber susceptibility obtained by injection and riddle-abrasion methods. *Annals of Applied Biology 39*, 339-350.

Boyd, A. E. W. (1952d). Dry-rot disease of the potato. VII. The effect of storages temperature upon subsequent susceptibility of tubers. *Annals of Applied Biology 39*, 351-357.

Boyd, A. E. W. (1967). The effects of length of the growth period and nutrition upon potato-tuber susceptibility to dry-rot (*Fuasrium coeruleum*). *Annals of Applied Biology 60*, 231-240.

Boyd, A. E. W. and Logan, J. W. H. (1967). The effect of planting seed tubers affected by dry rot (*Fusarium coeruleum*) and gangrene (*Phoma foveata*). The Edinburgh School of agriculture, Experimental Work, p. 25-27.

Boyd, A. E. W. and O'Donnell, B. H. (1968). The effect of planting seed tubers affected by dry rot (*Fusarium coeruleum*) and gangrene (*Phoma foveata*). The Edinburgh School of agriculture, Experimental Work, p. 37-38.

Boyd, A. E. W. and Tickle, J. H. (1972). Dry rot of potato tubers caused by *Fusarium sulphureum* Schlecht. *Plant Pathology 21*, p. 195.

Brian, P. W., Dawkins, A. W., Grove, J. F., Hemming, H. G., Lowe, D. and Norris, G. L. F. (1961). Phytotoxic compounds produced by *Fusarium equiseti*. *Journal of Experimental Botany 12*, No. 34, p. 1-12.

Brown, W. (1915). Studies in the physiology of parasitism. I. The action of *Botrytis cinerea*. *Annals of Botany 29*, 313-348.

Burton, W. G. (1989). The Potato. Third edition, Longman Scientific & Technical. Copublished in the United States with John Wiley & Sons, Inc., New York

Bush, D. A. and Codner, R. C. (1968). The nature of the macerating factor of *Penicillium digitatum* Saccardo. *Phytochemistry 7*, 863-869.

Bush, D. A. and Codner, R. C. (1970). Comparison of the properties of the pectin *trans*-eliminase of *Penicillium digitatum* and *Penicillium italicum*. *Phytochemistry 9*, 87-97.

Butler, E. J. & Jones, S. G. (1955). Plant Pathology. Macmillan & Co Ltd. London. p. 532-535.

Chatterjee, A. K. and Vidaver, A. K. (1986). Genetics of pathogenicity factors: application to phytopathogenic bacteria. *Advenced Plant Pathology 4*, 1-218.

Chesson, A. (1980). Maceration in relation to the post-harvest and processing of plant material. *Journal of Applied Bacteriology 48*, 1-45.

Chesson, A. and Codner, R. C. (1978). Maceration of vegetable tissue by a strain of *Bacillus subtilis*. *Journal of Applied Bacteriology 44*, 347-364.

Clarston, P. S. (1973). Methionine sulfoximine resistant mutants of tobacco. *Science 180*, 1366-1368.

Cole, M. & Wood, R. K. S. (1961). Pectic enzymes and phenolic substances in apples rotted by fungi, *Annals of Botany 25*, 435-452.

Cole, M. (1958). Oxidation products of leuco-anthocyanins as inhibitions of fungal polygacturonase in rotting apple fruit. *Nature, London 181*, 1596-1597.

Cooper, R. M. (1983). The mechanisms and significance of enzymic degradation of host cell walls by parasites. In: Biochemical Plant Pathology. Ed. by J. A. Callow, pp 101-135. New York: John Wiley & Sons Ltd.

Cooper, R. M. (1984). The role of cell wall-degrading enzymes in infection and damage. In Plant Diseases: Infection, Damage and Loss, ed R. K. S. Wood, G. J. Jelis, Oxford: Blackwell.

Cooper, R. M. and Wood, R. K. S. (1975). Regulation of synthesis of cell wall degrading enzymes by *Verticillium albo-atrum* and *Fusarium oxysporum* f. sp. *lycopersici*. *Physiological Plant Pathology 5*, 135-156.

Cooper, R. M. Wardman, P. A. and Skelton, J. E. M. (1981). The influence of cell walls from host and non-host plants on the production and activity of polygalacturonide-degrading enzymes from fungal pathogens. *Physiologica Plant Pathology 18*, 239-255.

Cooper, R. M., Rankin, B. and Wood, R. K. S. (1978). Cell wall-degrading enzymes of vascular wilt fungi. II. Properties and modes of action of polysaccharidases of *Verticellium albo-atrum* and *Fusarium oxysporum* f. sp. *lycopersici*. *Physiological Plant Pathology 31*, 101-134.

Corsini, D., Pavec, J. and Fenwick, G. (1977). Characterization of fusarium dry rot resistance in potato breeding clones (Abstr.) Proc. *American Phytopathological Society 4*, 203.

Daly, J. M. and Deverall, B. J. (1983). Toxins and plant pathogenesis. Academic Press, Sydney, New York, London.

Darvill, A. G., McNeeil, M., Albersheim, P. and Delmer, D. P. (1980). The primary walls of flowering plants; in the Biochemistry of Plants, vol 1 Ed. N. E. Tolbert, Academic Press, New York, p. 91-162.

Daub, M. E. (1986). Tissue culture and the selection of the resistance to pathogens. *Annal Review of Phytopathology 24*, 159-186.

Davis, D (1969). Fusaric acid in selective pathogenecity of *Fusarium oxysporum*. *Phytopathology 59*, 1391-1395.

Davis, D. (1967). Cross-protection in *Fusarium* wilt disease. *Phytopathology 57*, 311-314.

DeBary, A. (1886). Ueber einige Sclerotinien und Sclerotienkrankheiten. *Botanisches Zeitung 44*, 377-474.

Desjardins, A. E. and Plattner, R. D. (1989). Trichothecene toxin production by strains of *Gibberella pulicaris* (*Fusarium sambucinum*) in liquid culture and inpotatotubers. *Journal Agricultural and Food Chemistry 37*, 388-392.

Deverall, B. J. and Wood, R. K. S. (1961). Infection of bean plants (*Vicia faba* L.) with *Botrytis cinerea* and *B. fabae*. *Annals of Applied Biology 49*, 461-472.

Drysdale, R. B. and Langcake, P. (1973). Response of tomato to infection by *Fusarium oxysporum* f. sp. *lycopersici*. In: Fungal Pathogenicity and The Plant's Response (R. J. W. Byrde and C. V. Cuting, eds.), pp. 423-436. Academic press, Lodon and New York.

Durbin, R. D. (1981). Toxins in Plant Disease. Academic Press, New York.

Earle, E. D. (1982). Application of plant tissue culture in the study of host-pathogen interaction. In: Application of Plant Cell and Tissue Culture; to Agriculture and Industry. Ed. by D. T. Tomes, B. E. Ellis, P.M. Harney, K. J. Kasha, and R. L. Peterson. Plant Cell Culture Centre, University of Guelph, Published by the University of Guelph. Guelph. Ontario. Canada.

Ebel, J. (1986). Phytoalexin synthesis: The biochemical analysis of the induction process. *Annals Review of Phytopathology 24*, 235-264.

El-Banna, A. A., Scott, P. M., Lau, P., Sakuma, T., Platt, H. W. and Campbell, V. (1984). Formation of thrichothecenes by *Fusarium solani* var. *coeruleum* and *Fusarium sambucinum* in potatoes. *Applied and Environmental Microbiology 47*, 1169-1171.

Farkas, G. L. and Kiraly, Z. (1962). Role of phenolic compounds in the physiology of plant diseases and disease resistance. *Phytopathologische Zeistschrift 44*, 8-150.

Foister, C. E. (1940). Dry rot diseases of potatoes. *Scotish Journal of Agriculture 23,* 63-67.

Fontaine, T. D., Irving, G. W., MA., JR., R. MA., Poole, J. B. Doolittle, S. P. (1948). Isolation and partial characterisation of cristalline tomatine, an antibiotic agent from the tomato plant. *Archeves of Biochemistry 18*, 467-475.

Friend, J. (1973). Resistance of potato to *Phytophthora.* In Fungal Pathogenicity and the Plant's Response R.J.W. Byrde and C.V. cuttings, Eds. Academic Press, London and New York.

Friend, J. (1976). Lignifacation in infected tissue. In Biochmical Aspects of plant Parasite Relationships Friend and D.R. Threlfall, Eds. Academic Press, London and New York.

Friend, J. (1985). Phenolic substances and plant disease. *Annual Proceedings of the Phytochemical Society 25*, 367-392.

Friend, J. and Knee, M. (1969). Cell-wall changes in potato tuber tissue infected with *Phytophthora infestans* (Mont.) De Bary. *Journal of Experimental Botany 20,* 763-775.

Friend, J., Reynolds, S. B. and Aveyard, M. A. (1971). Phenolic metabolism in potato tuber tissue after infection with *Phytophthora infestans. Biochemical Journal 124,* 29 p.

Fushtey, S. G. (1957). Studies in the physiology of parasitism. XXIV. Further experiments on the killing of plant cells by fungal and bacterial extracts. *Annals of Botany 21*, 273-286.

Gäumann, e. (1950). Principles of Plant Infection. London, Crosby Lockwood & Son, Ltd. English translation edit by Brierley, W. B.

Gäumann, E. (1957). Fusaric acid as a wilt toxin. *Phytopathology 47*, 342-357.

Gäumann, E. (1958). The mechanism of fusaric acid injury. *Phytopathology 48*, 670-686.

Garett, S. D. (1960). Inoculum potential. In: Plant Pathology, J. G. Horsfall and A. E. Dimond, ed., Vol. III. Academic Press, New York.

Garibaldi, A. and Bateman, D. F. (1971) Pectic enzymes produced by *Erwinia chrysanthemi* and their effects on plant tissue. *Physiological Plant Pathology 1*, 25-40.

Gilchrist, D. G. and Grogan, R. G. (1976). Production and nature of a host-specific toxin from *Alternaria alternaria* f. sp. *lycopersici*. *Phytopathology 66*, 165-171.

Goodman, R. N., Kiraly, Z. and Wood, K. R. (1986). The Biochemistry and Physiology of Plant Disease. University of Missouri Press, Colombia.

Graniti, A., Durbin, R. D. and Ballio, A. (1989). Phytotoxins and Plant Pathogenesis. Berlin, Springer.

Greer, J. E. (1956). A study of potato tuber rots occurring in Northern Ireland with special reference to those caused by species of *Fusarium* and *Cylindrocarpon*. Ph. D. Thesis, Faculty of Agriculture Queen's University of Belfast.

Hagar, S. S. and McIntyre, G A. (1972). Pectic enzymes produced by *Pseudomonas fluorescence*, an organism associated with 'pink-eye' disease of potato tubers. *Canadian Journal of Botany 50*, 2479-2488.

Hall, J. A. and Wood, R. K. S. (1970). Plant cells killed by soft-rot parasites. *Nature 227*, 1266-1267.

Hall, J. A. and Wood, R. K. S. (1973). The killing of plant cells by pectolytic enzymes. In: Fungal Pathogenicity and the Plant's Response. R. J. W. Byrde and C. V. Cutting Eds. Academic Press. London p 19-38.

Hancock, J. G. (1968). Degradation of pectic substances during pathogenesis by *Fusarium solani* f. sp. *cucurbitae*. *Phytopathology 58*, 62-69.

Hancock, J. G. and Millar, R. L. (1965). Association of cellulolytic, proteolytic, and xylolitic enzymes with Southern anthracnose, spring black stem, and *Stemphylium* leaf spot of alfalfa. *Phytopathology 55*, 356-360.

Hancock, J. G., (1966). Pectic lyase production by *Colletotrichum trifolii* in relation to change in pH. *Phytopathology 50*, 1112-113.

Hide, G. A. and Cayley, G. R. (1980). Tests of fungicides for controlling gangrene (*Phoma exigua var. foveata*) and dry rot (*Fusarium solani var coeruleum* and *F. sulphureum*) on potatoes during storage, *Potato Research 23*, 395-403.

Hornok, L. (1982). Dry rot of potato tubers caused by *Fusarium trichothecioides* Wollenweber, a fungus newly recorded in Hungary. *Acta Phytopathologica Academae Scientiarrum Hungar icea 17*, 81-83.

Horton, J. C. and Keen, N. T. (1966). Sugar repression of endopolygalacturonase and cellulase synthesis during pathogenesis by *Pyrenochaeta terrestris* as a resistance mechanism in onion pink root. *Phytopathology 56*, 908-916.

Ishizaka, N. and Tomiyama, K. (1972). Effect of wounding or infection by *Phytophthora infestans* on the contents of terpenoids in potato tubers. *Plant and Cell Physiology 13*, 1053-1063.

Jarvis, M. C., Hall, M. A., Threlfall, D. R. and Friend, J. (1981a). The polysaccharide structure of potato cell walls: Chemical fraction. *Planta 152*, 93-100.

Jarvis, M. C., Threlfall, D. R. and Friend, J. (1981b). Potato cell wall polysaccharides: Degradation with enzymes from *Phytophthora infestants. Journal of Experimental Botany 32*, 1309-1319.

Jellis, G. J. (1975). Screening potato clones for resistance to dry rot (*Fusarium solani* var. *coeruleum*). *Annals of Applied Biology 81*, 417-418.

Jellis, G. J. and Boulton, R. E. (1984). Damage and loss caused by potato diseases. In: Plant Diseases: Infection, Damage and Loss. Ed by Wood, R. K. S. and Jellis, G. J. Blackwell Scientific Publications, Oxford.

Jellis, G. J. and Starling, N. C. (1983). Resistance to powdery dry rot (*Fusarium sulphureum)* in potato tubers. *Potato Research 26*, 295-301.

Jellis, G. J., Gunn, R. E. and Boulton, R. E. (1984). Variation in the disease resistance among potato somaclones. In Abstr. conf. Pap. Triennal Conf. EAPR, Interlaken, ed. F. A. Winiger, A. Stöckli. pp. 380-381. Wagningen:EAPR.

Johansson, L. (1986). Improved methods for induction of embryogenesis in anther cultures of *Solamum tuberosum. Potato Research 29*, 179-190.

Johnson, L. J. and Curl, E. A. (1972). Methods for Research on the Ecology of Soil-Borne Plant Pathogens. Burgess Publishing Company, pp 247.

Jones, L. R. (1909). The bacterial soft rots of certain vegetables. II. Pectinase, the cytolitic enzyme produced by *Bacillus carotovora* and certain other soft-rot organisms. *Vermont University Agricultural Sta. Tech. Bull. 147*, 281-360.

Kamoun, P. (1977). Appareils et Methodes en Biochimie. Flammarion Médecine Sciences, Paris.

Kebabti, A. (1986). Etude de la mycoflore responsable de la pouriture des tubercules de pomme de terre *Solamun tuberosum* en stockage. M.Sc. thesis, Oran University. Algeria.

Kenning, L. A. and Hanchey, P. (1980). Ultrastructure of lesion formation in *Rhizoctonia*-infected bean hypocotyl. *Phytopathology 70*, 997-1004.

Kern, H, (1972). Phytotoxins produced by fusaria. In Wood R. K. S., Ballio A., Graniti A., Phytotoxins in Plant Diseases, pp. 530. Academic Press, London, New York.

Kern, H. and Neaf-Roth, S. (1965). Zur Bildung phytotoxischer Farbstoffe durbstoffe durch Fusarien der gruppe Martiella. *Phytopathologische Zeistschrift 53*, 45-64.

Kern, H. and Neaf-Roth, S. (1967). Zwei neue, duech *Martiella* Fusarien Naphthazarin-Derivate. *Phytopathologische Zeistschrift 60*, 316-324.

Kern, H. and Neaf-Roth, S., and Item, H. (1970). Parasitogene Naphthazarin-Derivate als Hemmstoffe der decarboxylierung von α-ketoarbonsäuren. *Phytopathologische Zeistschrift 67*, 1-14.

Kern, H., (1978). Les naphthazarines des *Fusarium*. *Annal of Phytopathology 10*, 327-345.

Kern, H., Naef-Roth, S. and Ruffner, F., (1972). Der Einfluss der Ernährung auf die bildung von Naphthazarin-Derivaten durch *Fusarium martii* var. *pisi*. *Phytopathologische Zeistschrift 74*, 272-280.

Knee, M. and Friend, J. (1970). Some properties of the galactanase secreted by *Phytophthora infestans* (Mont.) DeBary. *Journal of General Microbiology 60*, 23-30.

Knoche, H. W. and Duvick, J. P. (1987). The role of fungal toxins in plant disease. In: Fungal infection of plants Ed. G. F. Pegg and P. G. Ayres, pp. 158-192. Cambridge University Press.

Kosuge, T. (1969). The role of phenolics in host response to infection. *Annual Review of Phytopathology 7*, 195-222.

Kuc, J. (1966). Resistance of plants to infection agents. *Annual Review of Microbiology 20*, 337-370.

Kuc, J. (1973). Metabolites accumulating in potato tubers following infection and stress. *Teratology 8*, 333-338.

Kuc, J. (1982). Phytoalexins from the Solanacae. In: Phytoalexin eds by J. A. Bailey and J. W. Mansfield. Blackie. Glasgow and Lodon.

Lafon, P. Girard, T., Payen, J., Sarfati, J. and Gaillardin, M. (1983). Contamination de pomme de terre de consommation par des Fusariotrichothecenes. *Microbial. Aliment. Nutrition 1*, 147-152.

Langcake, P., Bratt, P. M. and Drysdale, R. B. (1973). Pectin methyl esterase in Fusarium infected susceptible tomato plants. *Physiological Plant Pathology 3*, 101-106.

Langerfeld, E. (1983). Verhalten von Kartofflsorten gegenüber drei Trockenfäuleerrgern. *Potato Research 26*, 403-404.

Lansade, M. (1950). Recherches sur la Fusariose ou pourriture séche de la pomme de terre. *Annales Institut national de la Recherche agro*nomique, *Paris, (Ann. Epiphyt.)*, I, 157.

Lapwood, D. H. and Hide, G. A. (1971). Potato. In: Diseases of Crop Plants. Ed by Western, J. H. The Macmillan Press Ltd., p 89.

Larkin, P. J., Scowcroft, W. R. (1981). Sonaclonal variation-a novel source of variability from cell cultures for plant improvement. *Theoretical* and. *Applied. genetics 60*, 197-114.

Latus, D. Perkowski, J. and Chelkowski, J. (1987). Pathogenecity of nine *Fusarium* species towards tubers of Polish potato cultivars. *Fusarim*-Mycotoxins, Taxonomy, Pathogenecity. *Agricultural University of Warsaw: Warsaw*, Poland, *Abstract.*

Leach, S. S. (1975). Control of post harvest *Fusarium* tuber dry rot of white potatoes. P. 1-17 in: ARS-NE-55. U.S. Dep. Agric., Washington, D. C.

Leach, S. S. and Nielsen, L. W. (1975). Elimination of fusarial contamination on seed potatoes. *American. Potato Journal 53*, 364.

Leach, S. S. and Webb, R. E. (1981). Resistance of selected potato cultivars and clones to *Fusarium* dry rot. *Phytopathology 71*, 623-629.

Livingston, R. S. and Scheffer, R. P. (1981). Isolation and characterisation of host-selective toxin from *Helminthosporium sacchari. The Journal of Biological Chemistry 256,* 1705-1710.

Longman, S. (1909). The dry rot of potatoes. *Journal of Linnean Society. XXXIX. Bot. IX. 270.* p. 120.

Lounsbury, C. P. (1909). Dry rot of the potato. *Agriculture. Journal. Cape of Good Hope,* XXXV. p. 42.

Lund, B. M. (1973). The effect of certain bacteria on ethylene production by plant tissue. In: Fungal Pathogenicity and the Plant's Response. Ed by R. J. W. Byrde and C. V. Cutting Academic Press, New York, pp. 69-86.

Lyon, G. D. (1989). The biochemical basis of resistance to potato soft rot Erwinia spp. a review. *Plant Pathology 38*, 313-339.

Lyon, G. D. and Mayo, M. A (1978). The phytoalexin rishitin affects the viability of isolated protoplasts. *Phytopathologische Zeitschrift 92*, 298-304.

Maheshwari. S. C., Tyagi, A. K., Malhotra, K., and Sopory, S. K.(1980). Induction of haploidy from pollen grains in angiosperms. the current status. *Theoretical Applied Genetics 58*, 193-206.

Malmberg A. G. and Theander, O. (1980) Two phytoalexin glycosides from potato tubers infected with *Phoma. Phytochemistry 19*, 1737-1742.

Mann, B. (1962). Role of pectic enzymes in the *Fusarium* wilt syndrome of tomato.*Transaction British mycological Society 45,* 169-178.

Manner, J. G. (1993). Principles of Plant Pathology. Second edition. Cambridge, University Press, 343 p.

Massee, G. (1904). Some diseases of the potato. *Gardeners' Chronicle*. p., 257.

McDonnell, K. (1962). Relationship of pectic enzymes and pathogenicity in the *Fusarium* wilt of tomatoes. *Transactions of the British Mycological Society 45*, 55-62.

McGuire, R. G. and Kelman, A. (1984). Reduced severity of *Erwinia* soft rot in potato tubers with increased calcium content. *Phytopathology 74*, 1250-1256.

McGuire, R. G. and Kelman, A. (1986). Calcium in potato tuber cell walls in relation to tissue maceration by *Erwinia carotovora* pv. *atroseptica*. *Phytopathology 76*, 401-406.

McKee, R. K. (1952). Dry rot disease of the potato II. Fungi causing dry rot of seed potatoes in Britain. *Annals of Applied Biology 39*, 38-43.

McKee, R. K. (1954). Dry rot disease of the potato. VIII. A study of the pathogenicity of *Fusarium coeruleum* (Lib.) Sacc. and *Fusarium avenaceum* (Fr.) Sacc. *Annals of Applied Boilogy 41*, 417-434.

McKee, R. K. (1955). Host-parasite relationships in the dry rot disease of potatoes. *Annals of Applied Boilogy 34*, 147-148.

McKee, R. K. (1959). Factors affecting the toxicity of solanine and related alkaloids to *Fusarium coerulem*. *Journal of General Microbiology 20*, 686-696.

McKee, R. K. (1964). Observations on infection by *Phytophthora infestans*. *Transactions of the British Mycological Society 47*, 365-374.

McKee, R. K. and Boyd, A. E. W. (1952). Dry rot disease of the potato III. A biological method of assessing soil infectivity. *Annal of Applied Biology 39*, 44-53.

Mclendon, J. H. (1964). Evidence for the pectic nature of the middle lamella of potato tuber cell walls based on chromatography of macerating enzymes. *American Journal of Botany 51*, 628-633.

McMillan, G. P., Hedley, D. Fyffe, L. and Pérombelon, M. C. M. (1993). Potato resistance to soft-rot erwinias is related to cell wall pectin esterification. *Physiological and Molecular Plant Pathology 42*, 279-289.

Misaghi, I. J. (1982). Physiology and Biochemistry of Plant pathogen Interactions. Plenum Press. New York and London.

Mooi, J. C. (1950). Het *Fusarium*-rot of droogrot bij aardappelen. *Landbouwk. Tijdschr., 's-Grav.*, 62, 712-724.

Moore, F. J. (1945). A comparison of *Fusarium avenaceum* and *Fusarium caeruleum* as causes of wastage in stored potato tubers. *Annals of Applied Biology 32*, 304-309.

Mount, M. S, Bateman, D. F. and Bashman, H. G. (1970). Induction of electrolyte loss, tissue maceration, and cellular death of potato tissue by an endopolygalacturonate trans-eliminase. *Phytopathology 60*, 924-931.

Mullen, J. M. and Bateman, D. F. (1971). Production of an endo- polygalacturonate trans- eliminase by a potato dry-rot pathogen, *Fusarium roseum 'Avenaceum'* in culture and in diseased tissue. *Physiological Plant Pathology 1*, 363-373.

Mullen, J. M. and Bateman, D. F. (1975a). Polysaccharide degrading enzymes produced by *Fusarium roseum 'Avenaceum'* in culture and during pathogenesis. *Physiological Plant Pathology 6*, 233-246.

Mullen, J. M. and Bateman, D. F. (1975b). Enzymatic degradation of potato cell walls in potato virus X-free and potato virus X-infected tubers by *Fusarium roseum Avenaceum' Phytopathology 65*, 797-802.

Müller, K. O. (1940). Experimemtelle untersuchungen under die *Phytophthora*-resistenz der Kartoflel. Arb. Boil Reichsanstat. Land-U Forstwirtsch. Berlin 23, 189-231.

Müller, K. O. (1957). Wound heling of the potato tuber in relation to infection by *Phytophthora infestans* (Mont.) De Bary. *Australian Journal of Biologicl Science 10*, 189.

Mussel, W. H. (1973). Endopolygalacturonase: Evidence for involvement in *Verticilluim* wilt of cotton. *Phytopathology 63*, 62-70.

Mussell, H. W. and Green, R. J. (1970). Host colonisation and polygalacturonase production by two tracheomycotic fungi. *Phytopathology 60*, 192-195.

Neaf-Roth, S. (1959). Über die parasitogrnen und toxigenen veränderungen der Atmungsintensität bei Tomaten. *Zeitschrift Pflkrankh. Pflpath. Pflschutz. 64*, 421-426.

Niizeki and, H. and Oono, K. (1968). Induction of haploid rice plant from anther culture. *Proceedings of the Japan Academy 44*, 554-557.

Nitsch, J. P. and Nitsch, C. (1969). Haploid Plants pollen grains. *Science 163*, 85-87.

Nutman, F. G. and Roberts, F. M. (1963). Studies on the biology of *Hemileia vastatrix* Berk. & BR. *Transactions of the British Mycology Society 46*, 27-48.

Olsson, K. (1988). Resistance to gangrene (*Phoma exigua var. foveata*) and dry rot (*Fusarium solani var. coeruleum*) in potato tubers. 1. The influence of pectin-bound magnesium and calcium. *Potato Research 31*, 413-422.

Ouyang, T. W., Hu, H., Chuang, C. C. and Tseng, C.C. (1973). Induction of pollen plants from anthers of *Triticum aestivum* L cultured in vitro. *Scientia Sinica 16*, 79-95.

Page, O. T. (1959). Fusaric acid in banana plants infected with *Fusarium oxysporum* f. sp *cubense*. *Phytopathology 49*, 230.

Pagel, W. and Heitefuss, R. (1989). Calcium content and cell wall polygalacturonans in potato tubers of cultivars with different susceptibilities to *Erwinia carotovora* subsp. *atroseptica*. *Physiological and Molecular Plant pathology 35*, 11-21.

Panopoulos, N. J. and Staskawicz, B. J. (1981). Genetic of production. In: Toxins in Plant Disease chapter 4, p. 79-107, Durbin R. D. Academic Press, New York, London.

Papavizas, G. C. and Ayers, W. A. (1966). Polygalacturonate trans-eliminase production by *Fusarium oxysporum* and *Fuarium solani*. *Phytopathology 56*, 1269-1273.

Patil, S. S. and Dimond, A. E. (1968) Repression of polygalacturonase synthesis in *Fusarium oxysporum* f. sp *lycopersici* by sugars and its effect on symptom reduction in infected tomato plants. *Phytopathology 58*, 676-682.

Paxton, J (1980). A new working definition of the term 'phytoalexin', *Plant Disease 64*, 734.

Petersen, L. J. (1959). Relation between inoculum density and infection of wheat by uredospores of *Puccinia graminis* var. *tritici*. *Phytopathology 49*, 607-614.

Pethybridge, G. H. and Bowers, E. H. (1908). Dry rot of potato tuber. *Economic Proceedings of the Royal Dublin Society*, 547.

Pethybridge, G. H. and Lafferty, H. A. (1917). Further observations on the cause of the common dry rot of the potato tuber in the British Isles. *Scientific Proceedings of the Royal Dublin. Society 21*, 193.

Pillet, P. E. (1973). Effect of wall degrading enzymes on protoplasts transminase activity. *Experientia, 29*, 1206-1207.

Price, K. R., Howard, B. and Coxon, D. T. (1976). Stress metabolite production in potato tubers infected by *Phytophthora infestans, Fusarium avenaceum* and *Phoma exigua*. *Physiological Plant Pathology 9*, 189-197.

Pringle, R. B. and Scheffer, R. P. (1963). Purification of the selective toxin of *Periconia circinata*. *Phytopathology 53*, 785.

Pringle, R. B. and Scheffer, R. P. (1967a). Isolation of the host-specific toxin and a related substance with nonspecific toxicity from *Helmenthosporium carbonum*. *Phytopathology 57*, 1169-1172.

Pringle, R. B. and Scheffer, R. P. (1967b). Multiple host-specific toxins from *Periconia circinata*. *Phytopathology 57*, 530-532.

Pringle, R. B. and Scheffer, R. P. (1967c). Purification of the selective toxin of *Periconia circinata*. *Phytopathology 53*, 785-787.

Quantick, P. Cervone, F. and Wood, R. K. S. (1983). Isoenzymes of a polygalacturonate trans-eliminase produced by *Erwinia atroceptica* in potato tissue and in liquid culture. *Physiological Plant Pathology 22*, 77-86.

Reinke, J., and Berthold, G. (1879). Die Zersetzung der Kartoffel durch Pilze. Berlin, p 39.

Rich, A. E. (1983). Potato Diseases. Academic Press New York and London.

Ride, J. P. and Drysdale, R. B. (1972). A rapid method for the chemical estimation of filamentous fungi in plant tissue. *Physiological Plant Pathology 2*, 7-15.

Ring, S. G. and Selvendran, R. R. (1981). An arabinogalactoxyloglucan from the cell wall of *Solamun tuberosum*, *Phytochemistry 20*, 2511-2519.

Salam, M. A. (1948). A study of the tuber diseases of the potato with special reference to dry rot (*Fusarium* spp.) gangrene (*Phoma foveata* foister) and a form of rot caused dry rot *Cylindrocarpon* sp. Thesis submited for Ph.D., Queen's University, Belfast.

Scheffer, R. P. and Ullstrup, A. J. (1965). A host-specific toxic metabolite from *Helminthosporium carbonum*. *Phytopathology 55*,1037-1038.

Schmidt, E. (1928). Schädigungen der Kartoffel durch Pilze der Gattung *Fusarium* (LK.). *Arbeiten biologischen Abt.* (*Anst. Reichsanst.*), *Berl., 15*, 537.

Selvendran, R. R. Stevens, B. J. H. and DuPont, M. S. (1987). Dietary fiber: chemistry, analysis and properties, *Advances in Food Research 31*, 117-209.

Seppänen, E. (1981a). Fusarium of the potato in Finland I. On the *Fusarium* species causing dry rot in potatoes. *Annales Agriculturae Fenniae 20*,156-160.

Seppänen, E. (1981b). Fusarium of the potato in Finland II. On the growth optima of Fusarium species in tubers of cv. Bintje. *Annales Agriculturae Fenniae 20*,161-176.

Seppänen, E. (1981c). Fusarium of the potato in Finland III. Varietal resistance of potato tubers to some Fusarium species. *Annales Agriculturae Fenniae 20*, 177-183.

Seppänen, E. (1982a). Fusarium of the potato in Finland IV. Variation of tuber resistance to storage diseases before and after harvasting. *Annales Agriculturae Fenniae 21*, 123-130.

Seppänen, E. (1982b). Fusarium of the potato in Finland V. Further investigation into the growth optima of *Fusarium* species in potato tubers. *Annales Agriculturae Fenniae 21*, 162-168.

Seppänen, E. (1983). Fusarium of the potato in Finland VIII. Occurrence of the pathogens causing potato dry rot and Gangrene. *Annales Agriculturae Fenniae 22*,115-119.

Sheiman, M. I., Macmillan, J. D., Miller, L. and Chase, T. (1976). Corrdinated action of pectinesterase and polygalacturonate lyase complex of *clostridium multifermentans*. *European Journal Biochemistry 64*, 565-572.?

Sherbankoff, C. D. (1915). Fusaria of potatoes. *Men. Cornell Agricultural Experiment Station No. 6.*

Simmons, R. P. (1973). Reaction of wounds and lenticels to invading fungal pathogens, *Proc. 5th trienn. Conf. Eur. Assn. Potato Res., Norwich, England, 1972*, p. 44.

Sinden, S. L., Goth, R.W. and O' Brien, M. J. (1973). Effect of potato alkaloids on the growth of *Alernaria solani* and their possible role as resistance factors in potatoes. *Phytopathology 63*, 303-307.

Singh, R. K. and Wood, R. K. S. (1956). Studies in the physiology of parasitism. XXI The production and properties of pectic enzymes secreted by *Fusarium moniliforme* Sheldon. (1956). *Annals of Botany, N. S. XX,. 77*,89-103.

Small, T. (1944a). Dry rot of potato (*Fusarium caeruleum* (Lib.) Sacc.). Investigation on the sources and time of infection. *Annals of Applied Biology 31*, 290.

Small. T. (1944b). The soil as source of infection of dry rot of potato. *Nature, 153*, 436.

Smith, W. L. Jr. and Smart, H. F. (1955). Relation of soft rot development to protective barriers in Irish potato slices. *Phytopathology 45*, 649-654.

Soliday, C. L., Flurkey, W. H., OKita, T. W. and Kolattukudy, P. E. (1984). Cloning and structure determination of cDNA for cutinase, an enzyme involved in fungal penetration of plants. *Proceedings of the National Academy of Science, USA 81*, 3939-3943.

Stahl, C., Vanderhoeff, L. N., Siegel, N. and Helgeson, J. P. (1973). *Fusarium tricinctum* T-s toxin inhibits auxin-promoted elongation in soybean hypocotyl. *Plant Physiology 52*, 663-666.

Steiner, G. W. and Byther, R. S. (1971). Partial characterisation and use of a host-specific toxin from *Helminthosporium sacchari* on sugarcane. *Phytopathology 61*, 691-695.

Stephens, G. J. and Wood. K. S. (1974). Killing of plant cells by *Erwinia carotovora* Ph.D. thesis. London University, London.

Steyn, P. S.. Vleggar, R., Robie, C. J., Kriek, N. P. J. and Harington, J. S.(1978). Trichothecene mycotoxins from *Fusarium Sulphureum. Biochemistry 17*, 949-951.

Stoessl, A. (1981). Structure and biogenetic relations: Fungal nonhost-specific toxins. Chapter 5. In: R. D. Durbin, in Toxins in Plant Disease. Academic Press, London New York.

Strand, L. L., Corden, M. E., and MacDonald, D. L. (1976). Characterisation of two endopolygalacturonase isozymes produced by *Fusarium oxysporum* f. sp. *lycopersici. Biochimica et Biophysica Acta 429*, 870- 883.

Strobel, G. A. and Steiner, G. W. (1972). Runner lesion formation in relation to helminthosporoside in sugarcane leaves infected by *Helminthosporium sachari*. *Physiologycal Plant Pathology 2*, 129-132.

Strobel, G. A. Hess, W. M. and Steiner, G. W. (1972). Ultrastructure of cells in toxin-treated and *Helminthosporium sacchari*-infected sugarcane leaves. *Phytopathology 62*, 339-345.

Sturdy, M. L. (1973). Studies on the infection of potato tubers by *Fusarium coeruleum* (Lib.) Sacc. and *Phytophthora erythroseptica* Pethyb. Ph.D. thesis, University Hull.

Talmadge, K. W., Keegstra, K., Bauer, W. D., and Albersheim, P., (1973). The structure of plant cell walls. *Plant Physiology 51*, 158-173.

Templeton, G. E. (1972). *Alternaria* toxins related to pathogenesis in plants In: Microbial toxins. Fungal toxins. S. Kadis, A. Ceigler, and S. J. Ajl (eds). Academic Press. New York, 8, 169192,

Theron, D. J. and Holz, G. (1989). *Fusarium* species associated with dry rot and stem-end rot of potatoes in South Africa. *Phytophylactica 21*, 175-181.

Theron, D. J. and Holz, G. (1990). Effect of temperature on dry rot development of potato tubers inoculated with different *Fusarium* spp. *Potato Research 33*, 109-117.

Thomas, E. and Wenzel, G. (1975). Embryogenesis from microspores of *Brassica napus*. *Zeitschrift für Planzenzüchtung 74*, 77-81.

Thornton, R. C., Jarvis, B. C. and Cook, R. C. (1991). A chitin assay for the enumeration of *Plasmodiophora brassica* resting spores in clubroot tissue. *Mycological Research 95*, 879-882.

Tivoli, B. and Jouan, B. (1981). Inventaire, fréquence et agressivité des différentes especes ou variétés de *Fusarium* responsables de la pourriture séche des tubercules de pomme de terre. *Agronomie 1*, 787-794.

Tivoli, B., Abdul Razzaq, K. Jouan, B. et Lemarchand, E. (1986). Etude comparée des capacités de fusarium responsables de la pourriture séche des tubercules de pome de terre. *Potato Research 29*, 13-32.

Tribe, H. T. (1955). Studies in the physiology of parasitism. XIX On the killing of plant cells by enzymes from *Botrytis cinerea* and *Bacterium aroideae*. *Annals of Botany, N. S XIX*, 351-372.

Tseng, T. C. and Mount, M. S. (1974). Toxicity of endo-polygalacturonate trans-eliminase, phosphatidase and protease to potato and cucumber tissue. *Phytopathology 64*, 229-236.

Turner, J. G. (1984) Role of toxins in plant disease. In: Plant diseases: infection damage and loss. Ed. Wood, R. K. S. and Jellis, G. J., Blacwell Scientific Publications, Oxford.

Ueno, Y. Sato, N. Ishii, k. Sakai, K. Stunoda, H. and Enomoto, M. (1973). Biological and chemical detection of trichothecene mycotoxins of *Fusarium* species. *Applied Microbiology 25,* 699-704.

Ueno, Y., Sawano, M., and Ishii, K., (1975). Production of trichothecene mycotoxin by *Fusarium* species in shake culture. *Applied Microbiology 30,* 4-9.

Van Etten, H. D. and Bateman, D. F. (1969). Enzymatic degradation of galactan, galactomannan, and xylan by *Sclerotium rolfsii*. *Phytopathology 59*, 968-972.

Ven der Plank, J. E. (1975). Principles of plant infection. Academic Press, New York, London.

Waites, M. J, Reynolds, S. B. and Friend, J. (1978). The metabolisim of chlorogenic acid in tuber discs of a resistant and a susceptible potato cultivar after inoculation with *Fusarium solani var. coeruleum. Biochemical Society Transactions 6*, 441-442.

Wastie, R. L. (1962). Mechanism of action of an infective dose of *Botrytis* spores on bean leaves. *Transactions of the British Mycology Society 45,* 473-473.

Wastie, R. L. and Bradshaw, J. E. (1993). Inheritance of resistance to *Fusarium* spp. in tuber progenies of potato. *Potato Research 36*, 189-193.

Wastie, R. L., Stewart, H. E. and Brown, J. (1989). Comparative susceptibility of some potato cultivars to dry rot caused by *Fusarium sulphureum* and *F. solani* var. *coeruleum. Potato Research 32*, 49-55.

Wellving, A. (1976) Studies on the resistance of potato to storage rots caused by *Phoma exigua* var. *foveata* and *Fusarium* spp. Ph.D. thesis, Lambrukshogskolan, Uppsala, Sweden.131 pp.

Wenzel, G. (1985). Strategies in unconventional breeding for disease resistance. *Annual. Review of Phytopathoogy 23*, 149-172.

Wollenweber, H. W. (1913). Studies on the fusarium problem. *Phytopathology.III.* p. 24.

Wollenweber, H. W. and Reinking, O. A. (1935). Die *Fusarien.* Berlin: Paul Parey.

Wood, R. K. S. (1967). *Physiological Plant Pathology* (Blackwell Scientific Publications Oxford, 570 pp.

Woolley, D. W. (1948). Studies on the structure of lycomarasmin. *Journal of Biological Chemistry 176,* 1291-1298.

Wu, L. C. and Scheffer, R. P. (1962). Some effects of *Fusarium* infection of tomato on growth, oxidation and phosphorylation. *Phytopathology 52*, 354-358.

Wyllie, D. and Morehouse, L. G. (1977). Mycotoxins fungi, Mycotoxins, Mycotoxicoses. An Encyclopaedic Handbook, Marcel Dekker. INC. Printed in the United States of America.

Zimmermann, W. and Seemüller, E. (1984). Degradation of raspbery suberin by *Fusarium solani* f. sp *pisi* and *Armilaria mellea*. *Phytopathologische Zeitschrift 110*, 192-199.

APPENDICES

APPENDICES

CULTURE MEDIA

All Agar media were autoclaved at 15 lbs for 15 minutes

a-Potato sucrose agar (PSA)

Potato extract	500 ml
Sucrose	20 g
Agar	20 g
Distilled water	500 ml

The potato extract was prepared according to (Booth, 1971). The potatoes (1800g) were peeled, diced and enclosed in muslin in 4500 ml boiling water for 30 min the potato was then discarded. The potato extract was then added to the appropriate amounts of water sucrose and agar heated slowly until the agar had dissolved. The pH was adjusted to 6.5 before autoclaving.

b-Potato dextrose agar (PDA)

Potato	200 g
Dextrose	15 g
Agar	20 g
Distilled water	1.0 litre

Potatoes were prepared as above, boiled for 1 hr and then the extract was filtered through cheese-cloth. The agar and dextrose were dissolved and the mixture was made up to 1 litre with distilled water. The pH was adjusted to 6.5 before autoclaving at 15 lbs in^{-2} for 15 minutes.

c-Carrot potato dextrose agar (CPDA)

Carrot	100 g
Potato	100 g
Dextrose	15 g
Agar	20 g
Distilled water	1.0 litre

The carrot potato dextrose agar were prepared in the same way as the potato dextrose agar. The pH was adjusted to 6.5 before autoclaved at 15 lbs in^{-2} for 15 minutes.

d-Oatmeal agar

Oatmeal (powdered)	15 g
Agar	20 g
Distilled water	1 litre

The Oatmeal was added gradually to the water and heated for 1 hr in a boiling water bath. After straining through cheese-cloth, the liquor was made up to 1 litre with water and then the agar added and dissolved before autoclaving.

e-Soil extract agar

Agar	15 g
K_2HPO_4	0.2 g
Soil extract	1 litre

Soil extract was prepared according to (Johnson and Curl, 1972) by autoclaving 1000 g soil with 1 litre of tap water for 20 min at 15 lbs in^{-2} pressure. The soil was filtered off and the extract made up to 1 litre. The medium was adjusted to pH 6.5 prior to autoclaving

f-Czapek-Dox agar

Sucrose	30 g
Agar	20 g
$NaNO_3$	2 g
KH_2PO_4	1 g
$MgSO_4\,7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4\,7H_2O$	0.01 g
$ZnSO_4\,7H_2O$	0.01 g
$CuSO_4\,5H_2O$	0.005 g
Distilled water	1 litre

Liquid media

In the preparation of all the liquid media the mineral salts and the carbon source were dissolved separately in distilled water. The pH of the mineral salts solution was adjusted to pH 6.5 with 0.1 M NaOH or 0.1 M HCl and then the solution was autoclaving in 15 ml portions in 100 ml flasks, The carbon source was autoclaved in 10 ml portions in test tubes and, after cooling, one portion of mineral salts solution was mixed aseptically with one portion of carbon source solution.

a-Czapek-Dox liquid medium

$NaNO_3$	2.0 g
KH_2PO_4	1.0 g
$MgSO_4\,7\,H_2O$	0.5 g
KCl	0.5 g
$FeSO_4\,7H_2O$	0.01 g
$ZnSO_4\,7H_2O$	0.01 g
$CuSO_4\,5H_2$	0.005 g
Carbon source: Sucrose	30.0 g
or: Pectin	10.0 g
or: Sodium polypectate	10.0 g
Distilled water	1.0 litre

b-Mineral salts liquid medium (Pringle & Scheffer, 1963)

Sucrose	30.0 g
Ammonium tartarate	5.0 g
NH_4NO_3	1.0 g
KH_2PO_4	1.0 g
$MgSO_4\,7H_2O$	0.5 g
NaCl	0.1g
$CaCl_2\,2H_2O$	0.13 g
Difco yeast extract	1. g
Distilled water	1. 0 litre

c-Baker *et al*'s, medium (1981)

Glucose	20.0 g
NH_4NO_3	400.0 mg
Na_2PO_4	100.0 mg
KCl	300.0 mg
$MgSO_4\ 7H_2O$	40.0 mg
$CaCl_2\ 2H_2O$	40.0 mg
H_3BO_3	1.0 mg
$FeS_4\ 7H_2O$	1.0 mg
$MnSO_4\ 7H_2O$	1.0 mg
Na_2MoO_4	1.0 mg
$ZnSO_4\ _7H_2O$	1.0 mg
$CuSO_4\ 5H_2O$	0.1 mg
Distilled water	1.0 litre

In this medium the $NaNO_3$ was sometimes replaced with 400.0mg $(NH_4)_2\ SO_4$ or 400.0 mg $NaNO_3$.

d-Potato broth medium

Potato broth extract was prepared from 500 g of potato tuber tissue (as described earlier) The extract was made up to 1 litre with distilled water and then dispensed in 25 ml portions in 100 ml flasks and autoclaved. The pH after autoclaving was between pH 6.0 - 6.5.

e-Tuber tissue medium

Potato tubers were washed with tap water and then dried with tissue paper. The tubers were then diced with a knife into small cubes (1 x 0.5-1 x 1 cm). Fifty grams of tissue were weighed and dispensed into 250 ml flasks and autoclaved twice at 15 lbs pressure for 15 min.

REGRESSION EQUATION

Table 1a. Regression equations of rates of dry rot development in potato tubers of cv Maris Piper inoculated with different numbers of conidia of *Fusareum coeruleum.* First experiment Marsh 1991.

Isolate	Log number of spores inoculated	Regression equations
Fc1	5.8	-12.10 + 2.31 X
	4.8	-14.10 + 2.22 X
	3.8	-14.00 + 1.98 X
	2.8	-15.10 + 1.64 X
	1.8	-13.20 + 1.30 X
Fc2	5.8	-9.50 + 2.06 X
	4.8	-12.10 + 1.85X
	3.8	-9.04 + 1.37X
	2.8	-9.50 + 1.12X
	1.8	-10.50 + 1.05X
Fc3	5.8	-6.44 + 1.68X
	4.8	-8.58 + 1.66X
	3.8	-7.31 + 1.49X
	2.8	-11.00 + 1.52X
	1.8	-10.70 + 1.27X
Fc4	5.8	-6.25 + 1.46X
	4.8	-7.70 + 1.45X
	3.8	-7.22 + 1.28X
	2.8	-10.10 + 1.33X
	1.8	-12.30 + 1.23X

X= Time in days

Table 1b. Regression equations of rates of dry rot development in potato tubers of cv Record inoculated with different numbers of conidia of *Fusareum coeruleum.* First experiment March 1991.

Isolate	Log number of spores inoculated	Regression equations
*Fc*1	5.8	-9.65 + 2.00X
	4.8	-11.60 + 1.95X
	3.8	-11.30 + 1.72X
	2.8	-13.50 + 1.45X
	1.8	-12.50 + 1.26X
*Fc*2	5.8	-10.70 + 2.06X
	4.8	-10.80 + 1.92X
	3.8	-9.03 + 1.61X
	2.8	-13.70 + 1.58X
	1.8	-6.04 + 0.77X
*Fc*3	5.8	-4.61 + 1.18X
	4.8	-1.87 + 0.75X
	3.8	-2.08 + 0.63X
	2.8	-1.06 + 0.48X
	1.8	-2.76 + 0.44X
*Fc*4	5.8	-3.48 + 1.11X
	4.8	-4.03 + 1.05X
	3.8	-3.68 + 0.88X
	2.8	-5.00 + 0.84X
	1.8	-5.21 + 0.66X

X = Time in days

Table 1c. Regression equations of rates of dry rot development in potato tubers of cv Maris Piper inoculated with different number of conidia of *Fusareum coeruleum.* Second experiment January 1992.

Isolate	Log number of spores inoculated	Regression equations
Fc1	5.8	-9.19 + 1.58X
	4.8	-9.91 + 1.52X
	3.8	-9.68 + 1.34X
	2.8	-15.90 + 1.45X
	1.8	-13.20 + 1.16X
Fc2	5.8	-6.63 + 1.47X
	4.8	-7.52 + 1.39X
	3.8	-8.64 + 1.36X
	2.8	-9.84 + 1.13X
	1.8	-10.30 + 1.01X
Fc3	5.8	-8.22 + 1.76X
	4.8	-6.05 + 1.34X
	3.8	-5.70 + 1.07X
	2.8	-10.60 + 1.14X
	1.8	-10.10 + 0.97X
Fc4	5.8	-5.00 + 1.25X
	4.8	-5.60 + 1.14X
	3.8	-7.43 + 1.19X
	2.8	-13.00 + 1.23X
	1.8	-10.00 + 0.99X

X =Time in days

Table 1d. regression equations of rates of dry rot development in potato tubers of cv Record inoculated with different number of conidia of *Fusareum coeruleum.* Second experiment January 1992.

Isolate	Log number of spores inoculated	Regression equations
Fc1	5.8	-2.55 + 0.84X
	4.8	-1.65 + 0.61X
	3.8	-2.18 + 0.56X
	2.8	-2.80 + 0.43X
	1.8	-1.56 + 0.29X
Fc2	5.8	-5.80 + 1.21X
	4.8	-3.41 + 0.88X
	3.8	-4.21 + 0.82X
	2.8	-0.91 + 0.97X
	1.8	-7.60 + 0.76X
Fc3	5.8	-4.85 + 1.07X
	4.8	-3.92 + 0.87X
	3.8	-3.88 + 0.74X
	2.8	-5.88 + 0.72X
	1.8	-5.10 + 0.58X
Fc4	5.8	-0.25 + 0.56X
	4.8	-1.24 + 0.55X
	3.8	-2.63 + 0.58X
	2.8	-3.64 + 0.54X
	1.8	-4.05 + 0.50X

X=Time in days

Table 2. Regression equations of rates of dry rot development in potato tubers of cv Maris Piper inoculated with different numbers of conidia of isolate (Fc1) of *Fusareum coerulium.* Third experiment April 1993

Isolate	Log number of spores inoculated	Regression equations
Fc1	5.8	-7.61 + 1.80X
	4.8	-9.85 + 1.77X
	3.8	-10.20 + 1.58X
	2.8	-12.90 + 1.52X
	1.8	-14.30 + 1.42X

X =Time in days

Table 3a. Regression equations of rates of dry rot development in potato tubers of cv Maris Piper inoculated with different numbers of conidia of *Fusareum avenaceum.* First experiment Marsh 1991.

Isolates	Log number of spores inoculated	The regression equations
Fa1	5.8	-5.65 + 2.23X
	4.8	-7.95 + 2.25X
	3.8	-9.03 + 2.20X
	2.8	-9.97 + 2.12X
	1.8	-11.50 + 1.99X
Fa2	5.8	-5.30 + 2.11X
	4.8	-7.15 + 2.08X
	3.8	-6.95 + 1.93X
	2.8	-8.11 + 1.84X
	1.8	-6.17 + 1.50X
Fa3	5.8	-3.50 + 1.77X
	4.8	-5.55 + 1.79X
	3.8	-5.91 + 1.72X
	2.8	-8.19 + 1.75X
	1.8	-4.67 + 1.42X
Fa4	5.8	-3.90 + 1.62X
	4.8	-3.83 + 1.50X
	3.8	-4.89 + 1.42X
	2.8	-6.02 + 1.32X
	1.8	-7.17 + 1.25X

X = Time in days

Table 3b. Regression equations of rates of dry rot development in potato tubers of cv Record inoculated with different numbers of conidia of *Fusareum avenaceum.* First experiment Marsh 1991.

Isolates	Log number of spores inoculated	The regression equations
Fa1	5.8	-4.25 + 1.58X
	4.8	-5.08 + 1.46X
	3.8	-4.43 + 1.27X
	2.8	-4.47 + 1.13X
	1.8	-5.97 + 1.07X
Fa2	5.8	-9.65 + 2.02X
	4.8	-9.64 + 1.78X
	3.8	-10.20 + 1.78X
	2.8	-12.80 + 1.72X
	1.8	-10.00 + 1.41X
Fa3	5.8	-5.95 + 1.63X
	4.8	-4.83 + 1.42X
	3.8	-6.44 + 1.37X
	2.8	-7.17 + 1.33X
	1.8	-10.10 + 1.30X
Fa4	5.8	-3.75 + 1.27X
	4.8	-5.25 + 1.29X
	3.8	-6.06 + 1.25X
	2.8	-6.48 + 1.17X
	1.8	-7.00 + 1.01X

X=Time in days

Table 3c. Regression equations of rates of dry rot development in potato tubers of cv Maris Piper inoculated with different numbers of conidia of *Fusareum avenaceum*. Second experiment January 1992.

Isolates	Log number of spores inoculated	The regression equations
Fa1	5.8	-8.75 + 2.16X
	4.8	-9.09 + 2.04X
	3.8	-11.50 + 2.08X
	2.8	-12.10 + 1.94X
	1.8	-14.40 + 1.88X
Fa2	5.8	-5.95 + 2.05X
	4.8	-8.05 + 1.10X
	3.8	-9.00 + 2.06X
	2.8	-12.10 + 2.09X
	1.8	-13.80 + 1.87X
Fa3	5.8	-6.90 + 1.85X
	4.8	-7.98 + 1.86X
	3.8	-10.90 + 1.82X
	2.8	-8.58 + 1.38X
	1.8	-11.50 + 1.41X
Fa4	5.8	-4.90 + 1.52X
	4.8	-6.35 + 1.51X
	3.8	-6.35 + 1.40X
	2.8	-7.68 + 1.26X
	1.8	-5.03 + 0.93X

X = Time in days

Table 3d. Regression equations of rates of dry rot development in potato tubers of cv Record inoculated with different numbers of conidia of *Fusareum avenaceum.* Second experiment January 1992.

Isolates	Log number of spores inoculated	The regression equations
Fa1	5.8	-5.60 + 1.31X
	4.8	-6.90 + 1.31X
	3.8	-7.96 + 1.32X
	2.8	-2.50 + 0.70X
	1.8	-3.85 + 0.65X
Fa2	5.8	-7.70 + 1.63X
	4.8	-8.65 + 1.52X
	3.8	-8.06 + 1.38X
	2.8	-5.96 + 1.09X
	1.8	-7.88 + 0.92X
Fa3	5.8	-3.50 + 1.22X
	4.8	-6.52 + 1.21X
	3.8	-6.91 + 1.15X
	2.8	-5.82 + 0.87X
	1.8	-3.02 + 0.54X
Fa4	5.8	-2.95 + 1.11X
	4.8	-3.60 + 1.01X
	3.8	-3.60 + 0.86X
	2.8	-6.00 + 0.83X
	1.8	-3.67 + 0.59X

X = Time in days

Table 4. Regression equations of rates of dry rot development in potato tubers of cv Maris Piper inoculated with different numbers of conidia of isolate (Fa1) of *F. avenaceum*. Third experiment April 1993

Isolats	Log number of spores inoculated	The regression equation
Fa1	5.8	0.70 + 1.24X
	4.8	-0.94 + 1.22X
	3.8	-0.58 + 1.13X
	2.8	-2.58 + 1.09X
	1.8	-3.80 + 1.05X

X = Time in days

Table 5a. Regression equations of rates of dry rot development in potato tubers of cv Maris Piper inoculated with different numbers of conidia of *C. Destructans.* First experiment Marsh 1990.

Isolates	Log number of spores inoculated	The regressions equations
Cd1	5.8	-1.85 + 0.541X
	4.8	-1.82 + 0.441X
	3.8	-0.96 + 0.288X
	2.8	-0.30 + 0.117X
Cd2	5.8	0.25 + 0.515X
	4.8	-0.89 + 0.503X
	3.8	0.03 + 0.318X
	2.8	0.65 + 0.148X
Cd3	5.8	0.02 + 0.389X
	4.8	-0.36 + 0.320X
	3.8	-1.10 + 0.354X
	2.8	-0.21 + 0.112X

X = Time in days

Table 5b. Regression equations of rates of dry rot development in potato tubers of cv Record inoculated with different numbers of conidia of *C. destructans*. First experiment Marsh 1990.

Isolates	Log number of spores inoculated	The regressions equations
Cd1	5.8	-0.99 + 0.535X
	4.8	-0.57 + 0.381X
	3.8	0.20 + 0.218X
	2.8	-0.57 + 0.150X
Cd2	5.8	0.26 + 0.597X
	4.8	-1.23 + 0.561X
	3.8	-2.27 + 0.524X
	2.8	-0.57 + 0.147X
Cd3	5.8	0.79 + 0.315X
	4.8	0.38 + 0.317X
	3.8	-0.51 + 0.288X
	2.8	-0.36 + 0.150X

X = Time in days

Table 5c. Regression equations of rates of dry rot development in potato tubers of cv Maris Piper inoculated with different numbers of conidia of *C. deastructans*. Second experiment January 1991.

Isolates	Log number of spores inoculated	The regressions equations
Cd1	5.8	-0.15 + 0.447X
	4.8	-1.36 + 0.498X
	3.8	-1.39 + 0.385X
	2.8	-0.12 + 0.123X
Cd2	5.8	-0.78 + 0.567X
	4.8	-1.72 + 0.535X
	3.8	-1.93 + 0. 462X
	2.8	-1.23 + 0.199X
Cd3	5.8	-0.49 + 0.486X
	4.8	-0.93 + 0.408X
	3.8	-1.40 + 0.373X
	2.8	-0.30 + 0.135X

X = Time in days

Table 5d. Regression equations of rates of dry rot development in potato tubers of cv Record inoculated with different numbers of conidia of *C. deastructans*. Second experiment January 1991.

Isolates	Log number of spores inoculated	The regressions equations
Cd1	5.8	0.29 + 0.491X
	4.8	-0.23 + 0.449X
	3.8	0.02 + 0.337X
	2.8	-0.58 + 0.168X
Cd2	5.8	0.76 + 0.565X
	4.8	-0.58 + 0.517X
	3.8	-0.48 + 0.336X
	2.8	-3.63 + 0.475X
Cd3	5.8	0.52 + 0.413X
	4.8	-0.07 + 0.409X
	3.8	0.11 + 0.332X
	2.8	0.48 + 0.079X

X = Time in days

Tables 1-58

Table 1. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fc1) of *F. coeruleum*. Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 8 5 5 7 8 0 0 0 0	6.3
	4.8	4 6 5 5 6 4 0 0 0 0	5
	3.8	4 3 5 4 3 0 0 0 0 0	3.8
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	20 10 17 17 19 10 20 10 10 17	15
	4.8	15 10 10 10 10 15 15 15 10 8	11.8
	3.8	15 10 15 15 10 10 5 5 5 8	9.8
	2.8	5 10 10 5 10 5 6 4 0 0	6.9
	1.8	5 7 6 7 4 3 4 4 0 0	5
18	5.8	35 30 30 30 30 35 35 40 25 25	31.5
	4.8	25 20 30 30 30 30 40 20 17 25	26.7
	3.8	20 25 30 25 20 20 20 25 15 18	21.8
	2.8	20 15 20 20 20 10 10 15 12 18	16
	1.8	8 12 10 15 7 8 15 13 20 5	11.3
24	5.8	45 45 55 50 45 55 40 45 40 40	46
	4.8	50 45 35 45 40 45 40 50 40 40	43
	3.8	40 45 35 45 40 35 40 25 40 30	37.5
	2.8	40 30 30 30 30 35 20 20 20 10	26.5
	1.8	25 25 20 25 20 15 15 30 20 15	20.5

In all experiments ten tubers were inoculated with different inoculum levels: Log 5.8 = $6x10^5$ spores, Log 4.8 = $6x10^4$ spores, Log 3.8 = $6x10^3$ spores, Log 2.8 = $6x10^2$ spores, Log 1.8 = $6x10^1$ spores.

Table 2. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Fc1) of *F. coeruleum*. Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Main diameters of dry rot lesion
6	5.8	6 6 6 5 5 7 6 5 5 5	5.6
	4.8	4 5 5 4 4 6 4 3 0 0	4.4
	3.8	3 4 3 3 5 3 3 0 0 0	3.4
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	10 15 10 20 10 10 20 8 17 15	13.5
	4.8	10 12 8 8 10 10 12 8 10 12	10
	3.8	10 8 8 10 7 6 8 10 6 0	8.1
	2.8	10 5 8 5 4 6 8 8 0 0	6.8
	1.8	5 4 3 5 6 7 0 0 0 0	5
18	5.8	35 40 30 25 25 30 35 40 15 25	30
	4.8	35 25 40 25 20 20 35 35 25 20	28
	3.8	25 15 30 25 10 20 25 25 20 30	22.5
	2.8	15 20 15 20 12 15 15 8 8 5	13.3
	1.8	10 15 10 15 20 8 8 7 0 0	11.6
24	5.8	45 40 35 30 45 35 45 35 45 45	40
	4.8	35 45 50 40 45 25 40 35 30 25	37
	3.8	40 30 45 35 25 25 25 35 25 40	32.5
	2.8	25 25 35 25 30 20 15 20 35 10	24
	1.8	20 35 15 25 30 35 15 10 10 5	20

Table 3. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fc2) of *F. coeruleum*. Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Main diameters of dry rot lesion
6	5.8	7 6 6 7 5 6 6 7 7 8	6.5
	4.8	5 3 7 5 5 5 4 3 5 0	4.7
	3.8	4 3 3 4 5 3 3 0 0 0	3.6
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	15 20 15 20 10 10 20 15 15 15	15.5
	4.8	10 8 5 12 8 6 6 7 10 10	8.2
	3.8	7 8 5 8 8 9 7 8 8 4	7.2
	2.8	5 6 7 4 5 8 5 4 0 0	5.5
	1.8	5 4 4 4 5 5 4 0 0 0	4.4
18	5.8	30 18 30 35 30 20 35 25 30 28	28.1
	4.8	25 30 20 20 25 15 25 15 20 15	21
	3.8	25 20 20 15 12 20 10 25 15 10	17.2
	2.8	10 12 25 15 20 10 8 8 5 20	13.3
	1.8	10 12 8 15 10 8 10 8 5 15	10.1
24	5.8	45 40 40 40 45 35 55 55 40 30	42.5
	4.8	35 30 35 35 40 30 40 40 45 40	37
	3.8	30 35 35 35 20 25 15 30 25 20	27
	2.8	20 25 15 15 25 20 25 25 10 10	19
	1.8	15 20 15 25 20 10 15 10 15 25	17

Table 4. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Fc2) of *F. coeruleum*. Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Main diameter of dry rot lesion
6	5.8	5 6 7 8 4 4 8 4 0 0	5.8
	4.8	5 4 3 3 5 4 6 5 0 0	4.3
	3.8	4 4 3 5 5 3 3 0 0 0	3.9
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	15 10 20 10 15 15 10 5 20 10	13
	4.8	10 15 10 15 15 12 5 8 5 18	11.3
	3.8	15 15 10 5 10 5 10 5 0 0	9.4
	2.8	5 8 7 4 10 15 5 0 0 0	7.7
	1.8	5 8 4 4 5 4 3 0 0 0	7.4
18	5.8	30 30 35 30 25 24 45 35 10 30	29.4
	4.8	25 35 35 40 35 45 10 25 15 10	27.5
	3.8	30 35 45 20 20 25 35 10 15 5	24
	2.8	15 20 10 10 15 25 30 10 5 20	16
	1.8	10 15 20 10 5 10 8 12 10 5	10.5
24	5.8	50 35 40 50 45 45 35 45 30 35	41
	4.8	40 45 50 45 35 20 35 45 20 35	37
	3.8	25 40 25 35 25 40 45 50 10 15	31
	2.8	30 35 30 20 40 20 25 15 15 35	26.5
	1.8	15 15 25 16 10 12 10 15 15 8	14.1

Table 5. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fc3) of *F. coeruleum.* Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 4 8 6 5 7 6 4 5 0	5.6
	4.8	5 3 4 4 3 5 6 5 0 0	4.4
	3.8	3 4 5 5 4 3 3 0 0 0	3.9
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	20 15 20 15 10 10 25 15 20 5	15.5
	4.8	10 10 8 12 15 10 15 20 15 5	12
	3.8	10 10 15 8 12 10 15 20 5 8	11.3
	2.8	10 10 15 10 10 10 15 8 5 5	8.8
	1.8	8 5 5 10 12 5 7 8 0 0	7.5
18	5.8	30 25 20 30 20 35 20 40 35 10	27.5
	4.8	15 20 25 30 20 30 15 30 20 30	27.5
	3.8	20 30 30 10 30 25 35 15 10 30	23.3
	2.8	15 15 20 30 30 20 10 15 15 20	19
	1.8	20 10 15 20 10 15 5 10 5 15	12.5
24	5.8	20 35 45 45 40 45 40 25 35 25	35.5
	4.8	35 30 25 45 45 30 25 35 30 35	33.5
	3.8	35 40 35 35 15 30 20 20 30 35	29.5
	2.8	30 35 40 15 20 35 20 20 30 25	27
	1.8	30 15 20 25 30 35 20 15 30 5	22.5

Table 6. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Fc3) of *F. coeruleum*. Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 6 7 4 3 5 6 5 0 0	5.1
	4.8	3 4 5 5 4 4 5 5 0 0	4.4
	3.8	3 4 4 3 3 3 3 0 0 0	3.3
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	15 10 8 12 10 12 8 15 8 10	10.8
	4.8	10 7 10 8 6 10 15 5 8 5	8.4
	3.8	5 10 12 8 5 10 5 5 8 7	7.5
	2.8	5 6 7 8 10 5 5 3 0 0	6.1
	1.8	3 4 5 6 7 3 3 4 0 0	4.4
18	5.8	20 15 20 15 20 20 20 20 20 15	18.5
	4.8	12 15 20 20 20 15 10 20 15 10	15.7
	3.8	10 10 15 15 10 10 15 15 15 8	12.3
	2.8	15 10 15 5 15 5 10 10 15 5	10.5
	1.8	10 5 10 5 15 5 4 5 10 8	7.7
24	5.8	30 30 30 25 20 25 20 20 30 30	26
	4.8	25 15 15 20 15 10 15 15 20 20	17
	3.8	15 10 15 15 10 20 10 15 20 15	14.5
	2.8	10 10 20 15 15 10 15 10 5 10	12
	1.8	15 10 8 8 10 12 10 8 9 8	9.8

Table 7. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fc4) of *F. coeruleum.* Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 4 6 7 8 4 5 8 5 0	5.8
	4.8	4 5 3 4 5 6 4 3 5 0	4.3
	3.8	3 4 4 5 3 3 4 0 0 0	3.7
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	10 10 15 5 10 8 12 15 12 15	11.2
	4.8	8 10 12 7 8 14 15 5 14 4	9.7
	3.8	5 10 10 10 8 6 14 5 6 10	8.4
	2.8	10 10 5 6 8 4 5 8 10 10	7.6
	1.8	4 5 4 3 7 8 3 4 0 0	4.8
18	5.8	25 25 15 25 20 35 15 25 10 35	23
	4.8	20 25 25 15 10 15 25 35 15 30	21.5
	3.8	20 20 20 10 10 15 15 30 20 25	18.5
	2.8	15 15 15 20 15 20 10 20 15 15	16
	1.8	15 15 15 10 5 8 12 5 15 15	11.5
24	5.8	35 35 30 30 25 40 25 20 40 30	31
	4.8	35 35 20 20 25 30 40 40 15 35	29.5
	3.8	30 35 30 30 25 15 20 20 25 25	25.5
	2.8	25 30 25 30 20 25 15 20 25 20	23.5
	1.8	25 15 15 30 20 15 25 15 20 15	19.5

Table 8. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Fc4) of *F. coeruleum*. Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 4 6 7 4 4 5 5 5 0	5
	4.8	4 5 6 4 5 5 3 4 3 0	4.3
	3.8	4 3 4 3 3 4 3 3 0 0	3.4
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	15 10 10 10 10 15 15 20 10 5	12
	4.8	10 8 15 10 12 15 10 15 5 8	10.8
	3.8	15 10 5 8 10 5 10 10 8 12	9.3
	2.8	5 5 5 10 6 8 10 8 5 0	6.9
	1.8	5 6 5 4 5 6 3 5 0 0	4.9
18	5.8	20 15 15 25 20 20 15 25 15 15	18.5
	4.8	15 15 20 15 20 15 10 25 10 15	16
	3.8	10 10 15 15 20 15 15 15 10 15	14
	2.8	10 10 15 5 15 15 8 12 15 20	12.5
	1.8	10 6 10 12 8 8 10 10 5 6	8.5
24	5.8	30 30 25 20 25 15 25 30 35 15	25
	4.8	25 15 25 20 35 10 25 25 30 25	23.5
	3.8	15 10 15 25 25 30 15 15 20 25	19.5
	2.8	10 10 20 15 15 20 25 25 5 25	17
	1.8	15 15 15 15 8 12 20 15 6 7	12.8

Table 9. The diameter of dry rot lesion measured internally in potato tubers of cv Maris Piper inoculated with isolates of *F. coeruleum* 24 days after inoculation. Experiment set up Marsh 1991.

Isolates	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
Fc1	5.8	45 40 45 45 55 50 45 35 55 40	45.5
	4.8	45 45 45 40 50 50 50 40 35 40	44
	3.8	50 50 35 45 45 40 40 45 40 40	43
	2.8	40 35 30 40 40 45 45 50 30 30	38.5
	1.8	40 40 40 35 20 40 35 35 10 10	30.5
Fc2			
	5.8	55 40 45 40 35 45 50 40 30 50	43
	4.8	40 40 45 45 45 35 35 40 30 40	39.5
	3.8	40 55 50 40 35 30 45 40 45 45	42.5
	2.8	35 30 30 35 25 30 20 30 10 25	27.7
	1.8	30 25 20 25 30 25 20 30 10 25	24.6
Fc3	5.8	45 40 40 40 40 35 50 45 40 40	41.5
	4.8	40 40 35 35 40 30 35 40 40 50	38.5
	3.8	40 40 30 40 35 50 40 40 40 45	40
	2.8	35 20 15 35 40 20 30 35 35 20	28.5
	1.8	15 35 20 35 10 30 35 25 35 15	25.5
Fc4	5.8	40 40 30 35 40 35 35 25 40 35	35.5
	4.8	35 40 35 30 35 35 40 35 30 35	35
	3.8	35 35 30 35 35 25 30 40 30 30	32.5
	2.8	10 25 15 25 30 10 25 30 30 15	21.5
	1.8	10 15 30 30 20 15 20 20 25 10	20

Table 10. The diameter of dry rot lesion measured internally in potato tubers of cv Record inoculated with isolates of *F. coeruleum* 24 days after inoculation. Experiment set up Marsh 1991.

Isolates	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
Fc1	5.8	45 40 35 40 40 40 50 55 50 45	44
	4.8	50 40 35 45 50 45 50 40 45 35	43.5
	3.8	40 45 45 40 45 50 35 50 25 40	41
	2.8	40 35 35 35 35 45 30 30 40 30	35.5
	1.8	15 30 25 20 30 40 30 40 20 30	28
Fc2			
	5.8	50 30 35 45 45 45 55 50 50 30	43.5
	4.8	35 45 45 40 30 55 35 45 40 40	41
	3.8	45 35 40 50 45 35 35 40 35 45	40.5
	2.8	30 20 35 35 30 20 15 25 25 35	27
	1.8	15 20 25 10 15 25 15 10 15 10	16
Fc3	5.8	30 30 25 40 30 30 35 40 30 25	31.5
	4.8	25 35 30 20 30 25 30 25 30 35	28.5
	3.8	15 30 35 20 30 30 35 20 30 25	27
	2.8	20 20 15 20 30 20 15 15 15 15	18.5
	1.8	15 20 15 10 15 15 15 10 10 10	13.5
Fc4	5.8	30 35 30 35 35 35 30 35 35 35	33
	4.8	30 30 20 30 30 35 35 30 30 30	30
	3.8	25 10 20 30 20 35 30 35 20 35	26
	2.8	25 10 20 20 25 15 10 20 20 15	18
	1.8	8 15 10 10 15 5 10 10 10 10	10.3

Table 11. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fc1) of *F. coeruleum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 6 4 5 4 6 7 5 0 0	5.3
	4.8	5 4 4 3 3 5 6 6 0 0	4.5
	3.8	4 3 3 4 3 3 4 0 0 0	3.4
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	10 15 10 10 8 5 5 15 8 12	9.8
	4.8	5 10 8 12 10 5 5 5 8 12	8
	3.8	10 5 6 4 5 6 7 8 7 0	6.4
	2.8	4 5 6 4 5 6 8 6 0 0	5.5
	1.8	4 5 3 4 4 4 3 4 0 0	3.9
18	5.8	20 30 15 20 10 15 30 20 10 10	18
	4.8	30 15 15 10 10 15 20 25 10 10	16
	3.8	15 10 25 10 10 15 20 8 10 10	13.3
	2.8	10 5 5 15 10 15 8 12 5 5	9
	1.8	10 8 12 5 5 8 9 4 10 5	7.6
24	5.8	35 40 30 40 35 45 35 25 20 30	33.5
	4.8	35 40 20 40 30 35 35 20 20 40	31.5
	3.8	30 20 30 30 30 20 35 40 20 15	27
	2.8	20 15 35 20 15 25 10 25 30 30	22.5
	1.8	20 15 20 15 15 25 30 10 10 15	17.5

Table 12. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Fc1) of *F. coeruleum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 6 3 4 7 8 3 4 4 7	5.1
	4.8	4 5 6 7 4 3 3 4 5 0	4.6
	3.8	3 4 5 5 4 4 3 3 3 0	3.8
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	8 6 10 8 6 12 9 10 8 10	8.7
	4.8	5 6 4 8 7 8 10 5 6 8	6.7
	3.8	5 6 7 8 4 5 6 7 4 5	5.7
	2.8	4 5 6 4 3 4 5 3 7 0	4.6
	1.8	3 4 5 3 3 4 4 3 0 0	3.6
18	5.8	10 15 8 16 12 15 20 15 8 25	14.4
	4.8	8 7 16 7 15 10 20 12 10 15	12
	3.8	16 15 7 10 12 14 8 7 5 6	10
	2.8	6 5 7 8 4 5 6 7 8 10	6.6
	1.8	4 5 6 4 5 6 6 7 8 3	5.4
24	5.8	20 15 10 20 20 25 20 25 15 30	20
	4.8	15 10 20 12 15 25 20 15 10 8	15
	3.8	15 10 12 14 8 10 9 25 12 20	13.5
	2.8	10 15 10 14 8 6 7 8 9 10	9.7
	1.8	8 7 7 5 8 8 10 8 6 5	7.2

Table 13. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fc2) of *F. coeruleum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	6 5 7 8 4 5 6 7 8 0	6.2
	4.8	5 6 7 4 4 3 5 5 5 0	4.9
	3.8	5 4 3 3 3 4 4 4 0 0	3.8
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	10 6 8 15 10 8 15 10 8 12	10.2
	4.8	6 10 5 10 8 7 10 9 15 6	8.6
	3.8	5 10 10 8 8 6 6 12 5 8	7.8
	2.8	5 8 7 8 10 4 3 5 4 0	6.
	1.8	4 5 4 3 4 5 5 3 4 0	4.1
18	5.8	20 15 25 15 25 25 30 10 25 30	22
	4.8	30 15 8 25 20 20 15 25 25 10	19.3
	3.8	15 20 10 15 25 10 15 10 25 20	16.5
	2.8	10 15 8 20 15 10 8 10 15 8	11.9
	1.8	10 8 10 6 12 15 7 8 10 8	9.4
24	5.8	40 15 30 25 35 25 30 45 35 35	31.5
	4.8	35 25 30 15 35 20 25 30 40 35	29
	3.8	30 20 35 35 20 30 40 30 12 25	28.2
	2.8	15 20 15 30 15 20 25 10 20 25	19.5
	1.8	25 20 15 12 20 15 10 15 10 20	16.2

Table 14. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Fc2) of *F. coeruleum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 4 5 8 6 5 7 5 4 0	5.4
	4.8	4 5 6 6 7 5 5 4 3 0	5
	3.8	4 5 3 4 4 3 3 4 0 0	3.8
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	10 10 6 8 12 5 15 10 8 5	8.9
	4.8	7 8 10 15 12 6 4 5 6 6	7.9
	3.8	6 7 5 4 5 10 8 12 4 4	6.5
	2.8	5 4 6 7 4 5 6 5 7 0	5.4
	1.8	4 3 3 3 4 5 5 4 0 0	3.9
18	5.8	20 15 10 12 8 25 10 25 20 20	16.5
	4.8	15 10 15 20 10 15 10 25 10 12	14.2
	3.8	10 15 10 10 12 20 12 15 10 10	12.4
	2.8	10 15 8 8 9 10 6 7 5 8	10.5
	1.8	5 8 10 5 7 10 12 5 5 8	7.5
24	5.8	35 25 20 25 15 30 20 35 40 25	27
	4.8	20 15 20 25 20 20 15 35 15 20	20.5
	3.8	15 25 20 15 20 15 15 20 15 20	18
	2.8	15 15 10 20 15 20 25 10 15 25	15.5
	1.8	10 15 10 15 12 20 15 10 8 15	13

Table 15. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fc3) of *F. coeruleum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 6 8 6 7 5 7 4 8 0	6.2
	4.8	6 7 8 6 4 5 5 5 4 0	5.6
	3.8	3 4 5 5 4 4 3 3 0 0	3.9
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	15 15 8 12 15 10 12 8 5 15	11.5
	4.8	10 15 12 10 10 15 8 6 5 5	9.6
	3.8	10 6 8 12 10 10 8 8 5 6	8.3
	2.8	5 6 5 4 3 4 6 8 4 8	5.3
	1.8	5 4 3 5 4 3 4 5 6 0	4.3
18	5.8	30 25 20 35 15 20 30 35 25 35	27
	4.8	35 25 20 15 20 30 15 15 10 20	20.5
	3.8	15 10 15 20 20 15 15 12 15 10	14.7
	2.8	10 8 15 12 8 10 6 15 20 10	11.4
	1.8	10 6 8 7 8 5 10 7 12 10	8.3
24	5.8	40 35 30 45 25 30 45 40 30 40	36
	4.8	45 30 30 25 25 40 20 25 20 25	28.5
	3.8	25 15 20 35 25 20 25 20 30 15	23
	2.8	15 15 25 20 15 15 10 30 25 20	19
	1.8	15 10 20 15 15 20 25 10 15 15	16

Table 16. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate of (Fc3) of *F. coeruleum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 4 6 7 6 5 8 4 0 0	5.6
	4.8	4 5 6 4 3 3 4 5 6 0	4.4
	3.8	3 3 4 4 3 4 4 3 0 0	3.5
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	10 8 12 15 10 5 8 6 4 10	8.8
	4.8	10 8 6 7 8 10 12 6 5 3	7.5
	3.8	5 8 6 6 4 5 7 8 9 4	6.2
	2.8	5 6 5 8 4 5 3 3 4 5	4.8
	1.8	5 4 4 4 3 3 4 4 5 4	4.0
18	5.8	15 16 10 15 20 25 15 20 10 10	15.6
	4.8	15 15 10 10 20 15 20 8 10 8	13.1
	3.8	10 12 15 10 8 12 10 10 15 10	11.2
	2.8	8 10 15 10 10 8 6 9 4 12	9.2
	1.8	8 5 6 10 10 8 10 5 4 6	7.2
24	5.8	25 30 15 25 30 30 20 25 15 20	24.5
	4.8	25 30 20 15 25 20 25 15 10 15	20
	3.8	15 15 20 15 10 20 15 15 20 20	16.5
	2.8	10 15 20 15 10 15 10 10 15 15	13.5
	1.8	10 8 10 15 15 10 20 8 6 8	11

Table 17. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fc4) of *F. coeruleum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 6 7 8 4 5 6 7 6 7	6.1
	4.8	4 5 6 7 4 5 5 4 6 4	5
	3.8	5 3 4 4 5 3 3 4 3 0	3.8
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	10 8 12 9 15 6 12 8 10 10	10
	4.8	8 6 10 6 8 7 5 10 12 6	7.8
	3.8	5 7 5 6 7 8 10 8 6 3	6.5
	2.8	4 5 5 4 5 6 3 3 0 0	4.4
	1.8	3 4 3 3 3 4 3 4 0 0	3.4
18	5.8	15 20 25 10 20 15 25 30 15 10	18.5
	4.8	15 25 20 15 10 10 20 25 15 10	16.5
	3.8	10 15 25 10 15 15 25 10 15 10	15
	2.8	10 8 12 15 12 15 10 6 6 8	10.2
	1.8	8 10 6 10 12 5 7 8 10 8	8.4
24	5.8	25 35 40 45 15 15 25 35 25 20	28
	4.8	20 30 15 45 25 35 25 15 25 15	25
	3.8	25 30 35 35 25 15 15 20 25 20	24.5
	2.8	15 25 30 15 10 15 20 15 10 35	19
	1.8	15 12 10 15 20 25 15 10 15 15	15.2

Table 18. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Fc4) of *F. coeruleum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	4 5 6 3 4 4 5 7 4 8	5
	4.8	4 5 6 4 3 5 4 4 3 0	4.2
	3.8	3 3 3 3 3 3 3 3 0 0	3
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	10 8 12 15 8 6 5 6 7 8	8.5
	4.8	10 6 8 12 5 10 6 7 5 5	7.4
	3.8	6 7 8 5 5 10 5 4 7 6	6.3
	2.8	4 5 6 5 6 7 8 3 3 0	5.2
	1.8	4 3 4 5 3 3 4 6 3 4	3.9
18	5.8	15 10 15 10 8 12 10 8 15 20	12.5
	4.8	15 10 12 12 15 10 6 8 8 9	10.5
	3.8	15 10 15 10 8 8 9 9 6 7	9.7
	2.8	6 6 8 10 8 10 6 7 8 6	7.5
	1.8	8 6 6 7 5 9 10 6 8 9	7.4
24	5.8	25 15 10 20 10 10 10 15 15 20	15
	4.8	20 15 10 25 15 10 15 10 8 15	14.3
	3.8	20 25 15 16 8 10 12 8 6 15	13.5
	2.8	10 15 8 15 12 16 8 8 10 15	11.7
	1.8	10 8 8 10 15 6 10 8 9 16	10

Table 19. The diameter of dry rot lesion measured internally in potato tubers of cv Maris Piper inoculated with different isolates of *F. coeruleum* 24 days after inoculation. Experiment set up January 1992.

Isolates	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
Fc1	5.8	40 45 45 35 40 35 35 30 30 40	36.5
	4.8	25 35 35 45 40 40 35 40 40 20	35.5
	3.8	35 35 40 20 40 35 35 30 30 30	33
	2.8	20 20 15 30 30 35 25 25 20 10	23
	1.8	10 20 20 20 30 20 20 30 35 10	21.5
Fc2			
	5.8	40 35 30 35 35 40 40 20 30 40	34.5
	4.8	30 35 25 45 40 30 40 25 45 25	34
	3.8	25 30 40 35 30 30 35 40 25 30	32
	2.8	15 40 30 20 35 20 30 15 35 20	26
	1.8	10 25 20 35 30 30 25 20 10 10	21.5
Fc3	5.8	25 45 40 45 35 50 20 40 35 35	37
	4.8	25 40 40 35 40 20 45 35 40 30	35
	3.8	20 35 40 20 40 35 40 35 30 35	33
	2.8	30 15 35 20 15 30 35 15 35 10	24
	1.8	15 15 35 40 30 10 30 35 15 10	23.5
Fc4	5.8	35 35 20 30 35 20 30 45 35 20	30.5
	4.8	20 35 35 40 35 30 30 35 30 20	31
	3.8	35 30 30 20 30 30 20 30 30 25	28
	2.8	15 10 30 30 15 30 10 15 30 20	20.5
	1.8	10 20 30 10 30 25 30 30 15 10	21

Table 20. The diameter of dry rot lesion measured internally in potato tubers of cv Record inoculated with different isolates of *F. coeruleum* 24 days after inoculation. Experiment sep up January 1992.

Isolates	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
Fc1	5.8	35 15 25 35 30 15 20 15 30 35	25.5
	4.8	25 35 30 15 10 20 20 20 30 25	23
	3.8	20 10 35 20 20 15 25 25 35 25	22.8
	2.8	10 10 10 5 20 10 10 20 15 5	11.5
	1.8	10 10 5 10 5 10 5 10 5 10	8
Fc2			
	5.8	20 20 10 30 25 35 15 20 35 20	23
	4.8	35 20 10 30 15 20 25 25 20 25	22.5
	3.8	20 25 15 20 25 10 20 25 30 20	21
	2.8	15 10 20 10 15 15 10 20 15 5	13.5
	1.8	10 10 10 10 15 15 10 15 15 15	11.5
Fc3	5.8	25 35 20 30 10 35 30 35 30 15	26.5
	4.8	20 15 30 20 20 15 25 30 30 20	22.5
	3.8	20 20 20 15 30 30 25 20 20 20	22
	2.8	10 20 10 15 15 10 15 15 10 10	13
	1.8	10 15 10 10 15 10 10 20 10 10	12
Fc4	5.8	10 15 25 30 30 30 30 30 30 10	24
	4.8	30 20 25 30 25 15 25 20 30 10	23
	3.8	10 25 30 25 30 25 30 20 20 15	22.5
	2.8	10 10 15 15 10 10 10 10 10 10	11
	1.8	10 10 20 15 10 10 10 15 5 10	11.5

Table 21. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fc1) of *F. coeruleum*. Experiment set up April 1993.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameterof dry rot lesion
6	5.8	5 5 5 8 10 6 7 8 5 0	6.6
	4.8	5 5 7 6 5 7 6 7 6 0	6
	3.8	5 6 4 5 5 4 5 4 0 0	4.8
	2.8	3 3 3 3 3 3 3 0 0 0	3
	1.8	0 0 0 0 0 0 0 0 0 0	0
9	5.8	11 12 10 8 8 12 7 9 10 8	9.5
	4.8	7 7 10 10 10 10 8 5 6 8	8.1
	3.8	6 5 7 5 5 5 6 7 5 5	5.6
	2.8	4 4 4 4 5 3 3 4 5 0	4
	1.8	3 3 3 3 3 3 3 3 0 0	3
12	5.8	20 15 15 25 15 15 10 15 20 10	16.5
	4.8	10 15 10 15 15 10 20 10 10 10	12.5
	3.8	8 12 10 8 15 10 8 8 10 7	9.6
	2.8	7 5 6 6 6 5 7 6 7 0	6.1
	1.8	4 8 4 5 5 3 4 5 7 0	5
15	5.8	20 20 18 25 20 23 25 23 20 17	21.1
	4.8	25 15 20 20 18 15 10 20 15 20	17.8
	3.8	16 20 10 15 15 15 10 10 10 10	13.1
	2.8	10 7 5 10 10 15 10 15 10 12	10.4
	1.8	5 7 10 6 10 8 8 10 8 0	8
18	5.8	25 30 30 20 35 25 20 25 22 27	26.5
	4.8	25 20 20 20 22 15 15 20 25 25	20.7
	3.8	15 20 20 15 20 15 15 25 20 15	18
	2.8	20 20 20 15 10 10 15 10 15 15	15
	1.8	20 20 20 15 10 10 15 10 15 15	15

21	5.8	30 45 35 30 35 30 25 35 30 30	32 .4
	4.8	30 25 35 30 25 30 20 20 30 30	27.5
	3.8	30 25 25 20 30 20 20 25 25 25	24.5
	2.8	15 20 15 15 20 15 20 25 25 20	19
	1.8	15 15 15 20 10 15 15 10 20 25	16
24	5.8	40 30 30 40 45 40 50 30 35 35	37.5
	4.8	30 30 35 35 35 35 35 35 40 40	35
	3.8	30 35 35 30 25 30 35 35 35 30	32
	2.8	15 25 25 30 30 25 20 25 25 20	24
	1.8	20 20 15 15 25 15 20 25 25 20	20
27	5.8	45 40 45 35 40 45 40 45 45 50	43
	4.8	45 35 45 45 45 45 40 45 30 45	42
	3.8	30 30 40 35 30 45 30 40 35 35	35
	2.8	30 35 25 35 25 35 25 35 35 30	31
	1.8	30 20 15 20 25 25 30 15 15 30	26.8
30	5.8*	45 50 45 50 45 50 50 50 45 55	48.5
	4.8*	55 40 50 50 40 35 50 50 45 45	46
	3.8	40 40 35 40 35 50 35 40 45 40	40
	2.8	30 35 40 45 30 35 30 35 30 35	35.5
	1.8	30 35 30 40 35 25 40 35 25 20	31.5
33	3.8	45 50 45 45 50 35 45 40 45 40	44
	2.8	50 35 45 40 40 45 45 40 35 35	41
	1.8	40 30 40 25 35 40 35 35 35 40	35.5
36	3.8*	45 50 50 50 55 40 50 45 45 45	47.5
	2.8*	50 45 45 45 45 50 50 45 40 40	45.5
	1.8	45 35 40 30 45 45 40 40 40 50	41
39	1.8	50 40 45 35 45 50 45 40 45 50	44.5
42	1.8	50 50 50 40 50 50 35 45 40 55	46.5

* Tubers completely decayed. No further measurements made.

Table 22. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fa1) of *Fusareum avenaceum.* Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	8 8 7 6 6 9 5 8 6 8	7.1
	4.8	6 7 8 6 4 5 5 6 5 6	5.8
	3.8	6 5 7 4 4 5 5 6 4 0	5.1
	2.8	4 5 4 3 6 4 3 3 3 0	3.9
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	30 35 25 25 25 20 15 30 20 35	26
	4.8	30 25 15 35 10 30 20 25 15 25	23
	3.8	20 25 20 15 20 15 25 30 10 15	19.5
	2.8	15 25 20 10 15 25 20 10 25 10	17.5
	1.8	10 15 20 15 8 10 8 20 8 18	12
18	5.8	40 35 45 25 45 40 35 50 45 25	38.5
	4.8	35 45 20 45 35 40 15 35 45 45	36
	3.8	40 45 15 35 40 30 45 20 45 35	35
	2.8	30 35 40 40 30 20 30 40 25 35	32.5
	1.8	35 35 35 15 25 20 25 30 35 45	30
24	5.8	50 55 30 45 65 65 30 45 50 40	47.5
	4.8	45 55 50 65 35 30 25 45 55 60	46.5
	3.8	35 55 20 35 65 50 55 45 45 35	44
	2.8	35 45 30 35 45 60 50 45 35 35	41.5
	1.8	40 45 40 25 30 25 30 40 40 50	36.5

Table 23. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with (Fa1) isolate of *Fusareum avenaceum.* Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	8 8 5 6 5 5 8 6 6 8	6.5
	4.8	5 6 4 7 8 3 4 5 5 0	5.2
	3.8	4 6 4 3 6 4 5 8 4 0	4.9
	2.8	3 4 3 5 3 5 3 3 4 0	3.7
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	15 20 15 20 25 15 10 20 10 25	17.5
	4.8	15 15 10 8 25 10 20 25 10 15	15.3
	3.8	10 15 10 8 15 10 15 15 20 15	13.3
	2.8	10 15 10 8 8 1520 10 8 8	11.2
	1.8	10 8 10 15 8 5 6 10 12 8	9.2
18	5.8	35 25 35 20 15 25 30 25 25 35	27
	4.8	20 30 15 30 10 15 35 30 20 20	22.5
	3.8	15 20 25 20 20 25 10 30 15 25	20.5
	2.8	20 15 15 25 25 20 10 15 20 25	18.6
	1.8	10 25 10 15 15 20 10 15 10 15	14.5
24	5.8	35 30 35 40 25 45 20 40 45 35	35
	4.8	30 20 40 35 35 20 45 20 40 35	32
	3.8	25 25 25 30 35 35 30 20 25 30	27.5
	2.8	35 20 10 25 25 30 20 15 35 25	23.2
	1.8	25 20 20 30 15 30 15 25 15 25	21.7

Table 24. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fa2) of *Fusareum avenaceum.* Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 8 9 7 6 4 5 8 7 8	6.7
	4.8	6 7 5 4 5 4 4 5 5 6	5.1
	3.8	5 5 6 4 5 4 4 5 4 6	4.8
	2.8	5 3 4 4 3 3 4 3 5 0	3.8
	1.8		
12	5.8	25 25 30 35 25 25 15 25 30 15	25
	4.8	15 20 15 30 25 15 25 30 25 20	22
	3.8	15 20 15 30 15 20 25 15 30 25	21
	2.8	20 15 10 25 15 20 20 15 15 20	17.5
	1.8	15 10 15 10 10 15 20 15 25 15	15
18	5.8	40 30 40 35 35 45 35 20 40 35	35.5
	4.8	35 25 35 35 35 30 40 35 35 40	34.5
	3.8	40 40 30 25 30 25 30 25 25 30	30
	2.8	35 30 20 35 35 20 30 20 25 20	27
	1.8	30 25 15 25 15 25 15 15 15 25	20
24	5.8	45 55 45 40 35 65 35 55 50 25	45
	4.8	40 55 35 50 55 35 40 35 35 45	42.5
	3.8	45 40 45 45 30 35 55 35 30 45	40.5
	2.8	40 50 25 30 45 35 45 35 30 40	37.5
	1.8	30 40 35 25 25 35 30 35 40 35	33

Table 25. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Fa2) *Fusareum avenaceum.* Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 8 5 7 5 5 5 4 5 0	5.4
	4.8	5 6 4 3 5 5 6 3 4 0	4.6
	3.8	5 3 4 3 4 4 3 3 0 0	3.6
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	20 25 20 15 20 8 15 15 10 7	15.5
	4.8	10 20 10 20 15 20 15 10 15 8	14.3
	3.8	20 10 10 8 10 15 15 8 15 12	12.3
	2.8	15 8 10 10 10 15 8 15 8 10	10.9
	1.8	8 15 10 8 8 7 10 10 8 5	8.9
18	5.8	20 30 20 40 35 25 35 30 30 20	28.5
	4.8	30 20 30 30 30 20 25 20 25 15	24.5
	3.8	35 20 20 30 15 25 15 30 15 20	22.5
	2.8	15 20 10 30 15 10 25 20 15 20	18
	1.8	20 25 10 10 25 15 20 15 20 10	17
24	5.8	45 45 50 35 40 35 45 30 50 40	41.5
	4.8	40 30 40 40 40 45 25 35 45 45	38.5
	3.8	45 40 45 30 35 30 25 35 40 30	35.5
	2.8	30 40 40 35 30 20 30 30 40 20	31.5
	1.8	20 20 35 30 35 15 35 35 20 15	27.2

Table 26. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fa3) of *Fusareum avenaceum.* Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 6 7 6 8 6 7 4 5 6	6
	4.8	5 5 6 7 8 4 4 4 5 4	5.2
	3.8	6 3 3 4 4 4 5 5 3 0	4.1
	2.8	3 4 3 3 3 4 3 4 0 0	3.4
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	20 30 15 25 25 30 25 25 15 25	23.5
	4.8	25 25 15 25 15 15 30 25 15 10	20
	3.8	10 15 25 25 30 15 10 20 15 30	19.5
	2.8	15 20 15 15 10 25 10 15 15 10	15
	1.8	10 10 15 15 15 15 15 20 10 15	14
18	5.8	45 30 40 30 30 35 30 35 25 25	32.5
	4.8	30 25 25 30 30 30 35 25 35 40	30.5
	3.8	35 30 45 35 25 15 20 20 30 20	27.5
	2.8	40 25 30 15 20 25 30 30 20 35	27
	1.8	25 20 25 30 30 25 25 15 25 15	23.5
24	5.8	40 35 45 50 35 35 30 35 45 35	38.5
	4.8	45 40 35 40 35 30 35 30 40 45	37.5
	3.8	30 30 35 45 40 35 40 40 35 30	36
	2.8	35 25 20 30 30 40 40 40 45 40	34.5
	1.8	25 40 30 35 25 35 45 20 25 30	31

Table 27. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Fa3) of *Fusareum avenaceum.* Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 6 4 5 6 4 7 5 6 5	5.3
	4.8	5 4 6 6 4 3 4 5 4 0	4.6
	3.8	3 4 3 5 4 4 3 3 0 0	3.6
	2.8	3 3 3 3 3 3 0 0 0 0	3
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	10 10 10 15 15 25 25 20 15 15	16
	4.8	15 10 15 20 15 20 15 20 10 15	15.5
	3.8	10 15 5 10 10 15 15 10 8 20	11.8
	2.8	15 10 10 15 12 8 8 10 5 15	10.8
	1.8	5 10 6 8 12 10 10 5 5 8	7.9
18	5.8	15 20 30 25 30 30 30 25 35 20	26
	4.8	30 25 20 25 25 30 20 15 25 20	23.5
	3.8	15 30 15 30 30 30 20 15 15 10	21
	2.8	20 25 15 25 15 15 10 25 20 15	18.5
	1.8	15 15 15 20 10 10 20 15 15 10	14.5
24	5.8	40 35 30 30 35 40 35 30 40 30	34.5
	4.8	30 25 25 25 35 30 35 25 35 40	30.5
	3.8	20 30 20 35 30 25 25 45 25 25	28
	2.8	20 15 25 25 30 35 30 35 25 30	27
	1.8	30 25 15 30 25 25 25 20 15 25	23.5

Table 28. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fa4) of *Fusareum avenaceum.* Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	8 5 7 6 8 7 5 4 7 4	6.1
	4.8	5 6 4 5 5 7 6 4 5 0	5.2
	3.8	3 4 5 5 6 3 3 4 5 0	4.2
	2.8	3 3 3 4 4 3 3 3 0 0	3.3
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	20 20 20 15 25 25 15 25 15 15	19.5
	4.8	15 20 15 25 20 15 20 20 20 15	18.5
	3.8	20 25 15 15 20 15 10 20 15 10	16.5
	2.8	15 10 8 12 15 12 15 20 10 10	12.7
	1.8	15 5 12 8 10 15 10 12 5 8	10
18	5.8	25 25 30 35 30 30 25 30 20 30	28
	4.8	20 25 30 35 30 20 25 30 25 20	26
	3.8	25 20 20 15 25 25 25 15 25 20	20
	2.8	20 20 15 15 25 20 20 15 25 20	19.5
	1.8	15 20 15 15 20 20 15 20 10 20	17
24	5.8	30 40 40 45 30 40 30 40 30 30	35.5
	4.8	30 30 35 30 40 35 45 30 25 30	33
	3.8	25 20 35 25 40 30 25 35 40 35	31
	2.8	30 25 20 30 20 20 30 35 30 35	27.5
	1.8	25 35 25 20 20 30 30 25 20 20	25

Table 29. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Fa4) of *Fusareum avenaceum.* Experiment set up Marsh 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 5 6 6 5 7 4 6 8 4	5.6
	4.8	5 6 4 5 6 4 3 4 5 0	4.7
	3.8	5 4 4 4 5 3 4 3 0 0	4
	2.8	3 3 3 3 3 3 3 0 0 0	3
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	15 10 15 20 10 15 20 15 15 8	14.3
	4.8	10 10 15 8 12 15 15 15 12 15	12.3
	3.8	10 15 10 10 15 10 8 8 12 8	10.9
	2.8	12 10 15 8 12 5 5 10 8 8	9.3
	1.8	5 7 8 12 10 6 7 4 8	7.4
18	5.8	25 25 15 20 25 15 20 12 20 25	20.2
	4.8	25 15 15 12 20 15 25 20 20 20	18.2
	3.8	25 15 12 20 15 25 12 15 20 20	17.9
	2.8	20 15 15 12 15 25 10 15 12 25	16.4
	1.8	10 15 10 8 15 15 10 15 12 15	12.5
24	5.8	25 35 30 40 20 30 30 30 20 30	29
	4.8	25 40 30 35 30 25 20 20 25 35	28.5
	3.8	25 25 30 30 35 20 30 35 20 15	26.5
	2.8	30 25 15 15 25 25 20 30 30 25	24
	1.8	25 15 20 15 20 20 20 20 25 15	19.5

Table 30. The diameter of dry rot lesion measured internally in potato tubers of cv Maris Piper inoculated with different isolates of *F. avenaceum* 24 days after inoculation. Experiment set up March 1991.

Isolates	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
Fa1	5.8	50 55 50 45 45 55 60 40 45 45	49
	4.8	40 50 50 60 60 45 45 45 45 45	48.5
	3.8	50 45 40 40 45 40 40 55 50 55	46
	2.8	35 40 45 35 40 45 40 40 40 40	40
	1.8	30 25 35 45 35 45 35 35 30 30	35
Fa2			
	5.8	45 45 50 50 60 35 45 50 30 50	46.5
	4.8	50 45 50 40 50 40 40 45 45 45	45
	3.8	50 45 45 35 35 40 35 50 50 45	43
	2.8	30 45 40 35 40 40 35 40 45 35	38.5
	1.8	35 40 35 25 30 35 40 35 35 30	34
Fa3	5.8	35 40 35 50 40 40 35 45 40 35	39.5
	4.8	40 40 35 40 50 45 45 30 40 35	40
	3.8	35 35 40 40 40 40 45 35 40 40	39
	2.8	25 35 30 30 30 40 45 35 40 40	35
	1.8	30 30 40 35 20 35 35 30 20 15	29
Fa4	5.8	35 40 40 45 35 45 35 30 35 50	39
	4.8	30 45 25 30 35 40 45 30 35 35	35
	3.8	30 35 40 35 35 35 35 40 35 40	36
	2.8	30 35 25 30 25 30 25 35 25 30	29
	1.8	30 30 20 30 30 30 25 35 30 40	30

Table 31. The diameter of dry rot lesion measured internally in potato tubers of cv Record inoculated with isolates of *F. avenaceum.* 24 days after inoculation. Experiment set up March 1991.

Isolates	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
Fa1	5.8	35 40 50 40 30 45 45 45 45 50	42.5
	4.8	45 40 40 40 40 45 45 35 40 45	41.5
	3.8	35 40 40 30 45 40 45 35 40 30	38
	2.8	35 20 30 30 35 40 20 40 35 20	30.5
	1.8	20 30 30 30 35 15 22 30 30 30	27
Fa2			
	5.8	50 45 45 45 40 50 40 40 40 50	44.5
	4.8	40 35 50 35 30 40 45 30 30 40	37.5
	3.8	30 40 20 45 30 35 35 35 35 50	35.5
	2.8	30 35 20 30 35 40 20 35 35 40	32
	1.8	20 40 30 25 25 30 35 35 30 30	30
Fa3	5.8	35 35 40 40 35 35 40 35 30 40	36.5
	4.8	40 25 35 30 30 40 40 30 40 35	34.5
	3.8	30 30 30 30 35 35 30 35 40 35	32
	2.8	35 20 30 35 30 35 30 30 20 15	28
	1.8	30 30 30 30 20 40 30 35 30 20	29.5
Fa4	5.8	40 35 35 35 20 20 40 35 40 30	33
	4.8	20 40 35 35 35 35 30 35 35 45	34.5
	3.8	30 35 35 20 35 40 35 35 20 35	32
	2.8	20 30 20 30 20 35 30 20 30 35	27
	1.8	30 30 35 20 15 30 30 20 25 15	25

Table 32. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fa1) of *F. avenaceum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	6 8 7 5 8 4 6 5 4 7	6
	4.8	5 4 7 6 6 5 5 4 7 0	5.4
	3.8	5 6 4 3 4 5 4 4 4 0	4.3
	2.8	4 3 3 3 4 3 3 0 0 0	3.3
	1.8		0
12	5.8	20 25 25 25 20 25 15 20 15 10	20
	4.8	20 15 25 10 20 15 25 10 20 25	18.5
	3.8	15 10 15 20 15 8 12 15 20 25	15.5
	2.8	10 15 15 10 8 12 8 15 10 15	11.8
	1.8	10 10 8 12 7 15 10 7 15 15	10.9
18	5.8	35 30 40 25 40 15 40 35 35 15	31
	4.8	30 35 35 30 40 25 15 20 25 30	28.5
	3.8	35 20 30 25 20 25 30 30 15 15	24.5
	2.8	30 30 30 30 15 20 15 20 25 25	23.9
	1.8	30 20 15 20 10 25 20 15 15 30	20
24	5.8	45 50 45 45 55 35 50 40 45 45	45.5
	4.8	45 50 45 35 45 40 35 45 50 55	44.5
	3.8	45 50 45 40 45 45 45 35 50 25	42.5
	2.8	30 45 45 30 30 25 40 45 45 40	37.5
	1.8	40 30 30 30 30 30 45 40 35 25	33.5

Table 33. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Fa1) of *F. avenaceum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 6 7 5 6 5 4 5 4 6	5.3
	4.8	5 5 4 6 4 4 5 6 4 0	4.8
	3.8	3 4 4 3 4 3 3 3 0 0	3.4
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	15 10 12 8 15 15 12 8 10 12	11.7
	4.8	8 10 12 15 10 10 12 8 7	10.2
	3.8	7 8 12 10 15 12 8 9 8	9.9
	2.8	8 10 6 7 4 10 6 12 10 8	8.1
	1.8	6 7 6 5 5 8 9 10 5 5	6.6
18	5.8	20 10 15 20 15 15 25 20 15 20	17.5
	4.8	15 25 20 10 10 15 25 10 10 15	15.5
	3.8	10 10 20 20 20 15 10 10 20 10	14.5
	2.8	10 15 12 8 15 12 10 8 12 15	11.7
	1.8	10 10 8 12 5 8 6 10 8 12	8.9
24	5.8	40 25 30 20 35 25 25 40 25 30	29.5
	4.8	30 25 30 35 35 35 20 25 35 20	29
	3.8	30 35 25 20 15 25 35 30 35 30	27.8
	2.8	20 15 20 15 20 10 25 15 15 10	16.5
	1.8	10 10 20 15 20 20 10 15 15 10	14.5

Table 34. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fa2) of *F. avenaceum*. Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	8 6 8 7 8 6 8 4 8 5	6.8
	4.8	5 6 7 8 4 6 4 5 6 6	5.7
	3.8	5 4 6 4 5 4 6 3 4 5	4.6
	2.8	4 4 3 3 4 3 3 3 0 0	3.4
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	25 15 25 25 20 20 25 25 25 30	23.5
	4.8	25 15 25 20 25 15 25 20 25 15	21
	3.8	15 25 25 20 15 10 25 15 25 25	20
	2.8	15 15 15 15 10 10 15 15 20 15	14.5
	1.8	10 15 10 15 15 10 10 10 8 12	11.5
18	5.8	40 35 40 35 25 30 20 35 40 20	32
	4.8	40 20 30 25 20 35 25 35 35 40	30.5
	3.8	35 35 25 20 25 25 15 30 30 35	27.5
	2.8	20 30 30 35 30 15 30 20 35 20	26.5
	1.8	25 30 20 35 15 20 20 15 15 10	20.5
24	5.8	50 45 50 45 50 45 35 55 40 35	45
	4.8	50 40 45 40 45 40 55 40 35 55	44.5
	3.8	50 4540 50 40 35 35 55 45 35	43
	2.8	40 40 45 45 50 30 30 40 45 45	41
	1.8	45 30 40 3040 45 30 30 30 20	35.6

Table 35. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Fa2) of *F. avenaceum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 5 6 7 5 8 4 4 5 5	5.4
	4.8	5 4 6 6 4 5 4 4 3 0	4.6
	3.8	3 4 5 3 3 3 4 4 0 0	3.6
	2.8	3 3 3 3 3 3 0 0 0 0	3
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	10 20 15 15 10 10 15 12 8 8	12.3
	4.8	6 8 8 10 10 8 15 10 10 15	10
	3.8	8 10 6 8 10 12 15 8 10 10	9.7
	2.8	10 6 10 6 12 10 8 10 8 9	8.9
	1.8	6 7 5 4 5 7 6 7 8 4	5.9
18	5.8	25 15 30 15 25 15 25 30 30 15	22.5
	4.8	10 15 15 20 15 15 25 25 15 30	18.5
	3.8	15 10 25 15 20 25 20 15 10 15	17
	2.8	10 20 15 20 15 10 15 20 15 15	15.5
	1.8	10 8 8 10 6 12 10 12 10 8	9.4
24	5.8	40 30 35 40 30 25 40 30 30 45	34.5
	4.8	35 35 25 30 25 20 40 35 40 35	32
	3.8	25 20 35 35 25 15 20 35 35 40	28.5
	2.8	35 20 15 20 20 20 20 15 30 30	22.5
	1.8	15 15 15 10 10 30 20 15 20 20	17

Table 36. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper inoculated with isolate (Fa3) of *F. avenaceum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	6 5 4 6 7 5 4 5 4 5	5.1
	4.8	5 4 4 6 7 4 3 3 4 0	4.4
	3.8	4 3 4 3 3 3 3 4 0 0	3.4
	2.8	0 0 0 0 0 0 0 0 0 0	3
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	20 10 15 25 25 20 15 25 15 10	18
	4.8	20 20 15 10 25 15 15 20 10 15	16.5
	3.8	15 15 10 15 8 12 10 15 8 12	12
	2.8	15 15 10 8 12 8 10 5 7 10	10
	1.8	10 12 8 7 6 7 6 7 5	7.6
18	5.8	30 30 20 35 25 30 40 35 25 30	30
	4.8	35 30 20 25 30 35 35 25 20 35	29
	3.8	30 20 15 25 20 15 30 25 20 25	22.5
	2.8	15 10 20 15 25 10 25 15 20 25	18
	1.8	15 20 20 15 10 15 15 15 20 10	15.5
24	5.8	45 40 40 45 35 30 35 30 35 45	38
	4.8	45 40 25 35 35 45 40 35 40 35	37.5
	3.8	35 35 40 45 35 40 35 35 30 30	36
	2.8	35 30 30 25 20 30 20 25 20 30	26.5
	1.8	20 25 20 15 30 30 30 20 25 30	24.5

Table 37. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Fa3) of *F. avenaceum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	6 7 8 5 4 4 5 6 3 0	5.3
	4.8	4 5 6 6 4 4 4 3 3 0	4.3
	3.8	3 4 4 3 3 4 4 3 0 0	3.5
	2.8	3 3 3 3 3 3 0 0 0 0	3
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	10 15 10 12 8 10 8 12 12 8	10.5
	4.8	6 7 10 10 12 8 12 10 12 8	9.5
	3.8	10 8 12 8 10 8 7 6 5 0	8.2
	2.8	6 7 8 12 10 6 7 8 6 5	7.5
	1.8	6 7 8 5 5 4 5 6 0 0	5.8
18	5.8	15 25 20 15 25 10 15 10 15 20	17
	4.8	10 15 10 10 25 20 15 15 10 15	15
	3.8	10 15 15 10 15 15 20 10 15 8	13.9
	2.8	8 10 8 12 8 10 15 10 12 10	10.3
	1.8	8 10 12 8 8 7 10 10 6 6	8.5
24	5.8	20 25 20 25 35 30 15 40 35 30	27.2
	4.8	20 30 20 15 35 35 15 40 20 35	26.5
	3.8	15 30 20 35 30 30 30 25 15 15	24.5
	2.8	15 25 15 15 15 20 25 15 20 15	18
	1.8	10 12 10 15 15 20 12 8 10 10	12.2

Table 38. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fa4) *F. avenaceum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	6 7 6 6 7 5 4 4 5 6	5.6
	4.8	5 6 6 7 5 4 4 3 4 5	4.9
	3.8	4 5 5 4 4 4 3 3 4 3	3.9
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	15 10 25 10 15 15 20 15 20 15	16
	4.8	10 20 20 15 10 15 10 15 15 10	14
	3.8	15 10 15 15 15 10 15 8 12 15	13
	2.8	10 8 9 12 10 12 5 10 10 8	9.4
	1.8	10 8 5 8 12 10 6 5 8 8	8
18	5.8	25 35 20 20 30 30 20 25 30 25	26
	4.8	30 25 20 15 15 25 25 30 15 20	22
	3.8	20 15 20 30 25 20 20 20 20 15	20.5
	2.8	25 15 20 15 10 20 15 15 20 15	17
	1.8	15 20 15 20 15 10 15 10 8 8	13.6
24	5.8	30 25 35 30 40 30 30 35 45 30	33
	4.8	40 30 40 20 30 40 30 30 35 30	32.5
	3.8	40 30 30 20 25 30 30 30 30 30	29.5
	2.8	30 25 20 25 25 25 25 30 20 20	21.8
	1.8	20 15 20 25 12 25 30 15 15 15	19.2

Table 39. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Fa4) of *F. avenaceum.* Experiment set up January 1992.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	6 5 7 5 5 4 5 6 6 5	5.4
	4.8	6 4 4 5 4 4 4 5 5 4	4.5
	3.8	3 4 5 4 4 3 3 3 3 4	3.6
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	15 15 10 15 10 15 10 12 8 8	11.8
	4.8	10 10 8 8 8 12 12 10 8 12	9.8
	3.8	8 6 8 10 10 9 12 8 10 10	9.1
	2.8	5 8 6 4 5 8 8 6 5 5	6.
	1.8	5 6 5 4 4 7 6 8 0 0	5.6
18	5.8	20 25 30 15 20 25 15 15 20 25	21
	4.8	20 15 25 15 15 20 15 30 15 10	17.7
	3.8	15 10 15 10 15 20 10 15 10 10	13
	2.8	10 15 12 10 8 8 10 10 12 15	11
	1.8	10 8 8 6 8 10 9 10 6 10	8.5
24	5.8	30 25 20 20 25 25 35 20 20 25	24
	4.8	25 20 30 20 20 25 15 35 15 15	21.8
	3.8	20 15 25 25 20 25 20 15 15 15	19.5
	2.8	20 15 25 15 20 15 15 10 10 15	16
	1.8	15 10 10 10 12 15 15 15 10 15	12.7

Table 40. The diameter of dry rot lesion measured internally in potato tubers of cv Maris Piper inoculated with different isolates of *F. avenaceum* 24 dayas after inoculation. Experiment set up January 1992.

Isolates	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
Fa1	5.8	50 45 45 40 35 35 45 40 40 35	41
	4.8	50 45 40 40 45 35 40 40 30 30	39
	3.8	35 45 40 35 25 35 45 40 35 45	38
	2.8	35 25 30 35 25 40 25 30 30 35	31
	1.8	25 30 30 30 35 35 20 20 30 25	28
Fa2			
	5.8	45 35 50 50 50 45 45 45 45 50	46
	4.8	45 45 40 40 50 50 45 45 45 50	45.5
	3.8	35 40 45 45 35 45 40 45 45 40	41
	2.8	45 35 40 25 40 35 25 40 35 40	36
	1.8	35 40 25 40 30 35 30 25 30 30	32
Fa3	5.8	35 40 35 45 30 30 40 30 40 40	36.5
	4.8	35 35 45 45 35 45 35 30 25 25	36
	3.8	35 30 35 30 40 35 35 40 30 30	34
	2.8	20 35 30 30 35 20 25 30 20 15	26
	1.8	25 15 20 15 30 30 20 25 35 30	24.5
Fa4	5.8	30 30 30 25 30 35 35 25 35 40	31.5
	4.8	30 35 40 25 25 40 40 35 20 35	32.5
	3.8	35 30 40 30 35 40 35 20 30 15	31
	2.8	20 30 30 30 35 35 20 30 25 35	29
	1.8	20 30 30 10 35 40 25 35 30 15	27

Table 41. The diameter of dry rot lesion measured internally in potato tubers of cv Record inoculated with different isolates of *F. avenaceum* 24 days after inoculation. Experiment set up January 1992.

Isolates	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
Fa1	5.8	40 35 35 30 45 25 30 20 45 45	35
	4.8	30 20 35 25 30 40 30 30 45 25	31
	3.8	25 30 30 30 20 45 35 15 35 40	30.5
	2.8	10 15 25 20 10 20 25 20 30 30	20.5
	1.8	25 15 25 15 20 15 10 10 15 15	16.5
Fa2	5.8	30 35 35 35 30 35 40 45 40 30	35.5
	4.8	40 40 30 30 35 30 35 30 35 35	34
	3.8	20 30 20 30 30 25 30 35 35 35	29
	2.8	10 25 30 30 20 30 30 20 20 20	23.5
	1.8	15 25 30 20 25 20 15 15 20 15	20
Fa3	5.8	35 30 25 35 30 25 30 35 30 35	31
	4.8	35 30 25 30 30 20 30 35 30 20	28.5
	3.8	30 35 30 25 35 30 40 35 30 15	30.5
	2.8	15 20 20 25 20 25 15 30 15 30	21.5
	1.8	10 15 15 20 15 15 20 15 20 10	15.5
Fa4	5.8	30 40 40 35 25 35 35 35 25 30	33
	4.8	33 25 40 25 40 35 30 30 40 40	34
	3.8	35 35 25 20 30 20 40 30 25 40	30
	2.8	15 10 10 25 15 15 20 25 30 20	18.5
	1.8	15 20 15 10 25 15 20 10 10 10	15

Table 42. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Fa1) of *F. avenaceum.* Experiment set up April 1993.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 10 8 8 10 8 5 8 5 8	7.5
	4.8	7 8 5 8 8 8 5 6 6 5	6.6
	3.8	6 5 5 4 5 5 5 7 4	5.1
	2.8	5 4 4 4 4 3 5 0 0 0	4.1
	1.8	4 3 3 3 3 4 5 0 0 0	3.6
9	5.8	12 18 15 15 15 10 18 10 15 12	14
	4.8	15 10 10 12 12 12 10 12 10 10	11.3
	3.8	10 10 12 10 10 10 10 8 12 12	10.4
	2.8	7 6 7 8 4 5 8 6 5 8	6.4
	1.8	5 5 5 4 5 6 7 4 5 4	5
12	5.8	25 15 20 15 15 20 25 20 20 20	19.5
	4.8	15 15 10 20 20 25 20 15 15 20	17.5
	3.8	20 15 20 10 15 10 20 15 18 25	16.8
	2.8	8 10 15 15 15 10 18 16 12 15	13.4
	1.8	6 10 12 8 12 8 10 12 15 12	10.5
15	5.8	25 30 18 30 25 20 20 15 20 20	22.3
	4.8	25 15 15 25 20 20 20 18 25 20	20.3
	3.8	25 15 30 15 15 20 18 16 15 20	18.9
	2.8	20 15 15 15 10 15 20 15 20 15	16
	1.8	12 15 16 10 15 12 12 16 18 8	13.4
18	5.8	25 20 25 20 25 30 35 25 35 20	26
	4.8	25 20 20 20 35 25 20 25 20 30	24
	3.8	20 15 20 25 20 35 20 25 20 25	22.5
	2.8	20 12 25 18 15 25 25 20 25 15	20
	1.8	25 20 12 25 18 20 15 12 15 20	18.2
21	5.8	25 25 40 25 20 25 40 30 30 25	28.5
	4.8	25 25 30 25 25 25 35 30 25 20	26.5
	3.8	25 20 25 25 25 40 25 30 25 20	26
	2.8	25 15 20 25 25 30 20 30 30 25	24.5
	1.8	30 25 15 18 20 25 18 15 25 30	22.1

24	5.8	35 30 45 30 25 26 40 35 30 30	32.6
	4.8	30 28 35 30 28 25 40 35 30 25	30.6
	3.8	30 25 28 27 25 43 30 35 30 25	29.8
	2.8	30 28 25 25 25 35 22 22 35 25	27.2
	1.8	20 30 18 35 25 25 20 20 25 35	25.3
27	5.8	40 33 46 35 30 30 45 35 35 30	35.9
	4.8	30 30 40 35 30 28 42 40 35 25	33.5
	3.8	30 30 30 30 30 45 35 40 30 30	33
	2.8	35 30 25 30 25 35 25 25 35 30	29.5
	1.8	25 30 20 22 40 25 25 35 25 20	26.7
30	5.8	45 35 50 40 35 30 46 40 40 35	39.6
	4.8	35 30 45 35 35 30 45 45 35 30	36.5
	3.8	35 35 30 35 30 47 35 45 35 30	35.7
	2.8	40 35 25 35 30 35 30 30 40 35	33.5
	1.8	30 35 25 45 30 30 25 40 35 25	32
33	5.8	50 35 55 45 40 35 50 45 40 40	43.5
	4.8	40 35 45 40 40 35 50 50 35 35	40.5
	3.8	35 30 35 35 35 50 40 40 35 35	37
	2.8	40 35 30 45 30 35 30 30 40 35	35
	1.8	30 35 25 25 35 35 25 45 30 50	33.5
36	5.8*	55 40 55 50 40 40 55 50 45 40	47
	4.8*	45 40 55 45 40 40 55 55 40 40	45.5
	3.8	40 35 35 40 40 55 40 45 40 40	41
	2.8	45 30 30 35 35 40 35 35 45 50	38
	1.8	55 40 30 45 40 30 30 35 35 25	36.5
39	3.8*	45 40 35 45 40 55 45 50 45 45	44.5
	2.8	50 35 40 45 50 40 40 35 40 35	41
	1.8	55 45 35 30 40 35 30 30 40 50	39
42	2.8*	55 40 35 45 40 40 50 40 55 40	44
	1.8	60 45 40 35 45 35 50 35 45 35	42.5
45	1.8	35 60 45 40 50 45 40 40 53 35	44.5

* Tubers completely decayed. No furtur measurement made.

Table 43. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Cd1) of *C. destrictans.* Experiment set up March 1990.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	4 5 3 4 5 4 0 0 0 0	4.2
	4.8	3 4 3 3 3 4 3 0 0 0	3.3
	3.8	3 3 3 3 3 0 0 0 0 0	3
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	7 8 6 5 6 6 5 0 0 0	6.1
	4.8	4 6 5 6 6 5 5 0 0 0	5.3
	3.8	5 4 5 4 3 3 0 0 0 0	4
	2.8	3 4 3 3 3 0 0 0 0 0	3.2
	1.8	0 0 0 0 0 0 0 0 0 0	0
18	5.8	10 15 8 6 13 8 5 7 10 0	9.1
	4.8	10 12 8 5 6 5 5 10 5 0	7.3
	3.8	5 8 8 5 5 6 5 10 0 0	6.5
	2.8	4 4 3 3 4 0 0 0 0 0	3.6
	1.8	0 0 0 0 0 0 0 0 0 0	0
24	5.8	12 15 12 16 15 16 8 12 18 0	13.8
	4.8	10 15 12 15 16 5 8 9 12 0	11.3
	3.8	8 8 10 5 8 9 5 10 0 0	7.9
	2.8	5 4 4 5 5 0 0 0 0 0	4.6
	1.8	0 0 0 0 0 0 0 0 0 0	0

Table 44. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Cd1) of *C. destructans*. Experiment set up March 1990.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 6 5 4 6 5 0 0 0 0	5.2
	4.8	4 5 4 3 4 0 0 0 0 0	4.
	3.8	3 3 3 3 0 0 0 0 0 0	3
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	7 5 6 7 5 7 8 0 0 0	6.4
	4.8	5 5 6 5 5 10 4 0 0 0	5.7
	3.8	5 4 6 7 5 5 0 0 0 0	5.3
	2.8	3 4 3 3 3 4 0 0 0 0	3.3
	1.8	0 0 0 0 0 0 0 0 0 0	0
18	5.8	15 12 8 15 10 8 6 9 10 0	10.3
	4.8	10 8 5 15 6 7 5 10 0 0	8.3
	3.8	5 6 8 6 5 8 5 0 0 0	6.1
	2.8	4 3 5 5 4 4 0 0 0 0	4.2
	1.8	0 0 0 0 0 0 0 0 0 0	0
24	5.8	15 14 12 20 18 8 13 14 15	14.3
	4.8	15 10 5 15 7 8 8 13 15	10.7
	3.8	5 7 8 6 8 12 5 0 0 0	7.3
	2.8	5 6 4 4 6 5 0 0 0 0	5.
	1.8	0 0 0 0 0 0 0 0 0 0	0

Table 45. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Cd2) of *C. destrictans.* Experiment set up March 1990.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	6 5 6 5 5 6 0 0 0 0	5.5
	4.8	5 4 5 5 4 0 0 0 0 0	4.6
	3.8	3 4 4 5 3 0 0 0 0 0	3.8
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	10 5 6 10 5 8 8 0 0 0	7.4
	4.8	6 5 5 6 6 5 10 12 8 0	7
	3.8	4 5 5 7 5 7 10 0 0 0	6.1
	2.8	3 3 3 3 0 0 0 0 0 0	3
	1.8	0 0 0 0 0 0 0 0 0 0	0
18	5.8	15 15 8 12 8 12 12 15 12 0	12.1
	4.8	10 8 5 10 8 6 15 15 10 0	9.7
	3.8	5 6 5 8 10 10 8 0 0 0	7.4
	2.8	4 3 4 5 3 0 0 0 0 0	3.8
	1.8	0 0 0 0 0 0 0 0 0 0	0
24	5.8	20 5 12 16 15 15 25 20 12 5	14.5
	4.8	15 15 6 15 12 7 20 20 12 0	13.6
	3.8	6 7 6 12 15 12 15 5 0 0	9.8
	2.8	5 5 6 4 4 0 0 0 0 0	4.8
	1.8	0 0 0 0 0 0 0 0 0 0	0

Table 46. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Cd2) of *C. destructans*. Experiment set up March 1990.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	8 7 8 4 6 6 5 0 0 0	6.3
	4.8	5 4 5 6 6 7 0 0 0 0	5.5
	3.8	5 4 4 3 5 3 3 0 0 0	3.8
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	12 8 12 10 10 7 5 10 6 0	8.9
	4.8	6 5 7 8 10 10 5 5 6 0	6.9
	3.8	6 5 5 4 7 5 5 6 0 0	5.4
	2.8	3 4 3 3 0 0 0 0 0 0	3.3
	1.8	0 0 0 0 0 0 0 0 0 0	0
18	5.8	12 12 16 15 15 10 18 15 5	12.6
	4.8	8 6 10 12 15 13 6 6 8 0	9.3
	3.8	8 6 8 5 10 8 6 12 0 0	7.9
	2.8	4 5 5 3 3 0 0 0 0 0	4
	1.8	0 0 0 0 0 0 0 0 0 0	0
24	5.8	18 15 20 25 18 25 10 12 20 8	17.1
	4.8	8 10 20 15 25 20 15 10 16	15.4
	3.8	8 10 10 12 15 25 15 12 0 0	13.4
	2.8	6 7 4 4 6 3 0 0 0 0	5
	1.8	0 0 0 0 0 0 0 0 0 0	0

Table 47. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Cd3) of *C. destrictans.* experiment set up March 1990.

Time days	Log number of spores inoculated	Mean size deameter of dry rot	Mean diameters
6	5.8	4 5 6 3 4 3 4 4 0 0	4.1
	4.8	4 5 3 3 4 4 0 0 0 0	3.8
	3.8	3 3 3 3 3 0 0 0 0 0	3
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	6 8 8 5 6 8 10 5 0 0	7
	4.8	5 6 6 5 5 6 0 0 0 0	5.5
	3.8	6 5 7 5 7 4 0 0 0 0	5.7
	2.8	3 3 3 0 0 0 0 0 0 0	3
	1.8	0 0 0 0 0 0 0 0 0 0	0
18	5.8	7 12 10 5 7 12 15 6 8 0	9.1
	4.8	4 6 10 10 6 5 8 5 0 0	6.8
	3.8	7 6 8 5 8 5 6 0 0 0	6.4
	2.8	4 3 5 4 0 0 0 0 0	4.
	1.8	0 0 0 0 0 0 0 0 0	0
24	5.8	10 15 10 6 8 15 20 7 10 0	11.4
	4.8	8 6 16 12 7 6 8 15 0 0	9.8
	3.8	8 12 8 6 9 5 10 0 0 0	8.2
	2.8	5 4 5 5 3 0 0 0 0 0	4.4
	1.8	0 0 0 0 0 0 0 0 0 0	0

Table 48. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Cd3) of *C. destructans.* Experiment set up March 1990.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 5 4 4 3 0 0 0 0 0	4.2
	4.8	4 4 4 3 3 0 0 0 0 0	3.6
	3.8	3 3 3 3 0 0 0 0 0 0	3.8
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	7 8 4 7 8 7 5 0 0 0	6.6
	4.8	5 6 5 8 7 6 0 0 0 0	6.2
	3.8	5 5 6 7 4 4 0 0 0 0	5.2
	2.8	3 4 3 3 4 0 0 0 0 0	3.4
	1.8	0 0 0 0 0 0 0 0 0 0	0
18	5.8	10 12 5 10 8 8 6 4 0 0	7.9
	4.8	8 8 5 10 12 7 4 0 0 0	7.7
	3.8	6 6 8 10 5 5 0 0 0 0	6.7
	2.8	4 4 5 5 4 0 0 0 0 0	4.4
	1.8	0 0 0 0 0 0 0 0 0 0	0
24	5.8	12 10 6 12 12 10 12 12 0 0	10.8
	4.8	10 10 6 12 15 8 10 0 0 0	10.2
	3.8	8 8 10 12 6 6 0 0 0 0	8.3
	2.8	5 4 5 6 6 0 0 0 0 0	5.2
	1.8	0 0 0 0 0 0 0 0 0 0	0

Table 49. The diameter of dry rot lesion measured internally in potato tubers of cv Maris Piper inoculated with different isolates of *C. destructans*. 24 dayas after inoculation. Experiment set up March 1990.

Isolates	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
Cd1	5.8	15 10 20 20 10 15 18 10 15 8	14.1
	4.8	15 8 12 10 10 16 8 12 8 14	11.3
	3.8	8 10 8 8 8 12 8 8 8 10	8.8
	2.8	7 8 6 8 7 4 3 3 4 3	5.3
	1.8	0 0 0 0 0 0 0 0 0 0	0
Cd2			
	5.8	15 20 8 15 25 15 30 12 15 25	18
	4.8	10 12 15 12 20 10 25 15 20 8	14
	3.8	10 8 15 10 12 16 15 12 8 10	11.6
	2.8	10 8 12 8 9 6 4 3 4 3	6.7
	1.8	0 0 0 0 0 0 0 0 0 0	0
Cd3	5.8	10 10 8 15 12 15 17 15 10 8	12
	4.8	10 8 10 10 12 15 8 12 8 10	10
	3.8	8 10 10 7 8 10 8 8 8 7	8.4
	2.8	8 6 10 7 8 4 3 3 4 3	5.6
	1.8	0 0 0 0 0 0 0 0 0 0	0

Table 50. The diameter of dry rot lesion measured internally in potato tubers of cv Record inoculated with different isolates of *C. destructans* 24 days after inoculation. Experiment set up March 1990.

Isolates	Log number of spores inoculated	Mean size deameter of dry rot	Mean diameters
d1	5.8	10 15 12 15 15 20 8 20 25 18	15.8
	4.8	10 12 8 15 10 12 16 10 20 12	12.5
	3.8	10 15 8 10 10 12 8 10 12 10	10.5
	2.8	10 8 15 10 8 6 4 3 3 4	7.1
	1.8	0 0 0 0 0 0 0 0 0 0	0
Cd2			
	5.8	10 15 30 15 20 12 25 30 35 25	21.7
	4.8	20 10 20 25 20 20 10 15 15 25	18
	3.8	20 25 8 20 10 15 20 15 20 10	16.3
	2.8	8 15 16 10 8 6 3 3 4 4	7.7
	1.8	0 0 0 0 0 0 0 0 0 0	0
Cd3	5.8	10 10 12 20 10 20 15 25 8 10	14
	4.8	10 15 10 10 10 10 15 15 8 15	12
	3.8	10 15 15 8 10 15 10 12 8 12	11.5
	2.8	10 8 10 8 6 4 3 3 3 4	5.9
	1.8	0 0 0 0 0 0 0 0 0 0	0

Table 51. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate of (Cd1) of *C. destrictans.* Experiment set up March 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	4 6 4 4 5 0 0 0 0 0	4.6
	4.8	4 3 5 3 3 4 4 0 0 0 0	3.7
	3.8	3 3 3 3 0 0 0 0 0 0	3
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	5 8 10 8 6 5 4 5 8 0	6.6
	4.8	5 7 4 8 10 6 7 4 0 0	6.4
	3.8	5 4 8 4 4 5 6 0 0 0	5.1
	2.8	3 4 3 3 3 0 0 0 0 0	3.2
	1.8	0 0 0 0 0 0 0 0 0 0	0
18	5.8	15 8 10 12 7 8 9 12 10 0	10.1
	4.8	8 10 8 12 15 10 10 6 0 0	9.9
	3.8	6 8 10 6 8 7 8 0 0 0	7.6
	2.8	4 5 3 4 5 5 0 0 0 0	4.3
	1.8	0 0 0 0 0 0 0 0 0 0	0
24	5.8	18 12 12 15 10 12 10 15 10 0	12.7
	4.8	10 15 12 15 16 12 12 8 0 0	12.5
	3.8	10 10 12 8 9 10 10 0 0 0	9.9
	2.8	6 6 3 4 6 5 3 0 0 0	4.7
	1.8	0 0 0 0 0 0 0 0 0 0	0

Table 52. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Cd1) of *C. destructans*. Experiment set up March 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	6 7 4 5 6 0 0 0 0 0	5.6
	4.8	4 3 5 6 0 0 0 0 0 0	4.5
	3.8	4 3 5 4 0 0 0 0 0 0	4
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	8 8 5 6 8 10 7 0 0 0	7.4
	4.8	5 8 8 8 10 4 5 0 0 0	6.9
	3.8	5 6 7 7 6 4 0 0 0 0	5.8
	2.8	3 4 3 3 4 0 0 0 0 0	3.4
	1.8	0 0 0 0 0 0 0 0 0 0	0
18	5.8	12 12 8 10 10 15 13 0 0 0	11.4
	4.8	8 8 10 12 12 15 8 0 0 0	10.4
	3.8	8 8 10 10 8 5 5 0 0 0	7.7
	2.8	5 5 4 4 4 5 0 0 0 0	4.5
	1.8	0 0 0 0 0 0 0 0 0 0	0
24	5.8	15 16 12 14 15 18 15 8 0	14.1
	4.8	10 10 12 15 15 16 8 0 0 0	12.3
	3.8	10 10 12 15 10 8 8 0 0 0	10.4
	2.8	7 6 5 5 6 6 3 0 0 0	5.4
	1.8	0 0 0 0 0 0 0 0 0 0	0

Table 53. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper inoculated with isolate (Cd2) of *C. destrictans.* Experiment set up March 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 4 6 4 5 4 0 0 0 0	4.7
	4.8	4 5 3 4 4 3 0 0 0 0	3.8
	3.8	3 3 3 3 3 0 0 0 0 0	3
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	8 6 10 8 7 6 12 5 0 0	7.8
	4.8	6 7 8 5 5 8 4 0 0 0	6.1
	3.8	6 8 5 5 6 3 0 0 0 0	5.5
	2.8	4 3 3 3 3 0 0 0 0 0	3.2
	1.8	0 0 0 0 0 0 0 0 0 0	0
18	5.8	12 10 15 10 10 8 16 14 0 0	11.9
	4.8	8 10 10 8 8 12 15 0 0 0	10.1
	3.8	7 10 8 8 8 10 5 0 0 0	8
	2.8	5 5 4 5 5 3 3 0 0 0	4.3
	1.8	0 0 0 0 0 0 0 0 0 0	0
24	5.8	15 12 18 12 16 10 18 16 0 0	14.6
	4.8	10 12 15 10 12 15 18 0 0	13.1
	3.8	10 12 10 10 10 15 12 0 0 0	11.3
	2.8	6 6 6 7 7 4 3 0 0 0	5.6
	1.8	0 0 0 0 0 0 0 0 0 0	0

Table 54. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Cd2) of *C. destructans* Experiment set up March 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	4 5 4 7 4 0 0 0 0 0	4.8
	4.8	4 5 4 4 4 3 0 0 0 0	4
	3.8	3 3 3 3 0 0 0 0 0 0	3
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	6 10 8 10 12 6 6 5 0 0	7.8
	4.8	7 7 8 8 10 12 6 0 0 0	8.2
	3.8	5 6 4 5 6 7 7 0 0 0	5.7
	2.8	3 4 3 3 3 0 0 0 0 0	3.2
	1.8	0 0 0 0 0 0 0 0 0 0	0
18	5.8	10 15 12 12 12 8 12 10 0 0	11.4
	4.8	10 12 12 10 15 10 6 0 0 0	10.7
	3.8	6 8 6 7 10 10 10 0 0 0	8.1
	2.8	4 6 4 4 3 3 3 0 0 0	3.9
	1.8	0 0 0 0 0 0 0 0 0 0	0
24	5.8	15 18 15 16 12 10 18 15 0 0	14.9
	4.8	10 15 15 12 18 18 15 6 0 0	13.6
	3.8	8 10 6 8 12 10 10 0 0 0	9.1
	2.8	5 6 5 5 4 4 4 0 0 0	4.1
	1.8	0 0 0 0 0 0 0 0 0 0	0

Table 55. Development of dry rot lesions measured externally on the tuber surface of cv Maris Piper, after inoculation with isolate (Cd3) of *C. destrictans*. Experiment set up March 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	4 5 5 4 3 6 7 3 0 0	4.6
	4.8	4 5 4 4 3 4 3 0 0 0	3.4
	3.8	3 3 3 3 3 0 0 0 0 0	3
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	5 7 7 5 6 7 10 5 0 0	6.5
	4.8	5 6 6 5 5 6 6 0 0 0	5.6
	3.8	5 5 5 6 5 4 0 0 0 0	5
	2.8	3 3 4 3 3 0 0 0 0 0	3.2
	1.8	0 0 0 0 0 0 0 0 0 0	0
18	5.8	8 10 16 8 8 10 15 16 0 0	11.4
	4.8	6 7 8 8 8 10 10 0 0 0	8.1
	3.8	6 6 7 10 8 8 5 0 0 0	7.1
	2.8	5 4 5 4 3 5 0 0 0 0	4.3
	1.8	0 0 0 0 0 0 0 0 0 0	0
24	5.8	12 15 12 10 16 13 17 10 0	13.1
	4.8	8 10 15 12 12 15 12 5 0 0	11.1
	3.8	8 7 10 15 10 10 8 0 0 0	9.7
	2.8	6 5 6 4 3 6 4 0 0 0	4.8
	1.8	0 0 0 0 0 0 0 0 0 0	0

Table 56. Development of dry rot lesions measured externally on the tuber surface of cv Record, after inoculation with isolate (Cd1) of *C. destructans* Experiment set up March 1991.

Time days	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
6	5.8	5 6 4 4 4 4 0 0 0 0	4.5
	4.8	6 4 3 4 4 5 5 3 0 0	4.3
	3.8	3 4 4 5 3 0 0 0 0 0	3.8
	2.8	0 0 0 0 0 0 0 0 0 0	0
	1.8	0 0 0 0 0 0 0 0 0 0	0
12	5.8	6 8 10 8 6 6 8 5 10 0	7.4
	4.8	6 7 7 5 5 8 8 10 0 0	7
	3.8	5 6 6 7 7 5 0 0 0 0	6
	2.8	3 3 3 4 0 0 0 0 0 0	3.3
	1.8	0 0 0 0 0 0 0 0 0 0	0
18	5.8	8 12 15 10 7 8 10 8 13 6	9.7
	4.8	10 10 9 8 8 10 10 8 5 0	8.7
	3.8	7 7 8 10 10 6 0 0 0 0	8
	2.8	4 4 3 5 4 5 0 0 0 0	4.2
	1.8	0 0 0 0 0 0 0 0 0 0	0
24	5.8	12 15 18 12 8 10 15 10 18 8	12.6
	4.8	15 12 11 10 12 12 10 12 15 0	12.1
	3.8	10 10 12 15 12 8 4 0 0 0	10.1
	2.8	5 5 4 6 6 6 4 0 0	5.1
	1.8	0 0 0 0 0 0 0 0 0	0

Table 57. The diameter of dry rot lesion measured internally in potato tubers of cv Maris Piper inoculated with different isolates of *C. destructans* 24 dayas after inoculation. Experiment set up March 1991.

Isolates	Log number of spores inoculated	Diameter of dry rot lesion in each tuber	Mean diameter of dry rot lesion
Cd1	5.8	10 12 10 8 15 20 10 15 20 8	12.8
	4.8	15 8 10 15 10 12 8 10 18 10	11.2
	3.8	10 8 8 10 12 8 7 12 8 8	9.2
	2.8	8 8 10 8 5 7 8 4 3 3	6.4
	1.8	0 0 0 0 0 0 0 0 0 0	0
Cd2			
	5.8	15 30 10 15 10 25 15 10 10 15	15.5
	4.8	10 15 15 20 15 10 10 15 10 15	13.5
	3.8	10 10 15 10 10 15 10 15 12 8	11.5
	2.8	10 8 8 10 8 6 4 4 3 3	6.4
	1.8	0 0 0 0 0 0 0 0 0 0	0
Cd3	5.8	10 10 10 15 8 12 15 8 12 18	11.8
	4.8	10 10 15 8 8 15 10 10 8 15	10.9
	3.8	8 8 10 10 8 7 10 8 15 12	9.8
	2.8	10 8 8 10 6 4 5 3 3 3	6
	1.8	0 0 0 0 0 0 0 0 0 0	0

Table 58. The diameter of dry rot lesion measured internally in potato tubers of cv Record inoculated with different isolates of *C. destructans* 24 days after inoculation. Experiment set up March 1991.

Isolates	Log number of spores inoculated	Mean size deameter of dry rot	Mean diameters
Cd1	5.8	10 15 15 15 15 10 8 8 12 10	11.8
	4.8	8 8 15 10 10 10 15 8 12 15	10.8
	3.8	8 10 8 10 10 12 15 7 12 8	10
	2.8	10 8 8 8 8 10 6 3 4 3	6.8
	1.8	0 0 0 0 0 0 0 0 0 0	0
Cd2			
	5.8	15 15 20 15 25 15 10 20 20 30	18.5
	4.8	10 15 15 20 10 10 25 30 10 10	15.5
	3.8	10 20 20 15 10 10 10 10 10 15	13.1
	2.8	10 10 15 6 6 10 6 4 3 3	7.3
	1.8	0 0 0 0 0 0 0 0 0 0	0
Cd3	5.8	10 20 10 25 15 15 25 8 8 12	14.8
	4.8	10 8 10 10 15 14 15 8 15 10	11.8
	3.8	10 12 8 6 7 8 8 10 10 15	9.6
	2.8	8 8 10 8 8 6 6 3 4 3	6.4
	1.8	0 0 0 0 0 0 0 0 0 0	0