RESISTANCE OF PLANTS TO SPECIES OF COLLETOTRICHUM

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ABSTRACT

The responses of French bean cultivars to races of C. lindemuthianum and of cultivars of cucumber and coffee to C. lagenarium and C. coffeanum, respectively, were examined. Bean hypocotyls inoculated with suspensions of spores of non-pathogenic Colletotrichum spp. reacted hypersensitively within 24 - 48 h. Conidia of Colletotrichum spp. germinated and produced appressoria equally and abundantly on host and non-host plants and conidia of C. lagenarium produced appressoria equally on susceptible and resistant cultivars. The ability of conidia of races of C. lindemuthianum, C. coffeanum and C.lagenarium to germinate and produce germ tubes or appressoria in solutions of carbohydrates is discussed.

There was no relation between production of appressoria in water extracts of healthy tissue of host plants and susceptibility and resistance.

Localised disease resistance was induced in bean hypocotyls and cucumber cotyledons by pre-inoculating them with non-pathogenic Colletotrichum spp., extracts of cultures, germination fluids and extracts of mycelial cell walls containing elicitors from Colletotrichum spp.

Systemic protection against C. lagenarium was induced in second true leaves of cucumbers by first inoculating the cotyledons or the first true leaves with C. lagenarium. The possible mechanisms of systemic protection are discussed.

Growth regulating substances also increased or decreased resistance in bean and cucumber plants depending on concentrations, time of and for treatments and mode of application.

In etiolated bean hypocotyls irradiated with ultra-violet (UV) light, resistance to avirulent races of C. lindemuthianum or to non-pathogenic Colletotrichum spp., C. lagenarium and C. coffeanum, was eliminated. But resistance was induced in hypocotyls irradiated with UV 24 or 48 h before inoculation. This resistance depended on synthesis of phytoalexins.

Phytoalexin production in susceptible bean cotyledons inoculated with non-pathogenic Colletotrichum spp. was higher than in cotyledons inoculated with the pathogen.

Synthesis of pectic enzymes in vitro by Colletotrichum spp. and the role of pectic enzymes produced in vitro by races of C. lindemuthianum in bean cultivar specificity are discussed.

LIST OF ABBREVIATIONS

Å	–	Angstrom
c.	–	about
C^o	–	Centigrade degrees
d	–	day(s)
cm	–	centimeter(s)
g	–	gram(s)
gdw	–	glass distilled water
h	–	hour(s)
LANS	–	Long Ashton Nutrient Solution
l	–	litre(s)
m	–	metre(s)
mg	–	milligram(s)
ml	–	millilitre(s)
mm	–	millimeter(s)
M	–	molar
MW	–	molecular weight
N	–	normal
nm	–	nanometre
ppm	–	parts per million
p.s.i.	–	pounds per square inch
R_f	–	rate of flow
RH	–	relative humidity
RVU	–	relative viscosity unit
TLC	–	thin layer chromatography
UV	–	ultra violet (light)
µg	–	micrograms
µl	–	microlitres
µ	–	micron

CONTENTS

I. INTRODUCTION

This investigation studied the induction of resistance in French bean and cucumber plants to C. lindemuthianum and C. lagenarium respectively. Although many of the earliest reports on induced resistance have not been re-investigated by modern methods, localised induction of disease resistance has been studied in depth by a number of workers for different diseases in recent years. Thus resistance in bean hypocotyls was induced by pre-inoculating with non-pathogenic races of C. lindemuthianum and other non-pathogenic Colletotrichum spp. (Elliston et al., 1976). Moreover, claims of systemic protection in a few diseases have also been made. Hammerschmidt et al. (1976) demonstrated systemic protection of cucumber to Cladosporium cucumerinum and C. lagenarium by prior inoculation with C. cucumerinum.

Non-microbial agents, such as plant growth regulators and radiation have also been widely used for inducing resistance in plants.

The object of the present investigation was to obtain more information on the mechanisms of induction of localised and systemic resistance by microbial and non-microbial agents, because in most cases induction of resistance seems to be a result of changes in host physiology. If this proved to be the case, then it would be feasible to induce similar changes by use of non-microbial agents. Further understanding of the natures of these changes in host physiology could, therefore, lead to the development of new procedures based on biological control or chemotherapy.

II. LITERATURE REVIEW

A. Anthracnose diseases

1. Symptoms of infection

1.1 French bean

C. lindemuthianum attacks all above-ground parts of Phaseolus vulgaris but rarely the roots (Walker, 1969). The lesions on hypocotyls are characteristically dark brown. Angular spots appear on veins on the lower side of the leaf, spreading slightly into surrounding tissue and eventually appearing on the opposite side. Lesions on pods are light dark brown and are formed by enlargement of small necrotic spots to a diameter of 1 cm. or more, the fungus often growing through the pod to infect the seeds. Pods and stem lesions are sunken when mature and bear erumpent acervuli on which spores are produced. Symptoms on resistant cultivars are much reduced, often limited to microscopic red-brown spots, or absent.

1.2 Cucumber

C. lagenarium causes different symptoms on different hosts in the Cucurbitaceae (Walker, 1952). On cucumber leaves lesions commonly start on a vein and expand into brown spots which are angular or roughly circular, reaching 1 cm or more in diameter. Growing leaves may be distorted, and coalescence of many spots may blight entire leaves. Petiole and stem lesions are shallow, elongate, and tan coloured. Lesions do not appear on fruits until they approach maturity.

1.3 Coffee

Two groups of symptoms have been distinguished in coffee (Firman and Walker, 1977); anthracnose of leaves, fruits and branches, and dieback, characterised by desiccation of the extremities of the branches. In anthracnose of leaves, necrotic brown lesions are produced usually on the margins and as they become older they may develop concentric rings on which the acervuli are visible as small black dots mostly on the upper surface. In anthracnose symptoms on young green twigs, lesions start as yellow, slightly depressed, elongated to oval spots which rapidly turn brown. The lesions can be several centimetres long and are delimited by a yellow halo. In severe attacks twigs may have lesions all along their length; these may develop rapidly and encircle the twigs, necrosis progressed downwards and the leaves and fruit go yellow, blacken and eventually shrivel and fall. Anthracnose in fruits usually occurs as sunken patches which spread rapidly and eventually cover the whole berry.

2. Etiology

2.1 C. lindemuthianum

The mycelium is branched, septate, hyaline at first, becoming dark coloured with age. On culture media, conidia may form singly at the ends of hyphal threads. Conidia (4.4 to 5.3 by 13 to 22 μ) are continuous, hyaline, oblong, cylindrical, with ends round or somewhat pointed at one end; a clean vacuole-like body often occurs near the centre. One to four, usually two, germ tubes arise from a conidium. Appressoria are formed at the tips of germ tubes, especially when they come into contact with the host surface (Walker, 1969).

2.1.1 Races of C. lindemuthianum

During differential reaction of bean cultivars, isolates of

C. lindemuthianum can be assigned to one of four races (α, β, γ and δ). Burkholder (1923) pointed out that race γ was pathogenically closely related to race β but with increased virulence and race δ had overall pathogenic abilities similar to race α (Hubberling, 1961).

2.2 C. lagenarium

The fungus is a typical anthracnose organism (Walker, 1952). The mycelium is septate, hyaline when young, dark when old. Setae are brown, thick walled, two to three septate 90 to 120 μ long, variable in number. Conidia are hyaline, continuous, oblong to ovate oblong, 4 to 6 by 13 to 9 μ. As they germinate and as germ tubes contact firm surfaces, dark, thick-walled, spherical appressoria are formed.

2.2.1 Races of C. lagenarium

Three physiological races were distinguished by their ability to attack certain cucurbit seedlings and fruits (Goode, 1958). The three races were indistinguishable culturally and morphologically. The races were designated 1, 2 and 3 depending on the type of hosts from the Cucurbitaceae family they attack.

2.3 C. coffeanum Noack

The conidia are large (6 - 33 x 4 - 8 μ) and vary much in shape and size from oval, through elliptic to somewhat irregularly clavate and have granular contents. Conidia are borne on aerial conidiophores and never in acervuli. In culture it grows slowly with profuse dark grey to greenish olivaceous grey mycelium. Older cultures are variable, often pale and sterile (Rayner, 1952).

2.3.1 Physiological groups of C. coffeanum

Small (1926) classified C. coffeanum into three physiological groups, the apparently parasitic, the weakly parasitic capable of initiating ripe rots or requiring a wound for extrance to host tissue, and the saprophytic.

B. Histology and physiology of infection

1. French bean

Spores of C. lindemuthianum normally germinate by a single germ tube, the end of which formed a spherical vesicle separated from the remainder by septum (Dey, 1919). As the appressorium matures, the walls become thicker, darkly pigmented with the base attached to the cuticle of the host epidermis. Rahe et al. (1969) found no difference in spore germination on resistant and susceptible cultivars.

The epidermal cells of bean hypocotyls are entered by a fine infection hypha passing from the base of the appressorium into the cell lumen (Dey, 1919; Mercer, 1970). Dey (1919) could find no evidence of enzyme action on the cuticle but penetration of the cellulose layers was accompanied by their swelling and dissolution suggesting that enzymes were active. Leach (1923) and Sinden (1937) found that penetration of epidermal cells took place in both resistant and susceptible cultivars.

Leach (1923) noticed that the hyphae in cells after infection were of wide diameter and named them "primary hyphae". They colonized the cells intracellularly and became constricted when passing through a cell wall, only to swell again on reaching the next cell.

Skipp (1971) found that cells of susceptible cultivars containing actively growing primary hyphae showed no signs of damage for two or more days after they were first penetrated. Further colonization of the cells achieved by "secondary hyphae" (Leach, 1923) which passed through cell

walls without constriction and the hypocotyls eventually collapsed, and produced brown lesions.

In the process of infection in resistant bean cultivars, Leach (1923) and Skipp (1971) found that soon after penetration, cells became granular and brown. Leach suggested that the growth of the hyphae stopped because of lack of specific and essential nutrient and death of the host cell was caused by products of lysis of the starving hyphae. Based on the changes preceding cell death, Skipp (1971) identified two types of resistant reactions; Resistant Type 1, where the cell death and granulation of cytoplasm were the first recognizable signs of response which Stakman (1915) referred to as "hypersensitivity" and Resistant Type 2 where increased streaming and the accumulation of cytoplasm at the site of penetration preceded cell death. Skipp (1971) concluded that the type of resistance depends on the fungal race used rather than on the cultivar of bean.

2. Cucumber

On leaves of resistant and susceptible cucumber cultivars, appressoria were formed within 8 h and were equally abundant within 24 h after inoculation. Appressoria were most numerous immediately above the side walls of epidermal cells and the appressorium forces the way into the radial wall of the epidermal cells dissolving the middle lamella. Penetration was affected by projection of the infection peg from a germ pore in the appressorial wall attached to the host cuticle (Busch and Walker, 1958). Penetration was similar in resistant and susceptible cucumber leaf cells though the percentage of infection hyphae that formed from the appressoria on leaves differed significantly among resistant and susceptible cultivars (Akai et al., 1958).

Busch and Walker (1958) found that in susceptible leaves, the penetration peg invaded the epidermal cell and mycelium proceeded to

invade cells of mesophyll intracellularly. The infection hypha was constricted to a very small diameter when passing through cell walls. The invaded cells collapsed promptly and the chloroplasts and cytoplasm broke down in uninvaded cells and adjacent to, or not far removed, from invaded cells. The mycelium invaded the vascular tissue as well as the mesophyll and spongy parenchyma. In resistant leaves, penetration occurred as well as in susceptible leaves but the growth of the hyphae was much slower in the resistant tissue. Morphological evidence of host reaction in resistant plants took the form of thickening of the cell walls in advance of the hyphae and red coloured deposits in the intercellular spaces; the chloroplasts and other cytoplasmic material tended to collapse more slowly.

Because cell wall reactions of resistant tissue suggested the possibility of the formation of a morphological barrier against the pathogen, Busch and Walker (1958) concluded that the true basis of resistance may well be biochemical with morphological response a secondary reaction. Yasumori (1964) also concluded that in resistant cultivars the chemical modification in the middle lamellae of the epidermal cell wall beneath the appressoria prevented the enzymic disintegration of middle lamellae by the fungus and as a result the hyphae could not invade the leaves.

3. Coffee

Most research has been on epidemiology of C. coffeanum and there is no detailed work on the histology of infection of susceptible and resistant coffee plants.

Bock (1956) found that C. coffeanum produced appressoria on the surface of resistant and susceptible berries within 4 - 5 h after inoculation and penetration was effected by an infection peg. In susceptible berries, active lesions developed while in resistant berries

scab lesions were developed and Bock suggested that this might represent a defence reaction by the plant. Firman (1964) postulated an "internal resistance" for cv. Rume Sudan in addition to the cuticular resistance which was considered by Nutman and Roberts (1960) to be the sole source of varietal resistance. Martin (1964) and Martin and Juniper (1970), however, have questioned in general the role of the cuticle as a mechanical barrier and have suggested that resistance factors associated with the cuticle are of little significance compared with others operating within the tissues.

Hocking (1967 a, b) examined the possibility of a chemical/biochemical basis for disease resistance and obtained some evidence that the tissues of hard green coffee berries may contain pre-formed antifungal materials and in some cultivars cuticular factor may be involved in resistance.

C. Biochemical aspects of disease resistance

Many workers have reported on interactions between plants and pathogens with regard to phytoalexin synthesis, accumulation and the role of phytoalexins as inhibitors of growth and development.

Phytoalexins were defined by Müller (1956) as antibiotics which are the result of an interaction of two different metabolic systems, the host and the parasite and which inhibit the growth of pathogenic fungi to plants. Ingham (1973) suggested another concept according to which a phytoalexin is an antibiotic formed in plants via a metabolic sequence induced either biotically or in response to chemical or environmental factors. According to Stoessl et al. (1976) phytoalexins must not only be fungitoxic but must accumulate sufficiently rapidly to protect the plant.

1. The role of antifungal compounds in anthracnose diseases

1.1 French bean

The phytoalexins of bean have been extensively examined for a possible role in resistance to avirulent races of Colletotrichum lindemuthianum.

Rahe et al. (1969) studied the relationship between changes in phenol metabolism and the appearance of visible symptoms on bean hypocotyls. In hypocotyls, inoculated with virulent or avirulent races phenylalanine ammonia lyase (PAL), which is involved in the biosynthesis of phenolic compounds, and total phenol content were found to increase after infection. These changes occurred in resistant and susceptible reactions except that the change was detected within 36 - 48 h in resistant and within 72 - 84 h in susceptible hypocotyls.

Cruickshank and Perrin (1963, 1971) detected phaseollin in inoculated beans; Bailey and Deverall (1971) also detected low concentrations of phaseollin in some healthy hypocotyls. A major increase in phaseollin concentration occurred after cells became brown, either when they reacted hypersensitively to penetration by incompatible races, or when anthracnose lesions appeared following the period of intracellular growth. Rahe et al. (1969) also reported that there was a close association between cellular browning and accumulation of phaseollin. The major difference between the two types of host-parasite interaction was the timing of this process, which was dependent upon the sensitivity of the cell to the race of the parasite (Deverall, 1972).

If resistant cells produce phaseollin faster than susceptible cells, it would be important to determine the amounts of phaseollin being produced. Bailey (1970) adapting the formula used by Müller (1958), calculated the phaseollin content of the individual brown cells in infected resistant tissue and found as high as 4250 µg phaseollin/cc of

cell compared to 57.5/cc for the susceptible reaction. Phaseollin completely inhibited the growth of germ tubes of C. lindemuthianum at a concentration of 5 µg/ml and established mycelial growth at 65 µg/ml (Bailey and Deverall, 1971).

Several phenolic compounds were detected in extracts from infected beans and some or all may have had antifungal activity (Rahe et al. 1969) and it was necessary to decide whether a single chemical, phaseollin, is responsible for resistance, particularly in a situation where large numbers of compounds are likely to be produced and to accumulate in high concentrations.

Inoculation of Phaseolus vulgaris with tobacco necrosis virus and also with C. lindemuthianum has been shown to lead to the production of phaseollin, phaseollidin, phaseollinisoflavan and kievitone (Bailey and Burden, 1973 ; Burden et al. 1972). These compounds are also inhibitory to spore germination of C. lindemuthianum (Bailey and Burden, 1973).

Bailey (1974) obtained low level of phytoalexins in the extracts of "spreading lesions" and the concentration of phaseollin was below 50 µg/g tissue, which was insufficient to have any effect on the growth of C. lindemuthianum. But in the extracts of "limited lesions", Bailey obtained much greater levels of the four phytoalexins, the major constituents being phaseollin and phaseollinisoflavan. The concentration of phaseollin, 366 µg/g tissue, was far in excess of that required to prevent growth of C. lindemuthianum. The four phytoalexins were also isolated from hypersensitive tissues and phaseollin was present at concentrations between 3000 and 4000 µg/cm^3 cell (Bailey and Deverall, 1971). From this data Bailey (1974) estimated phaseollidin, phaseollinisoflavan and kievitone would also be present in hypersensitive cells at high concentrations and thereby operate to promote an extremely antifungal environment.

Bailey concluded that phaseollin, along with phaseollidin, phaseollinisoflavan and kievitone play an important part in resistance to

C. lindemuthianum in causing anthracnose in beans is that compatible races penetrate and cause no cellular response until considerable intracellular growth of hyphae has occurred whereas in incompatible races, they induce an immediate cellular response on contact with germ tubes and phaseollin accumulation seemed to follow this response (Deverall, 1972). This was also shown in beans inoculated with an incompatible race of the bacterium Pseudomonas phaseolicola or by tobacco necrosis virus (Stholasuta et al., 1971; Bailey and Ingham, 1971).

1.2 Cucumber

Phytoalexin formation in Leguminosae and Solanaceae have been studied in detail. Deverall (1974) failed to detect post-infectional increase in amounts of certain uncharacterised antifungal substances in healthy fruits and leaves of cucumber, even after hypersensitive responses to C. lagenarium.

1.3 Coffee

The presence of antifungal activity towards C. coffeanum in the cuticular wax of hard green coffee berries was investigated by Lampard and Carter (1973). The correlation between the activity of cuticular wax extracts from many cultivars of arabica coffee and field resistance to coffee berry disease suggested that antifungal compounds in cuticular wax may determine disease resistance. Their findings supported the view expressed by Nutman and Roberts (1960) and Firman (1964) that the cuticle is an important factor in resistance to coffee berry disease. The activity of the cuticular extracts decreased as the berry ripens; Nutman and Roberts (1960) and Firman (1964) found that susceptibility increases with ripening.

The antifungal cuticular factor could prevent, or reduce, the extent to which the fungus penetrates, thus reinforcing the effect of

the post-penetration response in containing any further spread of the pathogen through the tissue. Lampard and Carter (1973) have not yet reported on the chemical nature of the antifungal compound present in the cuticular wax.

2. Phenols and related compounds

Phenols have often been associated with resistance. Apart from their function in healthy plants, changes in concentration, after infection, have been correlated with resistance (McLean et al., 1961). Hare (1966) associated phenols with defence mechanisms in plants because of their general occurrence at infection and wounding sites and because many phenols and their oxidation products (quinones) are highly fungitoxic. The role of phenolic compounds in the physiology of plant disease and disease resistance has been reviewed in detail by Farkas and Kiraly (1962) and Cruickshank and Perrin (1964).

Chlorogenic acid and its hydrolysis product, caffeic acid, act as defense mechanisms in potato peels against several non-pathogens(Kuć, 1957; Kuć et al. 1959). It is not present in healthy tissue in amounts lethal to the fungus and appeared to be localized in the areas of fungus penetration. But Uritani (1953) found that Ceratostomella fimbriata was not inhibited in growth in culture media containing chlorogenic acid at a concentration as high as that in infected tissue. Suzuki and Toyoda (1957) also found that the growth of Helicobasidium mompa in culture was not inhibited by the addition of chlorogenic acid or its oxidation product. Resistant potato cv. infected with Phytophthora infestans showed more intense accumulation of toxic oxidative derivatives of polyphenols than do leaves of susceptible cv. (Fuchs, 1956; Tomiyama et al., 1958; Valle, 1957). The periderm of tubers from a cv. resistant to scab showed much more chlorogenic acid and total phenols than a susceptible cv. (Johnson and Schaal, 1957).

Resistance to Verticillium wilt in potatoes is also correlated with phenolic metabolism (Lee and LeTourneau, 1958; McLean et al., 1956, 1961; Patil et al., 1964). Phenols, particularly chlorogenic acid, were much higher in root tips and the vascular system of resistant cv.

Chlorogenic acid was associated with resistance to coffee canker (Ceratocystis fimbriata)(Echandi and Fernandez, 1962) and to apple scab (Kirkham and Flood, 1956; Kirkham and Hunter, 1964). Sweet potato roots infected with black rot produce large amounts of chlorogenic and caffeic acids (Minamikawa et al., 1963) which were related to resistance.

Caffeic acid and increased levels of phenolic compounds were found in roots and stems of plants of Capsicum frutescens resistant to Colletotrichum capsici (Bhuller et al., 1972).

The presence of large amounts of phenolic compounds in a plant cannot be generally related with resistance because the toxicity of phenolic compounds is different to different parasites (Tomiyama, 1967) but in most cases the increase of phenolic compounds is more rapid in the resistant than in susceptible tissue (Farakas and Kiraly, 1962).

3. Cell walls and cell wall-degrading enzymes

Infection of plants by microbial pathogens usually involves some degradation of the cell walls of the plant and the pathogen-secreted polysaccharide-degrading enzymes play a fundamental role in pathogenicity in some diseases (Albersheim et al., 1969). Most plant pathogens that can be cultured have the ability to produce polysaccharide degrading enzymes and these enzymes, taken together, have an ability to degrade the glycosidic linkage known to occur in the polysaccharides of primary plant cell walls (Albersheim et al., 1969; Albersheim, 1976; Albersheim et al., 1973).

Albersheim et al. (1969) have proposed that cv. specificity might be determined by variations in the composition of cell walls of the hosts, and that these wall differences could cause differential induction of

synthesis of polysaccharide-degrading enzymes by the pathogen.

When a pathogen is grown in culture on cell walls of different dicotyledonous cultivars an endopolygalacturonase is the first wall-degrading enzyme to accumulate (English et al., 1971; Jones et al., 1972). Pathogenesis of dicotyledons may be affected by cell wall proteins which inhibit the action of endopolygalacturonases (Albersheim and Anderson, 1971; Fisher et al., 1973; Jones et al., 1972). These inhibitors act against the endopolygalacturonase, but not against other cell wall degrading enzymes (Albersheim and Anderson, 1971). Albersheim and Anderson (1975) suggested that a pathogen is incapable of attacking a plant unless the pathogen finds the proper environment to permit it to secrete sufficient amounts of endopolygalacturonase to overcome the amount of inhibitor present in the cell walls of the plant.

Although there are several endopolygalacturonase inhibitors in the cell walls of dicotyledons, each is able to inhibit the endopolygalacturonases of a variety of unrelated plant pathogens (Albersheim and Anderson, 1971; Fisher et al., 1973; Jones et al., 1972), as shown by the ability of homogenous protein preparation, purified from bean hypocotyls, to inhibit with almost equal efficiency the endopolygalacturonases secreted by C. lindemuthianum, Helminthosporium maydis and Aspergillus niger (Albersheim, 1965; Fisher et al., 1973).

Races of C. lindemuthianum secrete α-galactosidase, β-galactosidase and β-xylosidase when grown on cell walls isolated from the hypocotyls of different cultivars of bean hypocotyls (English and Albersheim, 1969). These enzymes effectively degrade cell walls isolated from susceptible 5-day old hypocotyls but degrade only slightly the walls isolated from resistant 18-day old hypocotyls. Secretion of β-glucosidase and β-xylosidase is not dependent upon the bean cv. from which the hypocotyl cell walls used as a carbon source were isolated. However, the fungal strains secrete greater amounts of α-galactosidase when grown on hypocotyl cell walls isolated from susceptible plants than when grown on walls from

resistant plants. The α-galactosidase secreted by the races of C. lindemuthianum removes galactose from the hypocotyl cell walls and they suggested that α-galactosidase might be a major cell wall degrading enzyme of C. lindemuthianum and if the ability to colonize bean tissue depended on high substrate (galactoside) level in cell walls then the decline in galactose in old tissue could explain the observed increase in resistance.

Modification of the primary cell walls is another way by which plants are protected against cell wall-degrading enzymes secreted by pathogens (Albersheim and Anderson, 1975). The changes that occur during secondary cell wall formation inhibit dramatically the ability of pathogen-produced enzymes to degrade the cell wall polymers of their hosts and this can account for the relatively high levels of resistance often displayed by older parts of plants (Albersheim, 1965; Bateman et al., 1969; Mercer et al., 1974; Tomiyama, 1967).

D. Induced resistance

Cross-protection is often applied to the phenomenon whereby a plant is protected from infection by earlier or simultaneous exposure to another organism (Deverall, 1977). Induced resistance was described by Lobenstein (1972) as (a) resistance developing in non-invaded tissue after viral or bacterial or fungal infection of other parts of the plants and, (b) the phenomena whereby non-multiplying substances interfere via a host-mediated process and as a result affecting the physiology of the host.

1. Microbial agents

1.1 Fungi and fungal fraction

Protection against diseases caused by fungi has been induced locally and systemically by fungi (Deverall, 1977). Müller and Börger (1941)

reported that when sporangia of an avirulent race of P. infestans were applied to the cut surface of a potato tuber some time before inoculation with a race which would normally cause a rot, the amount of tissue damage caused by the second race was much reduced. Effective cross-protection was only observed in areas inoculated with the avirulent race and was thought to be a localized phenomenon. From their work they introduced the phytoalexin theory. The first to report that cross-protection had been obtained with fungi was Ray (1901). He found that injection of weakly virulent cultures of Botrytis cinerea into their legume hosts before inoculating with virulent strains prevented the development of disease. Extracts of cultures of the virulent strain also had this protective effective when applied to the soil around the host plant.

Systemic resistance to tobacco mosaic virus (TMV) was induced in tobacco Xanthi-nc leaves by injecting spore suspensions of the fungus Peronospora tabacina into the stems of the plants (Mandryk, 1963). The plants were challenge-inoculated three weeks later to allow time for the development of the mycelium in the injected stems. TMV lesions in the fungus pre-inoculated plants were smaller and fewer than in the controls. Hecht and Bateman (1964) also demonstrated the induction of systemic resistance against TMV and tobacco necrosis virus (TNV) by injection of the stem or leaf tissue with the fungus Thielaviopsis basicola, which causes a localized reaction.

Non-pathogenic isolates of Alternaria, applied to leaves of tobacco before inoculation with the pathogen, Alternaria alternata, reduced tobacco brown spot by 60% and the level of disease reduction was related to the concentration of conidia applied as a protectant (Spurr, 1977). Protection was maximum when the protective conidia were applied to the leaves 2 - 3 days before inoculation.

Schnathorst and Mathre (1966) protected cotton plants from V. albo-atrum by amending soil with microsclerotial of an avirulent strain.

Davis in 1967 protected tomato plants by pre-inoculating them with several formae speciales of Fusarium oxysporum and Barrow in 1969 protected cotton plants in the field from Verticillium sp. by pre-inoculating them with an avirulent strain.

Rice seedlings raised from seeds treated with an extract of a culture of Pyricularia oryzae showed reduced susceptibility to blast disease and slightly better growth (Kumamato, 1948), and yield of the treated plants was also higher (Watanase, 1952). Ganguly and Padmanabhan (1962) reported that soaking of rice seeds in diluted extract of a culture of Helminthosporium oryzae markedly reduced the leaf symptoms at later stages of development and also helped such plants to achieve higher yields. Boiling of the extract reduced its ability to induce resistance. Sinha and Das in 1972 reported that cell-free spore germination fluids obtained from different concentrations of mild race spore suspensions induced different levels of resistance in rice plants to H. oryzae. Trivedi and Sinha (1976) demonstrated that when rice plants were sprayed or when seeds were soaked with germination fluid of avirulent or virulent race of H. oryzae resistance was induced. There was a direct relation between the concentration of germination fluid of the mild race and the degree of resistance. The fluids did not inhibit spore germination or germ tube length. They concluded that germination fluid contains some fungal metabolite which, when absorbed by the leaf cells, stimulates them to produce a fungitoxic principle.

Hypersensitive resistance to fungi in plants is generally characterized by the production of abnormal metabolites at the sites of infection (Farkas and Kiraly, 1962; Kuć, 1963, 1966) and these substances are inhibitory to a broad spectrum of microorganisms. Their synthesis is suggested as accounting for the resistance of the host to the inducing microorganism (Cruickshank, 1965). Rahe et al. (1969) reported that etiolated hypocotyls of Phaseolus vulgaris respond hypersensitively to H. carbonum race 1 and Alternaria sp. 24 - 36 h after inoculation and

60 - 72 h after inoculation with a cultivar non-pathogenic race of C. lindemuthianum. From their findings the times of appearance of induced resistance were the same following inoculation with each of the three fungi, and concluded that metabolic alterations occurring 0 - 24 h after inoculation reflect the primary protective response of bean hypocotyls to these fungi. Elliston et al. (1971) showed that resistance induced in Phaseolus vulgaris by a cultivar non-pathogenic race of C. lindemuthianum against a cultivar pathogenic race was exhibited by cells distant from the inducing interaction and the induced resistance was expressed after the cultivar non-pathogenic race has penetrated, and is microscopically indistinguishable from the resistant reaction to a cultivar non-pathogenic race.

Bernard et al. (1972) reported that bean hypocotyls were protected against cultivar-pathogenic races of C. lindemuthianum by a factor which diffused into water droplets over incompatible (resistant) interactions. The factor protected only the bean cultivar from which it was obtained. Hypersensitive flecking did not occur in the protected areas. The factor from an incompatible interaction, when placed on another cultivar did not alter the susceptible or resistant response. The diffusate from compatible (susceptible) interactions also did not alter the susceptible or resistant response of the cultivar from which it was prepared or a cultivar which is a reciprocal differential to the β and γ race of C. lindemuthianum and germination of conidia of the races of C. lindemuthianum in vitro was not inhibited by diffusates from compatible or incompatible interactions.

Protection in Phaseolus vulgaris was achieved by cultivar-non-pathogenic races of C. lindemuthianum and Elliston et al. (1976) reported that non-pathogenic Colletotrichum spp. were capable of inducing protection against anthracnose in cultivars apparently susceptible to all races of C. lindemuthianum and in cultivars resistant to one or more races. The non-pathogenic Colletotrichum spp. differed in their ability to protect. C. gossypii and C. phomoides were less effective. C. fragarie, C. lagenarium

and C. truncatum caused bean cells to react hypersensitively and individual cells or small groups of cells beneath appressoria of these fungi granulated and browned which is accompanied by phytoalexin accumulation. This accumulation of phytoalexins in response to inducer-fungi may contribute to or be responsible for local protection.

C. lagenarium was effective in inducing systemic protection in bean cultivars susceptible to all races of C. lindemuthianum (Elliston et al., 1976). Protection induced by races of C. lagenarium was not induced as rapidly nor as extensively as protection induced by cultivar non-pathogenic races of C. lindemuthianum. Systemic protection induced by C. lagenarium was effective earliest in the most mature region of the bean hypocotyl. Elliston et al. (1976) concluded that protection induced by Colletotrichum spp. non-pathogenic to bean appeared to be non-specific with respect to races of C. lindemuthianum as its protection induced by races of C. lindemuthianum.

Interactions that induce systemic protection are in general of the hypersensitive type which are accompanied by phytoalexin production. Cell-free filtrates from cultures of several pathogens were reported to elicit phytoalexin accumulation in their hosts (Keen et al.,1972; Klarman and Gerdemann, 1963; Rathmell and Bendall, 1971). Resistance of soybean seedlings to Phytophthora megasperma var. sojae is part due to accumulation in infected tissue of a phytoalexin called glyceollin which is triggered by infection. Cell wall fractions from P. megasperma called "elicitors" were able to trigger phytoalexin production (Ayres et al., 1976). Elicitors of a nature similar to those from P. megasperma var. sojae have been isolated from C. lindemuthianum (Anderson and Albersheim, 1975). Bean cotyledons and hypocotyls treated with the elicitors of C. lindemuthianum produced phytoalexins.

Protection of cucumber against Cladosporium cucumerinum by prior inoculation with C. lindemuthianum, a pathogen of bean, and the protection

of cucumber to C. cucumerinum and against C. lagenarium by prior inoculation with C. cucumerinum have been demonstrated by Hammerschmidt et al. (1976). Kuć et al. in 1975 demonstrated that cucumber cultivars susceptible to Colletotrichum lagenarium race 1, were systemically protected against disease caused by the pathogen, by prior inoculation with race 1 of the pathogen. Inoculation of a single leaf of young cucumber plants with a spore suspension of C. lagenarium protected the leaf above against disease caused by subsequent inoculation with C. lagenarium. After the unprotected leaf developed symptoms, it elicited protection for the leaf above and this leaf was often symptomless. But Jenns and Kuć (1977) failed to protect cucumber cotyledons against TNV by prior infection with C. lagenarium.

1.2 Bacteria and bacterial fractions

Hypersensitivity is a general mechanism in incompatible host parasite relationship whether the pathogen be a fungus, a bacterium or a virus (Klement and Goodman, 1967).

Lovrekovich and Farkas (1965) showed that when washed and heat killed cells of Pseudomonas tabaci, the agent of the wildfire disease of tobacco, were introduced into the intracellular spaces of one half of a tobacco leaf, the other half infiltrated with water, symptoms failed to appear in the leaf which was pretreated with the killed bacteria when entire leaf was inoculated one day later with living cells of P. tabaci. Living bacteria exhibited stronger protective effect than did killed ones and Klement and Goodman (1967) attributed this protection due to the inhibition of bacterial multiplication. A similar phenomenon has been reported by Goodman (1965, 1967) whereby a Erwinia-like saprophytic bacterium which is associated with the fireblight disease of apple trees, inhibited the multiplication of the virulent E. amylovora in apple shoots. He also reported that a saprophytic Erwinia-like organism, an avirulent strain of

E. amylovora and P. tabaci, induced an immune response in apple tissue which inhibited later infection with a virulent strain of E. amylovora. Apple and pear can be protected from E. amylovora by cell free sonicates of E. amylovora (McIntyre et al., 1973).

Lallyett (1977) showed that P. fluorescens and P. solanacearum inhibited hypersensitive reaction when injected 24 h before either P. phaseolicola race 1 or P. mors-prunorum in bean leaves.

Pre-treatment of plants with saprophytic or killed pathogenic bacteria resulted in a protective effect, not only against subsequent infection with bacteria, but also against TMV infection (Klement et al., 1966; Little and Groubough, 1946). Heat killed cells of P. syringae and P. fluorescens (Loebenstein and Lovrekovich, Klement et al., 1966) injected into the intercellular spaces of tobacco NN or Xanthi-nc leaves, 0 - 7 days before inoculation with TMV, markedly reduced lesion numbers. Furthermore, heat killed bacteria suppressed lesion formation even when applied 5 - 72 h after inoculation. Injection of a bacterial preparation 24 h after inoculation or later, resulted in a decreased effect on lesion number and size was evident. Injection of an extract from Escherichia coli into Xanthi or Samsun tobacco leaves, 1 or 4 h after inoculation, reduced TMV lesion number and virus concentration (Albovy et al., 1969). Iso-propanol extracts from Bacillus uniflagellatus reduced the number of local lesions when added to the soil or sprayed on leaves of Xanthi tobacco prior to inoculation with TMV (Mann, 1969). Cultures of the bacterium added to the soil also reduced the number of lesions on Xanthi and viral content in systemic host.

Phytoalexins have been implicated as an important component of resistance responses in plants to incompatible bacteria. Keen and Kennedy (1974) found that coumestrol and glyceollin were induced in soybeans inoculated with incompatible races of Pseudomonas glycinea and were active against these races at physiological concentrations. P. mors-prunorum

or incompatible races of P. phaseolicola when introduced into bean leaves, induced 10 times more phaseollin than did a compatible race of P. phaseolicola (Stholasuta et al., 1971).

O'Brien and Wood (1973) and Lyon and Wood (1975) obtained antibacterial extracts from bean leaves inoculated with P. mors-prunorum or with incompatible races of P. phaseolicola.

Although it is not clear whether the induced resistance is caused by a common mechanism, the above mentioned results suggests that heat-stable constituents of gram-negative bacteria may play an important role in plants in evoking defence reactions (Novacky et al., 1973).

1.3 Virus and viral fractions

The first observations that infection in one part of the plant induces resistance in other non-invaded tissues was reported by Gilpatrick and Weintraub (1952). When the lower leaves of two clones of Dianthus barbatus were inoculated with carnation mosaic virus, primary local lesions developed whereas in the upper non-inoculated leaves the presence of a virus could not be demonstrated. The upper leaves were resistant to infection and developed few lesions after a subsequent inoculation and no virus was found in protected leaves.

Resistance has been reported to develop in various hypersensitive hosts, both in tissues near the primary local lesions and in more distant parts of the plant (Ross, 1961; Loebenstein, 1972). Around primary TMV lesions on beans (Yarwood, 1960) and tobacco NN (Ross, 1961a), a zone of uninfected tissue became resistant to a second inoculation with TMV. In this area no lesions or only a few minute lesions developed. The resistant zone in tobacco NN increased in size and degree of protection for about 6 - 8 d after the first inoculation.

Further studies showed that this resistance is not restricted to the areas around lesions but develops also in other more distant tissues of the plant. Inoculation of half-leaves of tobacco NN plants with TMV

induced a high level of resistance to TMV in the opposite half-leaves (Ross, 1961b). Similarly, resistance could be induced in the basal part of a leaf by inoculating the apical half. Challenge inoculation with TMV of the resistant half-leaves 7 d after the first inoculation resulted in limited lesion formation. The lesions were only one fifth to one third as large in diameter as lesions in susceptible half leaves and they were fewer in numbers. Inoculation of the lower leaves on a plant induced resistance in the upper non-inoculated leaves.

The resistance induced by TMV in tobacco NN is not specific for TMV and leaves with TMV-induced resistance were also resistant to TNV, tobacco ring spot virus (TRSV) and tomato spotted wilt virus (TomSWV) (Ross, 1961b). A virus such as TNV which induce necrotic local lesions in tobacco NN plants will also induce both local and systemic resistance against TMV. Jenns and Kuć (1977) were also able to protect systemically susceptible cucumber cultivars against C. lagenarium race 1 by prior infection with TNV Infection of one cotyledon with the virus, protected the opposite cotyledon and the first true leaf against disease caused by a subsequent infection with C. lagenarium, and caused a reduction in the number and size of lesions caused by the fungus.

Systemic induced resistance was also demonstrated in Pinto beans and cowpeas (Ross, 1966). Various combinations of different viruses were used both for the first resistance-inducing inoculation and for the challenge inoculation in Pinto beans and cowpeas. Resistance developed in the opposite primary leaf regardless of the order in which any virus pair was used. Lesions developing in the resistant tissues were smaller (diameter x 0.5) than lesions in the appropriate control leaves, and lesion numbers were reduced. Wilson (1958) also showed that TMV would make tissues more resistant to the rust Uromyces phaseoli typica and the protection was thought to be due to the production of an inhibitor of uredospore germination.

The development of resistance has so far been reported only for

viruses that induce necrotic local lesions and no resistance was induced by mechanically or chemically induced necroses (Ross, 1961b). However, in Capsicum pendulum, heat-inactivated potato X virus induced systemic resistance affecting both lesion number and size (Nazaich and Singh, 1970).

It seems possible that the effects on size and number are caused by the same factor and that in highly resistant tissues the infection does not progress far enough to result in visible lesions and this might be the case also with localized induced resistance, developing near or around primary local lesions. Resistance there seems to be high and as a result lesion numbers and not only size are reduced considerably.

To elucidate the metabolic bases of induced resistance, several enzyme systems have been investigated. Simmons and Ross(1971) suggested that inoculation with a lesion-inducing virus elicits high peroxidase activity in resistant leaves, which leads to early appearance of necrosis. The lateral movement of some product(s) of necrosis or of reactions preceding necrosis, then causes an early limitation of lesion size. Gianinazzi et al. (1970) and Loon and Kammen (1970) observed the appearance of four new proteins in the leaves of tobacco cultivars Samsun NN and Xanthi-nc after infection with TMV and they suggested that the four additional proteins may play a role in suppressing the second virus infection by inhibiting either virus multiplication or virus spread to the surrounding plant tissue. New protein components and increased peroxidase activity were not restricted to the inoculated leaves, but were also found in the non-inoculated plant parts that neither show symptoms nor contain demonstrable virus (Loon and Kammen, 1970; Loon, 1975; Simmons and Ross, 1971; Loon and Geeler, 1971). Rohloff and Lerch (1977) found also four newly formed proteins seven days after inoculation of tobacco cultivar Xanthi-nc with TMV or TNV. Kassanis and White (1974) showed that the appearance of the new proteins and the formation of acquired resistance were inhibited by actinomycin D and they suggested that these proteins are

responsible for acquired resistance.

2. Non-microbial agents

2.1 Plant growth regulators (PGR)

Many PGR are known to influence the biochemical processes of higher plants, Davis and Di mond (1953) hypothesized that synthetic organic chemicals could increase resistance to disease by inducing changes in the host's metabolic processes unfavourable for disease development.

Indole acetic acid (IAA) reduced severity of Fusarium wilt of tomato plants when applied before inoculation (Davis and Di mond, 1956). Van Andel (1968) reported that IAA increased susceptibility of cucumbers to Cladosporium cucumerinum. Sinha (1964) demonstrated a decrease in severity of symptoms of Verticillium wilt of tomato with high concentrations of IAA whereas at low concentrations, IAA induced wilting. Emmanouil (1977) reported that after dipping tomato leaves in solution of IAA, colonisation of tomato plants infected with Verticillium dahliae decreased by 50% with similar results in egg-plants and peppers treated with IAA.

IAA applied to oats and wheat in various ways, had no effect on stem rust development in susceptible cultivars; IAA slightly increased resistance in a resistant variety (Gottlieb and Hart, 1943; Samborski and Shaw, 1957).

Dekker (1963) reported that kinetin fully protected leaf disks of cucumber from infection by Erysiphe cichoracearum.

Gibberellic acid (GA) increased wilting in Gem Resistant (GR) and Gem Susceptible (GS) tomato plants inoculated with Verticillium albo-atrum (Sinha and Wood, 1964). Kiraly et al. (1962) found that GA decreased susceptibility of wheat to Tilletia foetida whereas Petersen et al. (1963) reported that GA increased susceptibility of red kidney beans to infection by Rhizoctonia solani.

Maleic hydrazide, a growth inhibitor, increased the susceptibility of oats and wheat to stem rust and also induced susceptibility to Fusarium wilt in cotton when applied prior to inoculation but not when applied later (Sadasivan, 1961). Maleic hydrazide increased wilting whereas Cycocel reduced wilting in GS tomato plants inoculated with Verticillium albo-atrum (Sinha and Wood, 1964).

The role of ethylene in plant-resistance has been reviewed by Pegg (1976a, b) who reported control of Verticillium wilt in tomatoes by gassing them with ethylene. Chalutz and Stahman (1969) induced synthesis of isocoumarin in carrots and of pisatin in peas by treating plants with ethylene. Lisker et al. (1976) reported increases in concentrations of phytuberin and rishitin in potato tubers treated with ethylene as Ethrel before inoculation with P. infestans. Emmanouil (1977) reported that application of ethylene as Ethrel through tomato roots, depressed the effect of development of V. dahliae and it was suggested that the effect of ethylene could not be attributed to the production of phytoalexin because of its quantity but in pepper plants and eggplants treated with ethylene, capsidiol and lubimin were synthesized in considerable concentrations.

Brown and Barmore (1977) reported that in Robinson tangerine fruits treated with ethylene after inoculation with Colletotrichum gloeosporioides Penz., disease incidence was increased but the incidence was reduced when fruits were exposed to ethylene for 3 d prior to inoculation. Ethylene also induced resistance in green fruits when its application preceded inoculation. Stahman et al. (1966) induced resistance against Ceratocystis fimbriata by treating roots of sweet potato with ethylene. Lockhart et al. (1968) was also able to induce resistance in apples against Gloeosporium album by treating with ethylene.

Pre-inoculation gassing of tobacco leaves with ethylene resulted in 20 - 30% reduction in TMV lesion growth on treated leaves (Ross and Pritchard, 1972).

Loon (1977) induced resistance in Samsun NN tobacco leaves against

TMV by treating with ethephon, which generates ethylene. In cucumber and cotton cotyledons, application of Ethrel markedly enhanced TMV content (Cheo, 1971).

2.2 Irradiation

Vascular wilts have proved difficult to control and pathologists have been forced to develop new control measures such as chemotherapy (Waggoner and Dimond, 1952). In addition, tests of therapy by ionizing and non-ionizing radiation have been investigated (Waggoner, 1954; Waggoner and Dimond, 1952; Klein, 1967).

Ultraviolet irradiation (UV) which affects a variety of morphologenetic processes in plants may also be used to suppress localization of lesions in infected plants (Klein, 1967). Short-wave UV irradiation (2537Å) significantly enhanced TMV multiplication in cucumber cotyledons and lesion size in N. glutinosa plants if applied 24 h after inoculation (Loebenstein et al., 1970). Short-wave radiation applied 2 - 4 d after inoculation, or irradiation with long-waves (3660Å) one day after inoculation, did not increase virus concentration or lesion size. Larger lesions on primary leaves of Pinto beans irradiated one day after inoculation and incubated afterwards for 3 d in the dark were also observed (Wu and Dimitman, 1970).

Benda (1955) and Bawden and Kleczkowski (1952) have reported that UV irradiation of bean leaves before inoculation with TMV, number of lesions formed was reduced. Siegel and Wildman (1956) obtained the same results with Nicotiana glutinosa.

Irradiation of susceptible soybean plants with UV caused them to become more resistant to attack by P. megasperma var. sojae (Bridge and Klarman, 1973).

Irradiation of tomato plants with ionizing radiation increased and decreased resistance to Fusarium wilt (Waggoner and Dimond, 1956). Whole

irradiated before inoculation were more resistant, whereas the same treatment at inoculation decreased resistance. Root irradiation before inoculation increased resistance significantly, but the same treatment at inoculation caused only a small increase. Shoot irradiation at any time decreased resistance.

Nicotiana glutinosa and Samsun NN tobacco leaves were inoculated with TMV and immediately irradiated with gamma radiation for 24 and 48 h and after 48 h irradiation there was a lower reduction in the number of lesion (Halliwell and Langston, 1965). But plants irradiated 24 to 48 h prior to inoculation with TMV had an average increase of 56 and 54% more lesions respectively than the control leaves.

Decay of table grapes inoculated with Botrytis cinerea was reduced substantially by exposure to gamma radiation before infections had become established in the fruit (Covey and Bramlage, 1965).

The production of phytoalexins by a plant in response to infection or certain other types of physiological stimuli, is widely believed to be an important disease resistance mechanism. Langcake and Price (1977) have induced production of resveratol by grapevines in response to UV-irradiation. The phytoalexin, hydroxyphaseollin was detected in soybean hypocotyls 12 h after UV irradiation and pisatin and phenylalanine ammonia lyase, a key emzyme in the biosynthesis of pisatin, were induced in pea tissue by UV irradiation (Bridge and Klarman, 1973; Hadwiger and Schwochau, 1971). Cartwright et al. (1977) were also able to induce production of phytoalexins in rice plants by UV irradiation.

2.3 Temperature

Temperature exerts a profound influence on biological systems and it has been noted that heat treatment of plants was associated with alterations of disease resistance (Schnathorst and Mathre, 1965; Walker, 1965; Yarwood, 1956; Yarwood and Hooker, 1966).

Schulz and Bateman (1969) reported that treatment of bean seeds at 5°C during the first 24 h germination, increased susceptibility to infection by Rhizoctonia solani. Susceptibility of primary leaves of young bean plants to anthracnose and certain viruses was increased by immersion of leaves in hot water for a few seconds before inoculation (Yarwood, 1956). Rahe and Kuć (1970) observed that in etiolated bean hypotocylts inoculated with C. lindemuthianum, lesion size and number were decreased with increasing temperature of incubation between 28° - 32°C.

Leaves of bean, cowpea and sunflower were predisposed to infection with the cucumber mildew fungus, Erysiphe cichoracearum, by immersing in hot water before inoculation (Yarwood, 1963). Jerome and Müller (1958) reported that bean pods become susceptible to Sclerotinia fructicola and Botrytis cinerea after treatment for 2 h at 44°C. Cruickshank (1963) also reported that pea pods became susceptible to Monilinia fructicola after treatment at 40° - 45°C. Older leaves of lima bean which are generally resistant to rust, became susceptible when heated for 3 - 4 min at 45°C. before inoculation (Ikegami, 1968).

Resistant soybean plants became susceptible to Phytophthora megasperma var. sojae when subjected to heat treatment at 43 - 45°C. (Chamberlain and Gerdemann, 1966). After treatment at 44°C., soybeans became susceptible to two non-pathogens of soybeans, Phytophthora cactorum and Helminthosporium sativum. Resistance was recovered in plants treated at 45°C for 15 min but loss of resistance was irreversible in plants treated for 2 min at 50° or 55°C. (Chamberlain, 1970).

III. MATERIALS AND METHODS

A. Fungal Material and its Preparation

1. Sources of Fungi

Three races of Colletotrichum lindemuthianum (Sacc. and Magn.) Bri. and CAV. were obtained from the stock cultures collection of the Department of Botany, Imperial College.

Colletotrichum lagenarium (Dass.) Ell. and Halst, (causative agent of cucumber anthracnose) cultures were kindly supplied by Professor A. Touze, L.D.V.P., Toulouse, France, which were isolated from single spores from infected muskmelon fruits, and by Wye College, University of London.

Cultures of Colletotrichum coffeanum Noack (Glomerella cingulata) (Stonem) (Spauld and Schrenk), the causative agent of coffee berry disease, were obtained from the Institute of Agricultural Research, Ethiopia, Commonwealth Mycological Institute and from the Coffee Research Institute, Ruiru, Kenya.

Cladosporium cucumerinum (Ellis and Arth.) cultures were obtained from the Imperial College Stock Cultures Collection.

Races of C. lindemuthianum and C. lagenarium were reisolated from diseased parts of bean and cucumber plants respectively.

2. Growth of the Fungi

Throughout the research, acidic vegetable juice agar used for growth of the above Colletotrichum spp. was prepared as follows :

Vegetable juice (V_8)	200 ml
Agar	20 g
Distilled water	800 ml

Cladosporium cucumerinum was grown on sucrose casamino acid agar (SCA) which was prepared as follows :

Sucrose	15.0 g
KH_2PO_4	1.0 g
$MgSO_4.7H_2O$	0.5 g
Vitamin free casamino acid	4.6 g
Micronutrients	1 ml
Glass distilled water to	1 l

The trace element solution was made up as follows :

Salt	Stock sol. (mg/100 ml)
$FeSO_4.7H_2O$	249
$CuSO_4.5H_2O$	40
$ZnSO_4.7H_2O$	44
$MnSO_4.4H_2O$	41
$Na_2Mo_4.2H_2O$	51

For the growth of C. cucumerinum, 20 g/l agar was added.

Dense spore suspensions in sterile distilled water were spread over the surface of 20 - 25 ml of agar media in 9 cm. (diameter) Petri dishes or 250 Erlenmeyer flasks. The spore suspensions were spread uniformly over the surface and then excess was poured off. The Petri dishes were incubated at 22° - 24°C under fluorescent tubes for 3 - 4 days and then at 22° - 24°C under "Philips Black Light" fluorescent tubes giving maximum emission at 360 nm. The photoperiod was 12 h per day.

3. Inoculum Preparation and Application

Cultures of C. lindemuthianum and C. coffeanum were used within 9 - 10 days and C. lagenarium was used within 5 - 8 days. Cultures were

rinsed with distilled sterile water and spores were removed into water with a sterile loop from the surface of the cultures. The suspension was filtered through four layers of muslin and washed by two centrifugations each at 830 x g for 10 min. Suspensions were adjusted to the required concentration with sterile water after haemocytometer counts.

A 5 µl drop of a suspension of C. lindemuthianum conidia (5×10^5/ml) (2500 spores/drop) was the inoculum used in detached hypocotyls of French bean (Phaseolus vulgaris) and a 5 µl drop of a suspension of C. lagenarium conidia ($5 \times 10^4 - 1 \times 10^5$/ml) (250 - 500 spores/drop) was used in inoculation of cucumber cotyledons, true leaves and hypocotyls. Drops were applied from an Agla Micrometer Syringe (Burroughs Wellcome Ltd., London).

B. Plant Material

1. Sources of Plant Material

a) French bean (Phaseolus vulgaris)

Seeds were supplied by Processors and Growers Research Organisation (Great North Road, Thornhaugh, Peterborough). Cultivar Kievit Koekoek came originally from the Netherlands and was then produced at Imperial College Field Station, Ascot, Berks. Seed of several cultivars were supplied by the Institute of Agricultural Research, Nazreth, Ethiopia.

b) Cucumber (Cucumis sativus)

Seeds of several cultivars were bought from Sutton and Sons Ltd. and cultivars resistant to C. lagenarium were kindly supplied by Clemson University, South Carolina.

c) Coffee (Coffea arabica)

Seeds of three cultivars were kindly supplied by the Institute of Agricultural Research, Ethiopia and seed of two cultivars were supplied by Coffee Research Station, Ruiru, Kenya.

2. Growth and Inoculation of Plants

a) French bean

Seeds of French bean which were free of damage or disease were washed under running tap water for 30 min, and then placed between layers of absorbent tissue, moistened with distilled water in plastic boxes (17 x 11.5 x 6 cm). The boxes were closed and seed were incubated at 24° - 25°C for 3 days. The covering tissue was changed once. Germinated seeds were then stripped of their testas and planted in boxes of Vermiculite (Micafil, Dupre Vermiculite Ltd.), saturated with Long Ashton Solution which was prepared as follows :

Stock Sol. No.	Salt	Conc. (g/200 ml)	
1	$Ca(NO_3)_2.4H_2O$	110.0	
2	KNO_3	50.5	
3	$NaH_2PO_4.2H_2O$	20.0	
4	$MgSO_4.7H_2O$	36.0	
5	FeNaEDTA	1.22	
6	Micronutrients		
	$MnSO_4.4H_2O$	0.223	dissolved in 200 ml of H_2O
	$CuSO_4.5H_2O$	0.024	
	$ZnSO_4.7H_2O$	0.029	
	$(NH_4)_6Mo_7.4H_2O$	0.0035	
	H_3BO_3	0.186	

FeNaEDTA was adjusted to pH 5.6 with 1N NaOH and kept at 4°C. 5 ml of each solutions 1 - 6 were made up to 2.5 litres with glass distilled water.

Boxes were transferred to a growth cabinet (150 x 60 x 50 cm) fitted with an extractor fan and Philips fluorescent tubes. A 12 h light period was applied and the temperature was maintained at 24° - 25°C. The light intensity was between 450 - 600 lumens/sq. ft. Hypocotyls were harvested 9 - 10 days from germination. Etiolated bean hypocotyls were prepared in a similar way from plants except grown in the dark.

Excised hypocotyls were trimmed to 5 - 8 cm from 0.5 cm below the cotyledonary node and 1 cm. from the surface of the Vermiculite. The hypocotyls were then washed under running tap water for 20 - 30 min, dried, both ends smeared with vaseline (to prevent drying) and mounted on perspex holders (Plate 1), in a plastic box at c. 100% RH. The box lids were lined with moist tissue to prevent formation of droplets of condensed water. Hypocotyls after being inoculated by the drop method were incubated at 17° - 19°C with 12 h light/day in the growth cabinet.

Intact plants were sprayed with spores suspension by a Shandon Laboratory Spray Gun (No. 2046) and placed in polythene bags at c. 100% RH for 48 h. They were then kept uncovered on glasshouse benches.

b) Cucumber Plants

Cucumber seeds were washed under running water for 30 min and planted in John Innes No. 2 compost in plastic trays (34 x 20 x 5) in the glasshouse under Philips 400w mercury vapour lamps as a source of light and heat. The t° varied from 20°C during the winter to 36°C in summer. Fifteen days after planting or when true leaves began to develop, seedlings were detached along with 1.5 - 2 cm

hypocotyls. The cut ends were immersed in tap water and the cuttings were supported in a wire mesh.

Cotyledons were inoculated by the drop method. Hypocotyls of cucumber were inoculated in the same way as were hypocotyls of French bean plants. For other studies, seedlings were transplanted to John Innes No. 2 compost in pots.

c) Coffee Plants

Plants were grown from seed at the University of London, Botanical Supply Unit, Egham and in a glasshouse at Imperial College, London. Seeds with the parchment removed were planted in moist sterilized sand in plastic boxes with closely fitting transparent lids kept at room t° (22° - 24°C). Seedlings were ready for inoculation when they had hypocotyls of 3 - 5 cm. long, usually after 5 - 6 weeks growth.

Before inoculation, the lid was removed and hypocotyls were sprayed with C. coffeanum spores (2×10^6/ml) from a Laboratory spray gun and the boxes immediately closed again. The inoculation was repeated 48 h later.

The boxes were incubated at 22° - 24°C for the first 4 days and then at 19° - 20°C with the lids removed.

C. Pathogenicity Tests

a) French bean

Bean cultivars were screened for their reaction to races of C. lindemuthianum using the drop inoculum and spraying methods. After the plants were prepared as described above, spore suspensions of appropriate races and concentrations were applied as 5 µl/drop on

hypocotyls or intact plants were sprayed (hypocotyls and leaves) to run off. The sprayed plants were placed in polythene bags for 48 h. Disease symptoms on plants were scored 6 - 7 days after inoculation by either method.

b) Cucumber

Cucumber cotyledons and true leaves of different cultivars were tested against C. lagenarium as follows :

1. Cotyledons

Cotyledons of cucumber cultivars were grown as described above were used. They were inoculated with C. lagenarium (1×10^5 conidia/ml) each with 6 x 5 µl/drop from an Agla micrometer syringe. They were then incubated in boxes at 24° - 25°C and lesions were measured every other day for 10 - 12 days.

2. True leaves

Seedlings were transplanted to compost in pots (c. 20 cm. diameter). Fully expanded true leaves were removed 5 — 8 days later, i.e. 20 - 23 days after planting seed. The leaves were sprayed with C. lagenarium (1×10^5 conidia/ml). Numbers of lesions developing per unit area were counted.

c) Coffee plants

Coffee seedlings were grown as described above.

Reactions of hypocotyls to C. coffeanum were assessed by the methods used by Vossen et al. (1976).

D. Isolation of C. lindemuthianum from bean seeds

Seeds of French bean supplied by the Institute of Agricultural Research, Ethiopia were used for the isolation of C. lindemuthianum by the method described by Schneider et al. (1974) with some modification.

Ten seeds were taken at random from cultivars susceptible to different races of C. lindemuthianum and washed separately in 2 ml sterile distilled water by vigorous shaking. The washings from each seed were plated, in a series of dilutions, separately on vegetable juice medium containing 30 µg streptomycin sulphate/ml. The washed seeds were then surface treated by immersing in 0.25% sodium hypochlorite solution for 4 min., then in 70% ethyl alcohol for 2 min. and finally rinsed in sterile distilled water. Each seed was aseptically dissected into seed coat, cotyledons and embryo. Each section was plated on vegetable juice medium.

E. Spore germination test (SGT)

The method adopted by Purkayastha and Deverall (1965) was used with some modification. Used microscope slides were preferred to new ones. They were cleaned by rubbing with cotton wool in hot water and detergent (Stergene) and by repeated washings in tap water. The washed slides were immersed in distilled water for 48 h and finally in acetone for a few hours before drying at 50° - 60°C. This technique gave slides which had no deleterous effect on spore germination and did not allow test drops to spread unduly.

Three 20 µl drops of each solution to be bioassayed were put on a slide from an Agla micrometer syringe. One µl of spore suspension (5×10^5 conidia/ml) was added to the centre of each drop. If any of the drops spread, the slide was discarded. Slides were placed on Perspex holders on moist filter paper in plastic boxes which were sealed. The boxes were incubated in the dark for appropriate periods. At the end of incubation the

germination was stopped and spores were stained by adding a drop of aniline blue in lactophenol (Amann's Medium) to each drop. The Amann's Medium was prepared as follows :

Aniline blue (water soluble)	1 g
Glycerol	25 ml
Lactic acid	25 ml
Phenol	25 g
Distilled water	25 ml

Percentage of germination and lengths of germ tubes were measured. Germ tube lengths were determined by a calibrated micrometer eyepiece. Germination and germ tube length was generally used to assess the effects of the solution being bioassayed. Other characteristics were recorded if they appeared to be significant.

F. Sterilization

Glassware and media were sterilized by autoclaving at 15 psi for 15 min. Solutions of substances which would have been altered by autoclaving were sterilized by passage through a membrane filter (0.22 μ).

G. pH measurements

The pH of large volumes was measured with a 'Pye 290' pH meter (Pye Unicam, Cambridge). For smaller volumes BDH Narrow Range indicator papers were used.

H. Preparation of healthy plant extracts

Water extracts from different parts of plants were prepared as follows. One g fresh weight of tissue was homogenized in 10 ml of sterile

distilled water in a Sorval Omnimixer. The homogenates after being filtered and centrifuged, were reduced to $^1/10$ of the original volume under reduced t^o in a vacuum evaporator. These were sterilized through a membrane filter (0.22 μ) and bioassayed on glass slides against the Colletotrichum spp.

I. Preparation of diffusates

(a) Water diffusates

The diffusate technique was adapted from Muller (1958). Sterile distilled water was applied from an Agla micrometer syringe on to cotyledons of susceptible (S) and resistant (R) cucumber plants in boxes which were sealed with petroleum jelly and incubated in the dark at 24^o - 25^oC for 48 h.

The droplets, collectively referred to as water diffusates, were collected in a Pasteur pipette and centrifuged at 850 x g for 15 min. to remove bacteria and debris. The diffusates were reduced to $\frac{1}{2}$ of the original volume under low t^o in oxygen (free from nitrogen). The diffusates were either bioassayed immediately or after storage at -20^oC.

(b) Spore diffusates

Spores of C. lindemuthianum (race β), C. lagenarium and C. coffeanum (5×10^5/ml) were applied to cotyledons of R and S cucumber from an Agla syringe and incubated for 48 - 60 h. Cotyledons treated with race β were incubated in the dark at 17^o - 19^oC and the rest were incubated at 24^o - 25^oC. The collected diffusates were centrifuged at 850 x g for 15 min to remove spores and debris, and then reduced to $\frac{1}{2}$ of the original volume under low t^o in oxygen free nitrogen. The diffusates were either bioassayed immediately or after storage at -20^oC.

J. Culture extracts

Vegetable juice agar prepared in 250 ml Erlenmeyer flasks as described above was seeded with a spore suspension (1×10^6 conidia/ml) and excess suspension was poured off. The flasks were incubated at 22° - 24°C for 7 - 8 days and 20 ml of sterile distilled water was added and shaken for 2 - 3 min. The flasks were left at 22° - 24°C for 48 h. The liquid was removed, filtered and centrifuged for 30 min at 850 x g to remove the spores and other suspended material. The final preparation is referred to as culture diffusate. It was reduced to $^1/10$ of the original volume and sterilized by passage through a membrane filter (0.22 μ). The diffusates were either bio-assayed immediately or after storage at -20°C.

K. Mycelial cell wall extracts

Spores of C. lindemuthianum (race β), C. lagenarium and C. coffeanum were grown in liquid culture as described by Anderson and Albersheim (1975). The liquid medium was prepared as follows :

Sucrose	15 g
KH_2PO_4	1 g
$MgSO_4$	0.25 g
$Fe\ Cl_3$	0.027g
Casein hydrolysate	10 g
Distilled water	900 ml

The cultures were grown for 8 days at 23°C on a rotary shaker at 100 rpm. The mycelia were removed and comminuted in a Waring blender for 60 sec. using 5 ml of water per g wet weight of mycelia. The homogenate was filtered through a coarse sintered glass plate. The residue was homogenized first in distilled water then in a mixture of chloroform and methanol (1 : 1) after discarding the supernatant fluid, it was homogenized

in acetone, again after discarding the supernatant fluid when air dried was the cell wall fraction.

Elicitor was extracted from the cell walls by suspending 1 g of walls in 100 ml of water and autoclaving for 20 min at 121°C. The autoclaved suspension was then clarified by centrifugation and concentrated to 1/10 original volume under reduced pressure. The solution of elicitor was stored at - 20°C.

L. Lesion diffusate

Cucumber cotyledons of R and S cultivars were prepared and inoculated with C. lagenarium as described above. Four to five days after inoculation 4 cotyledons were detached and placed in a sterile Petri dish with 20 ml of sterile distilled water. Uninoculated cucumber cotyledons were used as controls. The inoculated side of the cotyledon was placed on the water. They were left for another 4 - 5 days at 24° - 25°C. The liquid referred to as lesion diffusate was filtered through a membrane filter and centrifuged for 30 min at 850 x g. The diffusate was finally reduced to 2 ml under reduced pressure and stored at - 20°C.

M. Treatments

1. Plant growth regulators

French bean plants and cucumber cotyledons were treated with plant growth regulators. The plant growth regulators used were :

Item No.

1. Indole-3-acetic acid (IAA) (M.W. = 175.18)
2. Maleic hydrazide (1,2-Dihydro-3,6-pyridazinedione) (M.W. = 112.09)
3. Gibberellic acid (GA) (M.W. = 346.37)
4. Cycocel (2-chloroethyl trimethyl ammoniumchloride)

5. Kinetin (6-Furfurylaminopurine) (M.W. = 215.21)

6. Ethrel (M.W. = 28.05)

1 - 4 were supplied by BDH, 5 by Koch-Light Co. and 6 by A.H. Macks & Co. Ltd. (Wyke, Bradford).

(a) French bean

Seeds of Canadian Wonder susceptible to all races of C. lindemuthianum, were planted in vermiculite, saturated with Long Ashton solution (LANS), for appropriate periods. Plants were then uprooted and the roots were washed in tap water before placing in solution of the plant growth regulators for 24 h or 72 h before inoculation.

Controls were dipped in water. Hypocotyls from the treated and control plants were prepared and inoculated with C. lindemuthianum (race β) as described earlier.

Similarly, solutions of plant growth regulators were applied in drops from an Agla syringe onto hypocotyls with drops of distilled water as controls. The sites where the plant growth regulators were applied were marked with a non-water soluble ink. Drops of solutions of the plant growth regulators were left for 24 h or 72 h and then removed with absorbent paper. The sites were inoculated with C. lindemuthianum (race β). The effects of plant growth regulators on disease expression were scored on the scale used by Skipp (1971).

1. Brown flecking - minute red brown spots in the inoculation site

2. Limited lesion - a lesion which is confined to part of the area of the inoculation site

3. Non-limited lesion - a lesion which is the same size as or larger than the area of the inoculation site.

(b) Cucumber plants

Cotyledons of cultivar resistant and susceptible to C. lagenarium were treated with solutions of plant growth regulators as follows.

(i) Absorption through roots

12 - 14 d old cucumber seedlings were uprooted, roots were washed and placed in solutions of plant growth regulators for 24 h and 72 h. Controls were placed in glass distilled water. Cotyledons were put in plastic boxes as described above. The cotyledons were inoculated with 5 µl drops of a suspension of conidia of C. lagenarium (1×10^5/ml). The effects of the growth regulators were assessed from as the lesion size after incubation for different periods.

(ii) Continuous absorption

15 d old cucumber cotyledons were prepared as described above. Cut ends were placed in modified plastic vials containing solutions of plant growth regulators. Controls were placed in glass distilled water. The cotyledons were inoculated with 5 µl drops of a suspension of conidia of C. lagenarium (1×10^5/ml) while they were exposed to the solution of the plant growth regulators. The effects were assessed from size of lesions.

(iii) Cotyledon disks

Cotyledon disks were cut with a cork borer (No. 9) from 15 d old resistant and susceptible cucumber cotyledons. The disks were placed on 20 ml of solutions of plant growth regulator solutions or distilled water in Petri dishes. Some disks had the lower others had the upper surface on the liquid. After appropriate periods, disks were removed, placed in boxes lined with moist tissue paper, and were inoculated with 5 µl drops of a suspension of conidia of C. lagenarium (1×10^5/ml) and incubated at 24° - 25°C. The effects were assessed from size lesions.

2. Non ionizing radiation (UV)

Etiolated hypocotyls of R and S cultivars of French bean were prepared as described above. Hypocotyls were irradiated with UV light from a "Philips TUV 30 watt" germicidal single tube with output of 8 watts at 2537 Å waveband. The hypocotyls were 140 cm from the UV source. After irradiation hypocotyls either were inoculated with Colletotrichum spp. or extracted for other purposes.

3. Temperature effect

Etiolated bean hypocotyls of R and S (C. lindemuthianum - race β) cultivars of French bean in boxes with lids on, were exposed to different t° for different periods. The boxes were then kept to attain room t° before hypocotyls were inoculated with Colletotrichum spp.

N. Histological techniques

Transverse and longitudinal sections of hypocotyls were cut by hand with a razor blade. Thin epidermal strips were taken from inoculated areas to observe germination of and mode of penetration from conidia. Material was sectioned either fresh or after storage in a fixative which was prepared as follows :

Formalin	13 ml
Glacial acetic acid	5 ml
Ethanol (50%)	200 ml

Leaves, after being fixed and cleared were observed by light microscopy.

1. Leaf fixative 24 - 48 h

Chloroform	30 ml
Ethanol (100%)	60 ml
Glacial acetic acid	10 ml

Then

2. Clearing agent

Xylene	100 ml
Phenol	5 g

Sections were viewed unstained or after staining with Trypan blue prepared as follows :

Lactophenol	100 ml
Trypan blue	0.2 g

O. Light microscopy and photomicrography

Sections, stained or unstained, were observed for reaction and penetration of cells and other features.

Photographs were taken with an Exacta VX 1000 camera mounted on the microscope. The camera was loaded with either Agfachrome 50L professional 35 mm reversal film or Kodak high speed Ektachrome (tungsten) reversal film (ASA125).

P. Stereo-scanning electron microscopy (SEM)

Longitudinal of fixed material sections of bean hypocotyls and leaf disks of beans and cucumber cotyledons were prepared for SEM as follows.

1. Dehydration

Sections of fixed material were taken through an acetone series consisting of single changes at 10 min. each in 10, 20, 30, 40, 50, 60, 70, 80, 90 and two changes at 100%. Sections were then removed from acetone and dried in a critical point dryer (Polaron E300) at $32^{\circ}C$ and 1200 psi.

2. Coating with gold

Dried sections were mounted on aluminium rivets on a thin coat of 'Durofix' adhesive (Rawlplug Co. Ltd., London), and coated with gold in a Polaron diode sputter coater (Polaron E5000) for 2 min. at an accelerating voltage of 1.2 kv and a current of 40 amp. A standard coating was 480 Å thick.

Coated sections were examined in a Cambridge Stereoscan Mk 2A stereo scanning electron microscope using an accelerating gun potential of 10 kv.

Photographs were taken with an Exa 1A camera loaded with Ilford Pan F film.

Coated sections, when not in use, were stored in a desiccator at room t°.

Q. Extraction techniques

Seeds of French bean, cultivars Canadian Wonder and Kievet, susceptible and resistant respectively to C. lindemuthianum (race β) were washed under running water for 30 min. Seeds were germinated as described earlier. Healthy, germinated seeds were selected and the testas and embryos were removed. Cotyledons were washed thoroughly with distilled sterile water and a portion of (c. 0.5 - 0.7 mm^2) the epidermis was removed with a razor blade. They were placed in a box lined with moist tissue paper and dried in a sterile stream of air in a Laminar flow cabinet. The cotyledons were sprayed from a laboratory spray gun, with suspensions (5 x 10^5/ml) of spores of C. lindemuthianum (race β), C. lagenarium and C. coffeanum. Cotyledons treated with race β were incubated at 17° - 19°C and the rest were incubated at 24° - 25°C. After incubating for appropriate periods, brown areas which developed were removed with a razor blade and stored at -20°C. or extracted immediately as described below.

1. Extraction of isoflavanoid compounds

Material was comminuted in ethanol in a Sorvall Omnimixer at high speed for 1 min (10 ml ethanol/g). The homogenate was filtered under suction through Whatman No. 1 filter paper. The filtrate was retained and the solid residue was re-extracted similarly. The filtrate was reduced to dryness in a Büchi rotary evaporator under reduced pressure at 40°C. The residue was taken up in 10 ml distilled water and centrifuged (1400 x g) for 15 min. The supernatant was retained and the residue washed in 10 ml water and centrifuged.

Supernatants were combined and the residue discarded. The supernatant was shaken with an equal volume of ethyl acetate in a separating funnel and allowed to separate for 20 min. The ethyl acetate phase was collected and the separation was repeated three times. The ethyl acetate fractions were combined and sodium sulphate (anhydrous) was added if the fraction was wet. This was reduced to dryness and stored at -20°C in nitrogen air or used immediately for thin layer chromatography.

2. Extraction for phenolic compounds

Tissues from inoculated and control cotyledons of cucumber were collected and extracted in a Sorval Omnimixer in 80% ethanol (2 ml/g). The extracts were filtered on a Buchner funnel through Whatman No. 1 paper and the residue washed excess 80% ethanol. The extracts were partitioned three times with equal volume of petroleum ether and a straw coloured liquid was obtained. The petroleum ether fraction was discarded and the ethanol fraction was evaporated to dryness under reduced pressure and t° in a rotary evaporator. Total phenols in the extract were determined by the Folin Ciacalteu method (Plummer, 1971).

R. Chromatography technique

Throughout this research, ascending thin-layer-chromatography (TLC) was used as a preparative technique. TLC plates were 20 cm square, glass, plastic or aluminium backed silica gel plates (Griffin and George Ltd.) with a layer thickness of 0.2 mm.

To the extracted and dried fractions, 0.5 ml of ethyl acetate was added and applied to the plates as bands using a drawn-out Pasteur pipette. This was applied at a loading equivalent to 1 g extracted tissue per cm. unless otherwise stated. Chromatograms were run in a Shandon Chromatank

after equilibration in a solvent saturated atmosphere for at least 1 - 2 h. The solvents used were :

a. Chloroform : Methanol (25 : 1)

b. Benzene : Ethylacetate : Methanol (25 : 8 : 4)

The plates were run first in solvent (a) and later the relevant compounds were re-run in solvent (b).

In preparation for quantitative analysis, silica gel at zones of the chromatogram relevant to the investigation were scraped off and placed in a small sintered glass funnel, covered with a piece of Whatman No. 1 filter paper. Compounds were then eluted with ethanol. The eluate was dried in a rotary evaporator and the compounds were redissolved in a known quantity of ethanol and measured in a Beckman spectrophotometer.

S. Bioassay technique

Chromatograms (TLC) were assayed for fungitoxic compounds by spraying them with a heavy suspension of spores of Cladosporium cucumerinum, suspended in sucrose casamino acid (SCA) liquid medium. Zones of inhibition appeared white against a dark background of sporulating C. cucumerinum mycelium in 2 - 3 days after incubating in a moist chamber at 24° - 25°C.

T. Phenylalanine-ammonia-lyase (PAL)

Enzyme activity was estimated spectrophotometrically (Hadwiger, 1970). One g of fresh plant material was extracted with 5 ml of 0.025 M borate buffer (pH 8.8) and centrifuged at 14,000 x g for 15 min. The supernatant was used as the enzyme extract. The reaction medium containing 1.5 ml of 0.05 M borate buffer (pH 8.8), 1 ml 0.06 M phenylalanine solution and 0.5 ml enzyme extract were incubated for 2 h at 37°C. The change in optical density ay 290 nm was read as a measure of enzyme activity. A change in absorption

of 1.0/h/cm was taken as a unit of activity. The control contained phenylalanine and autoclaved enzyme.

U. Enzyme production

1. Culture filtrate preparation

Colle totrichum spp. were grown in liquid media containing 1% carbon source prepared as follows :

(a) Czapex broth	Conc. g/l
$NaNO_3$	3 g
K_2HPO_4	1 g
KCl	0.5 g
$MgSO_4.7H_2O$	0.5 g
$FeSO_4.7H_2O$	0.01g
Micronutrients	1 ml
Pectin or NaPP	10 g

made up to 1 l with distilled water

The medium was adjusted to pH 5.

(b) Shake culture	
NH_4NO_3	3/li
$MgSO_4.7H_2O$	.5
$ZnSO_4$	.01
$FeCl_3$	.06
KH_2PO_4	2.5
Micronutrients	1 ml
Pectin or NaPP	10 g

made up to 1 l with distilled water

Media were incubated in 250 flasks in a rotary shaker at 1000 rpm.

After incubation the cultures were filtered through Whatman paper (No. 1) and centrifuged at 850 g for 30 min at 4°C and the supernatant was retained. The filtrate was then dialysed and assayed.

2. Dialysis

Culture filtrates were dialysed against distilled water. Visking tubing (H.M.C. Scientific Instrument Centre) 8/32 in. in diameter was soaked in sterile water until pliable, knotted at one end and filled with culture filtrate. The free end was tied and placed in a large volume of distilled water. Culture filtrates were dialysed at 4°C for 24 h and were either assayed immediately or after storage at -20°C.

3. Assay

The protein solutions prepared from culture filtrates were tested for endopolygalacturonase (PG) and pectic trans-eliminase (PTE). Enzyme activities were estimated by determining the reduction of viscosity of solutions of appropriate substrate in reaction mixtures prepared as follows.

Substrate was NaPP obtained from NBC Corp. Cleveland, Ohio. The NaPP was washed with acidified (0.05 M HCl) 70% ethanol.

For PG, the reaction mixture consisted of 5 ml of 2% (w/v) NaPP, 1 ml citrate buffer (0.5 M) pH 5 and 4 ml of enzyme solution. Depending on the activity of the enzyme solution the volume added was variable and made up to 4 ml with distilled water.

For PTE, the reaction mixture consisted of 8 ml of a sufficient solution NaPP at pH 9 - 9.1 (0.05 Tris-HCl), 1 ml of enzyme solution and 1 ml $CaCl_2$ (0.002 M) solution.

Viscometry was carried out at 37°C in Cannon-Fenske Viscometers

(size 200). Viscometers were calibrated by the flow time of 10 ml of distilled water at 37°C. Enzyme activities were expressed as relative viscosity units (RVU) defined as 1000/t, where t is the time (min) for a 50% decrease in viscosity of the reaction mixture.

4. Thiobarbituric acid test (TBA)

The reaction mixtures were as described for viscometry with NaPP as substrate. They were incubated at 37°C for 1 h. Samples of 1.0 ml were removed and added to 7.5 ml thiobarbituric acid solution (0.01 M) and 2.5 ml HCl (1 N) in a test tube and mixed well. The tube was covered with a cap and placed on a boiling water bath for 30 min. After cooling, the red colour produced was read at 515 nm. for PG and 550 nm. for PTE, against samples prepared in the same way but with an autoclaved enzyme.

5. Isoelectric focussing

This method was used for analytical separation of proteins according to their isoelectric point (pI) and for characterisation of proteins by their pI.

The principle of the method depends on the establishment of a pH gradient between two electrodes; the proteins in the sample migrate to the points on the gradient corresponding to their individual pI. The pH gradient is produced by the use of low molecular weight 'Ampholine' carrier ampholytes (LKB-Producter) giving gradients between pH 3 - 11 or fraction of this range. When preparing the column, sucrose gradient is used to obtain a convection free medium. An LKB 8101 'Ampholine' electrofocussing column (110 ml) was used.

Solutions were prepared as follows.

Cathode solution (light)

NaOH	0.2 g
Distilled water	14 ml

Anode solution (dense)

Sulphuric acid	0.2 ml
Distilled water	14 ml
Sucrose	12 g

Gradient solution (light)

Carrier ampholyte	0.75ml

diluted to 60 ml with the culture filtrate

Gradient solution (dense)

Carrier ampholyte	2.25ml
Sucrose	28 g

diluted to 42 ml with distilled water

The column has an inner and outer jacket through which circulated externally cooled water maintained at $4^{\circ}C$ by a water bath thermostat.

The column was set up as instructed by the suppliers and after the solutions were in place, it was connected to an LKB DC power supply and the voltage adjusted to c. 1000 V for 72 h.

To empty the column, the anode solution (dense) was first removed by siphoning as it interferes with the pH of the enzyme fraction. Fractions of 2 ml were collected in 10 ml graduated plastic test tubes. The pH values of the fractions were measured using 'Pye 290' pH meter.

The fractions were then dialysed against distilled water at

$4^{o}C$ for 24 h to remove the sucrose and carrier ampholytes and were assayed using viscometry or stored at $-20^{o}C$.

V. Gel electrophoresis

Protein extracts from cucumber leaves were prepared as follows. Leaf disks were extracted in a buffer (1 ml/g) with pestle and mortar under ice. Extracts were centrifuged at 40,000 for 30 min. The supernatant was retained and used for gel electrophoresis.

The buffer was 1M Tris-HCl (pH 8)

0.5 M Sucrose

3% Mercaptoethanol

Gel electrophoresis was used for the resolution of proteins in a LKB 2117 Multiphor apparatus. A 10% acrylamide gel (pH 8.9) was prepared as follows :

(a)

1. Gel buffer

1N HCl	12.0 ml	dissolved in 100 ml
Tris HCl	9.15g	of water. pH 8.9

2. Acrylamide stock

Acrylamide	22.2 g	in 100 ml of
Methylene Bis-Acrylamide		distilled water

3. NH_4 per sulphate (fresh)

0.15 g/100 ml distilled water

4. TEMED

(N N N 'N' - Tetramethylethylene diamine)

(b) 10% Acrylamide gel was prepared from (a) as follows :

30 ml of 1

26.6 ml of 2

3 ml of 3

0.04 ml of 4 added last

The acrylamide gel solution was moulded on a glass plate (125 x 260 x 1 mm) supported on both sides by glass plates (125 x 260 x 3 mm) and lined with a gasket. These were clamped except where the mixture was to be added. The mixture was added through the unclamped area and left at room t^{o} to polymerize. The gel was collected by inserting two spatulas between the glass plates.

The samples, soaked in Whatman paper (5 x 10 mm) were placed on the polyacrylamide gel at equal distances. Bromophenol blue (BPB) was used as an indicator. The buffer vessels were filled with 1 l each of Tris-HCl - Glycine buffer (pH 8.3). Whatman paper No. 1 was used as wicks touching the polyacrylamide gel.

(a) Bromophenol blue (BPB) 1.5 g/l

(b) Running buffer

Tris-HCl	6.0 g/l	pH 8.3
Glycine	28.8 g/l	

The apparatus was connected to an LKB power supply unit and the voltage was 200 - 400 V. The separation was completed within 3 - 4 h. The polyacrylamide gel was removed and stained with 0.03 % Coomasir blue for 24 h and destained with 10% acetic acid.

W. Carbohydrate determination

Quantitative determination of carbohydrates was by the Anthrone method (Plummer, 1971).

4 ml of anthrone reagent (0.2 % in conc. H_2SO_4) was added to 1 ml of the solution to be tested, and rapidly mixed. The tubes were placed in a boiling water bath for 10 min. To prevent loss of water by evaporation, the tubes were covered. When cool the solution was read at 620 nm against a reagent blank in a spectrophotometer. Standard curves were prepared from glucose and glycogen solution (10 mg/100 ml each).

X. Protein and phenol determination

Proteins and phenolic compounds were determined by the Folin-Ciacalteu reagent which was prepared as follows :

(a) 2% w/v Na_2CO_3 in 0.1 NaOH

(b) 1% w/v $CuSO_4.5H_2O$ solution and
2% w/v potassium sodium tartrate solution mixed in equal quantities on day of use

(c) Alkaline copper reagent : 50 parts of (a) to 1 part of (b)

(d) Folin Ciacalteu reagent diluted to 1N.

1 ml of solution and 5 ml of reagent (c) were mixed and kept at 37°C in a water bath for 15 min. 0.5 ml reagent (d) was then added and mixed. After a further 30 min. the absorbance of the protein solution was estimated at 750 nm against a blank reagent and the phenol solution was estimated using a Colorimeter (EEL, red filter). Calibration curves were prepared with solutions of crystalline bovine serum albumin (Koch-Light Co.) for protein estimation and catechol for phenol determination.

Y. Measurement of electrolyte loss

Loss of electrolytes from treated cucumber cotyledons was estimated by determining the change in conductivity of bathing solutions over a period of time with a 'Chandos' conductivity meter at 24-25°C. 10 disks (diam. 3 mm) were suspended in 10 ml distilled water in a glass vial.

Z. Chemicals

Unless otherwise stated, chemicals were of Analar grade and were obtained from the British Drug House (BDH) and Hopkins and Williams. Ethanol and methanol were obtained from James Burrough Ltd.

IV. EXPERIMENTAL WORK

A. Symptom development and histological observations

This section is concerned with the host responses which will lead to the visible expression of resistance or susceptibility of infected French bean, cucumber and coffee caused by their respective pathogens.

1. French bean

The ability of C. lindemuthianum races α, β and δ to form lesions on the varieties listed in Tables 1 and 2 was tested as described below.

Excised and intact hypocotyls from 10 d plants were inoculated with spore suspensions (5×10^5/ml) and incubated at 17° - 19°C.. The excised hypocotyls were inoculated at 4 sites/hypocotyl (5 µl/drop). Eight hypocotyls, 4 for microscopic observation and 4 for disease reaction, were used for each combination. Intact hypocotyls were inoculated by spraying from a spray gun with spore suspension until run off.

After incubation for 10 - 12 d, reactions of cultivars to the three races of C. lindemuthianum were scored as described by Smith (1967) and Skipp (1971) as follows :- (Plate 1)

a. Resistant reaction was described as hypocotyls (intact and excised) without visible lesions or with only light flecking at the points of inoculation.

b. Intermediate reaction in excised hypocotyls was described as hypocotyls with one or two limited lesions out of 4 inoculation sites/hypocotyl and 4 - 5 limited lesions/hypocotyl in intact hypocotyls sprayed with the spore suspension.

c. Susceptible reaction in excised hypocotyls was described as hypocotyls with more than 2 limited lesions out of 4 sites of inoculation sites/hypocotyl or general hypocotyl infection involving collapse and death. Intact hypocotyls were described as susceptible when numerous limited lesions developed all over the hypocotyl or infection involving collapse and death.

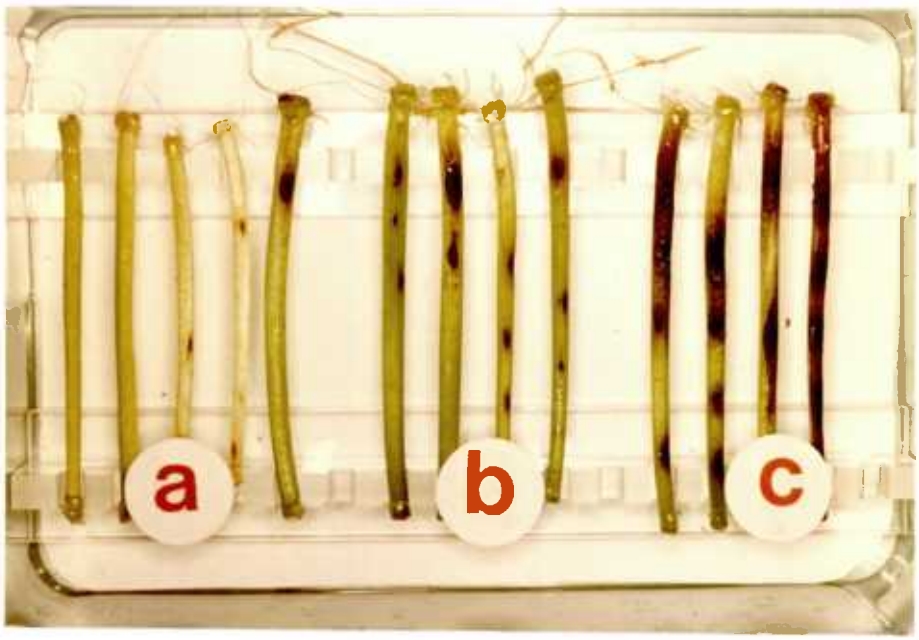


Plate 1. Disease reaction in French bean 10 d after inoculation and incubated at 17° - 19°C.

a = Resistant reaction

b = Intermediate reaction

c = Susceptible reaction

Results shown in Tables 1 and 2 are mean of two experiments.

Table 1. Differential reactions of French bean cultivars to races of C. lindemuthianum

Cultivar*	Races		
	α	β	δ
Chicobel	R/R	R/R	R/R
Purley king	R/R	R/R	R/R
Slenderette	R/I**	S/S	S/I
Cascade	S/I	S/S	S/S
Tender crop	R/I	S/S	S/S
Bina	S/S	S/S	S/S
Revenge	R/R	R/R	R/I
Lochness	S/S	S/S	S/S
Cavalier	S/S	S/S	S/S

* Cultivars supplied by Processors and Growers Research Organization

** R/ = Resistant on detached hypocotyls and

/I = Intermediate on sprayed plants

S = Susceptible reaction

Cultivars Chicobel and Purley king were highly resistant with no visible symptoms. The other cultivars which were classified as resistant produced flecks at the sites of inoculation. The reaction of all the cultivars agreed with those reported by the suppliers.

Table 2. Differential reaction of French bean cultivars to races of C. lindemuthianum

Cultivar*	Races α	β	δ
Nazreth selection 138	S/S	S/S	S/S
Mexican 142	R/R	R/R	R/R
Mangetot contender	R/R	R/R	R/R
Tangam 16	R/R	R/R	R/R
Ethiopia 10	R/R	R/R	R/R
Brown mottled	R/R	R/R	R/R
Negro mecentru	R/R	R/R	R/R
Nazreth selection 27	S/S	S/S	S/S
" " 203	R/I**	R/R	R/I
Tara	R/I	R/R	R/I
6R - 340	S/S	S/S	S/S
Small black caffer	R/R	R/R	R/R
Red wollamo	R/I	R/R	R/I
Pinto a	S/S	R/I	S/S
Black Dessie a	S/S	R/R	S/S
W-95 a	R/R	R/R	S/S
Stella a	R/R	R/R	S/S
Ethiopia Red a	R/R	S/S	R/R
6R - 395 a	S/S	R/R	S/I
Nazreth small a	S/S	R/R	R/R
Brown speckled a	R/R	R/R	S/S
Arroz 3 a	R/R	R/R	S/S
Total Resistant	15	18	12
Susceptible	7	4	10

contd...../

* Cultivars supplied by the Institute of Agricultural Research in Ethiopia

** R/ = Resistant reaction on detached hypocotyls

/I = Intermediate reaction on sprayed plants

S = Susceptible reaction

a Cultivars showing different reactions to races of C. lindemuthianum

The results given in Table 2 show that most of the cultivars were resistant to the three races used. Nine cultivars (out of 22) showed different reactions to the three races of C. lindemuthianum.

Attempts were made to isolate C. lindemuthianum from the different parts of seeds of susceptible cultivars. Ten seeds from each susceptible cultivar were washed in sterile gdw and the washings were spread into V_8 agar in Petri dishes. Then seeds were dissected asceptically into cotyledon, embryo and the testa. Each part of a seed was washed separately in sterile gdw and spread into V_8 agar in Petri dishes. 5 - 7 d after incubation the cultures were examined under the microscope. Washings from the cotyledon and embryo did not produce any microorganisms. Washings from the seed coat indicated the presence only of Alternaria spp. which is not pathogenic to French bean.

There was little evidence, therefore, that seeds of any of the many cultivars that were tested were contaminated or infected by C. lindemuthianum.

1.1 Histological observations

The reactions of epidermal cells of hypocotyls of cultivars Chicobel and Bina, resistant and susceptible respectively to race β were studied by light microscopy. Ten d old hypocotyls were inoculated with spore suspension of race β (5×10^5/ml) in 5 µl drop on 4 sites. Epidermal strips were removed from inoculation sites over a time course of the infection process.

In both R and S cultivars, appressoria were produced in about equal

numbers within 24 h. R cultivar showed a hypersensitive reaction (HR) of cells within 24 - 48 h in which 2 - 3 cells, directly beneath or very near the germinating spores, were killed after granulation of the cytoplasm (Plate 3).

In the S cultivar intracellular hyphae were first observed 50 - 60 h after inoculation as a hyaline ring at one side of the appressorium and this body expanded to form an elongate primary hypha, as described by Leach (1923) (Plate 4). The primary hyphae were 6 - 8 µm in diameter when examined soon after penetration. Penetration of the cells was continued by secondary hyphae which branched from the primary hyphae. The secondary hyphae are 3 - 4 µm in diameter and can be distinguished from the primary hyphae by the lack of any marked swelling or little constriction at the site of passage through a cell wall (Plates 5 and 6).

As the penetrating hyphae continued to grow intracellularly, cellular browning and granulation occurred 7 - 8 d after inoculation, a depression, grey green at first but which later became a light chocolate brown appeared at the inoculation site. With the change of colour, a drop of brown exudate appeared on the inoculation site. Complete collapse of hypocotyl tissue occurred later and was due to fungal colonization and bacterial contamination.

In a limited lesion, the hyphae penetrated only a few cells before its growth was stopped.

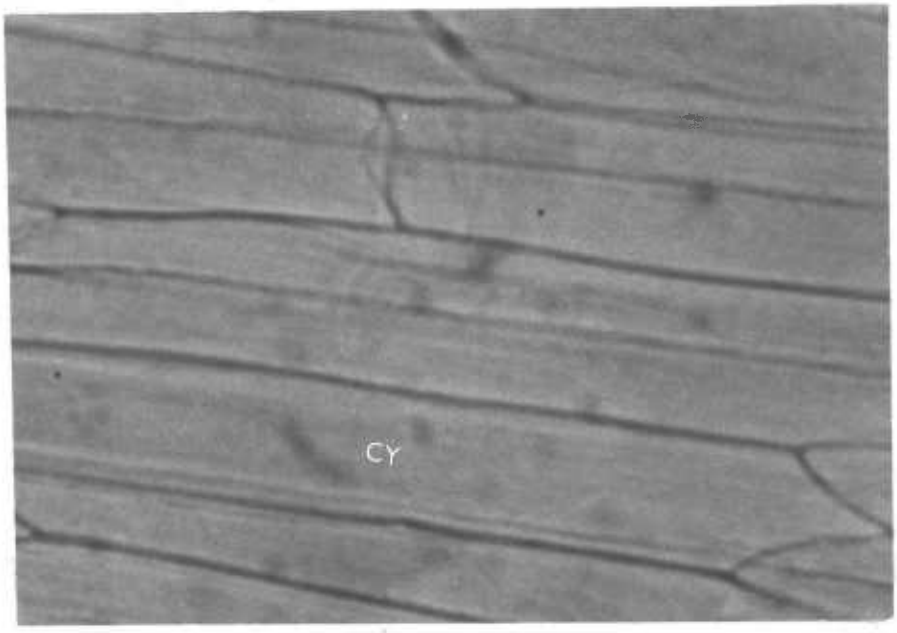


Plate 2. Healthy epidermal strips from Canadian Wonder Cv. of French bean hypocotyl showing normal cytoplasm (CY) x 600

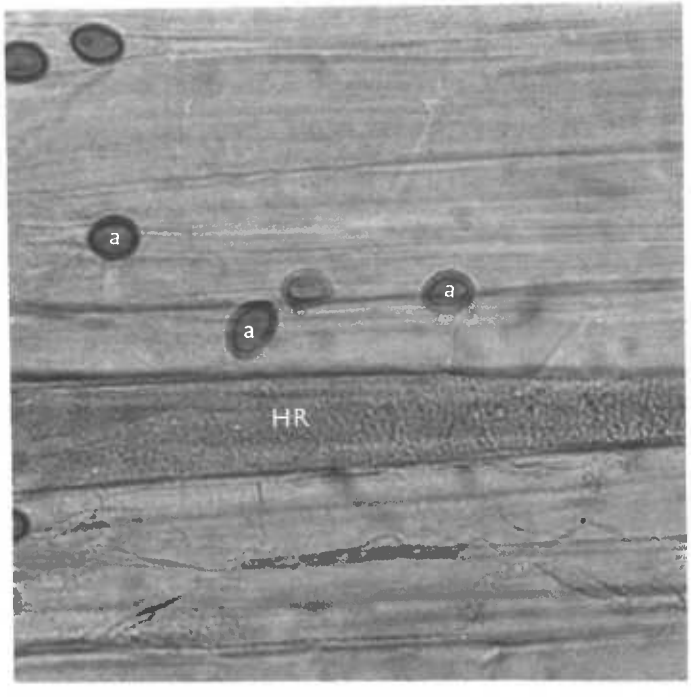


Plate 3. Hypersensitive reaction (HR) showing granulation of the cytoplasm 24 - 48 h after inoculation by Kievit Cv. of French bean inoculated with C. lindemuthianum (race β) a = appressorium

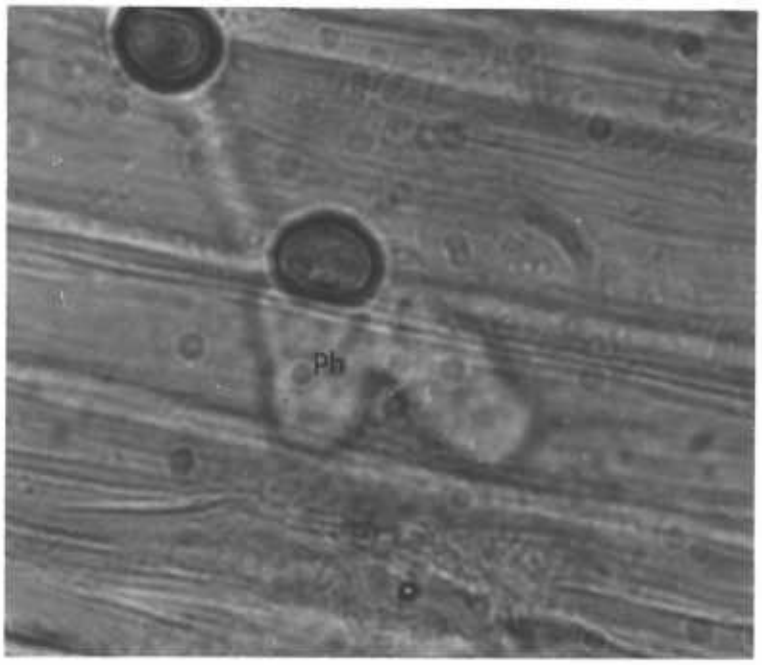


Plate 4. Primary hyphae (ph) originating from the appressorium (a) 72 - 96 h after inoculation of Canadian Wonder Cv. with race β. x 1500

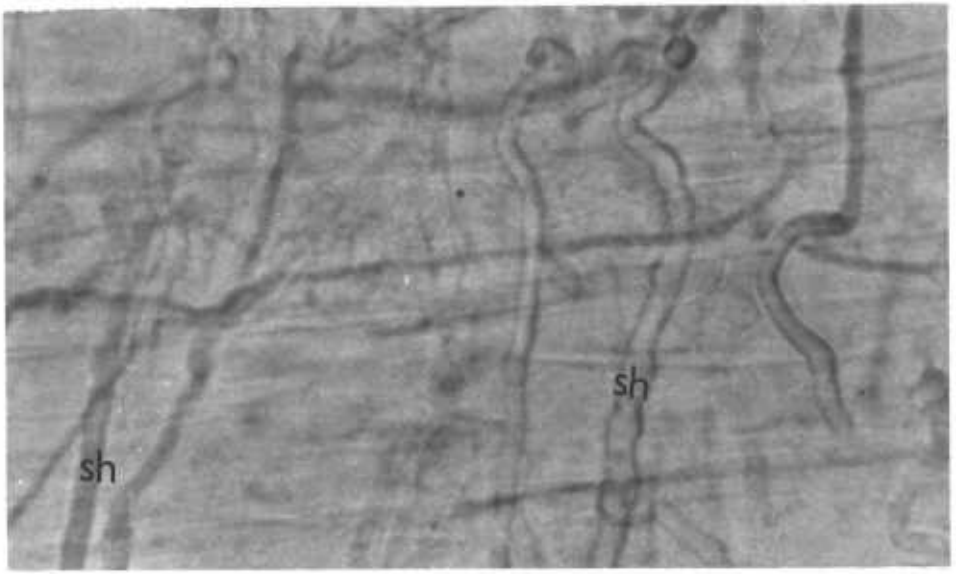


Plate 5. 144 h after inoculating of Canadian Wonder Cv. with race β. Branching of secondary hyphae (Sh). x 600

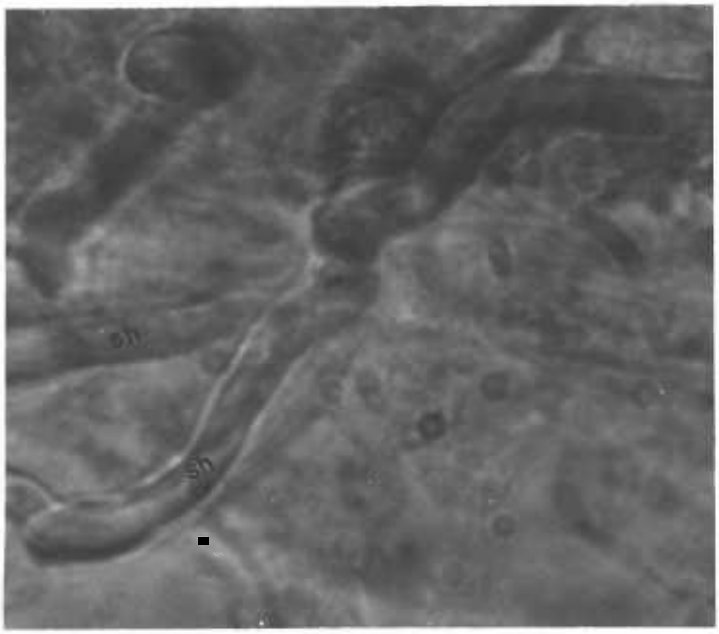


Plate 6. Secondary hyphae (Sh) magnified x 1500. Race β on Canadian Wonder Cv. hypocotyl 144 d after inoculation

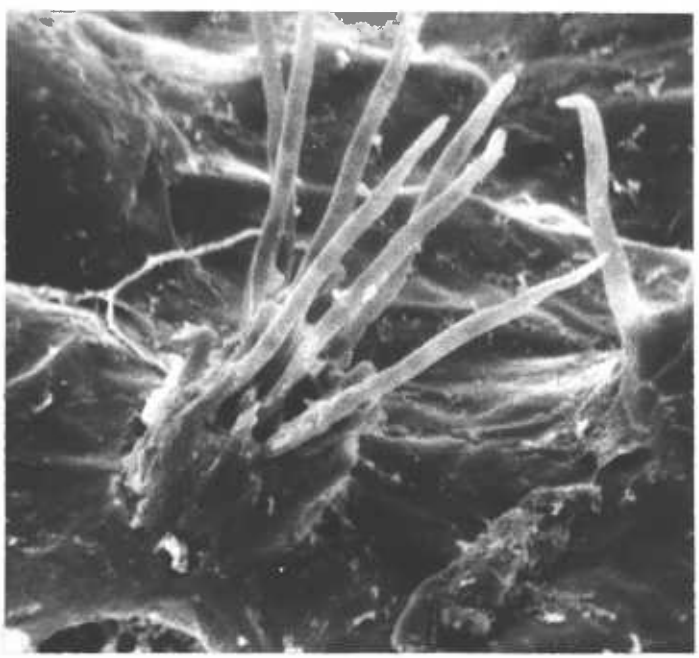


Plate 7. Acer vuli formation by race β on Canadian Wonder Cv. hypocotyl as observed under SEM. x 1050

2. Cucumber

Several cultivars of cucumber were tested for their reaction to C. lagenarium. Cucumber plants were growing in John Innes (No. 2) compost for 15 d in the greenhouse at 24° - 25°C. Cotyledons along with hypocotyls were detached, placed in boxes and supported by a wire mesh. 8 cotyledons of each cultivar were inoculated with 5 µl drop of suspension of spores of C. lagenarium (1×10^5/ml). Four sites/cotyledon were inoculated and incubated at 24° - 25°C.

Cucumber plants growing in pots (20 cm) for 21 d produced a pair of true leaves. Both the true leaves were sprayed with spore suspension of C. lagenarium (1×10^5/ml) from a Laboratory Spray gun until run off. 4 plants/cultivar were sprayed and covered with polythene bag for 48 h. Later the bags were removed and incubated at 24° - 25°C.

The cultivars were screened against C. lagenarium on the basis of the rate of expansion of lesions in mm/d on the detached cotyledons and on the number of lesions developing/unit area on the true leaves.

Table 3 shows that there was not always a good relationship between the rate of expansion of lesions on detached cotyledons and the number of lesions produced/unit area of true leaves. Thus Baton Vert was high in resistance on both assessments whereas Perfection appeared to be resistant on detached cotyledon but susceptible in leaves. But for the majority of the cultivars that was reasonably good agreement for the two methods of assessments (see ** on Table 3).

Table 3. Differential reaction of cucumber cultivars* to C. lagenarium (Wye isolate)

Cultivar	Lesion expansion in mm/d	No. of lesion on true leaves/cm^2
Baton Vert	** 0.70 [a](1)	1.07 (2)
Perfection	0.95 (2)	2.18 (9)
Burpless Tasty Green	0.98 (3)	1.74 (7)
Marion	** 1.22 (4)	0.72 (1)
Sigmaden	** 1.25 (5)	1.24 (3)
Telegraph Improved	** 1.30 (6)	1.30 (4)
Venlo Pickling	** 1.33 (7)	1.87 (8)
Telegraph	** 1.34 (8)	2.60 (11)
Long Green	1.36 (9)	1.36 (5)
Nadir	** 1.43 (10)	2.96 (12)
Butchers	** 1.46 (11)	2.52 (10)
Apple shaped	1.52 (12)	1.54 (6)

* Cultivars supplied by Sutton and Sons Ltd.

a () = shows rank of the cultivar on the rate of lesion expansion in mm/d on detached cotyledons and on No. of lesions on true leaves/cm^2

** = Rank differences 3 or fewer

Cultures of C. lagenarium which were obtained from Wye College and Toulouse, France (74 FR) were compared for their virulence on the same cultivars of cucumber. Detached cotyledons were prepared as described above. One of the cotyledons was inoculated with one isolate and the other cotyledon with the second isolate to avoid differences between plants.

The virulence of the two isolates was compared by the rate of lesion expansion in mm/d in each cultivar.

Table 4. Comparison of virulence of two isolates of C. lagenarium

Cultivar	Rate of lesion expansion in mm/d	
	Wye	Toulouse (74 FR)
Baton Vert	** 0.53[a](1)	0.69[a](3)
Burpless Tasty Green	** 0.68 (2)	0.83 (5)
Long Green	** 0.73 (3)	0.66 (2)
Nadir	** 0.75 (4)	0.65 (1)
Perfection	0.81 (5)	0.96 (9)
Marion	** 0.83 (6)	0.77 (4)
Telegraph Improved	** 0.94 (7)	0.97 (10)
Sigmaden	** 0.96 (8)	0.83 (5)
Venlo Pickling	** 1.00 (9)	1.06 (12)
Telegraph	** 1.04 (10)	0.86 (7)
Butchers	** 1.10 (11)	1.00 (11)
Apple shaped	1.25 (12)	0.88 (8)

Variation was 0.06 - 0.12

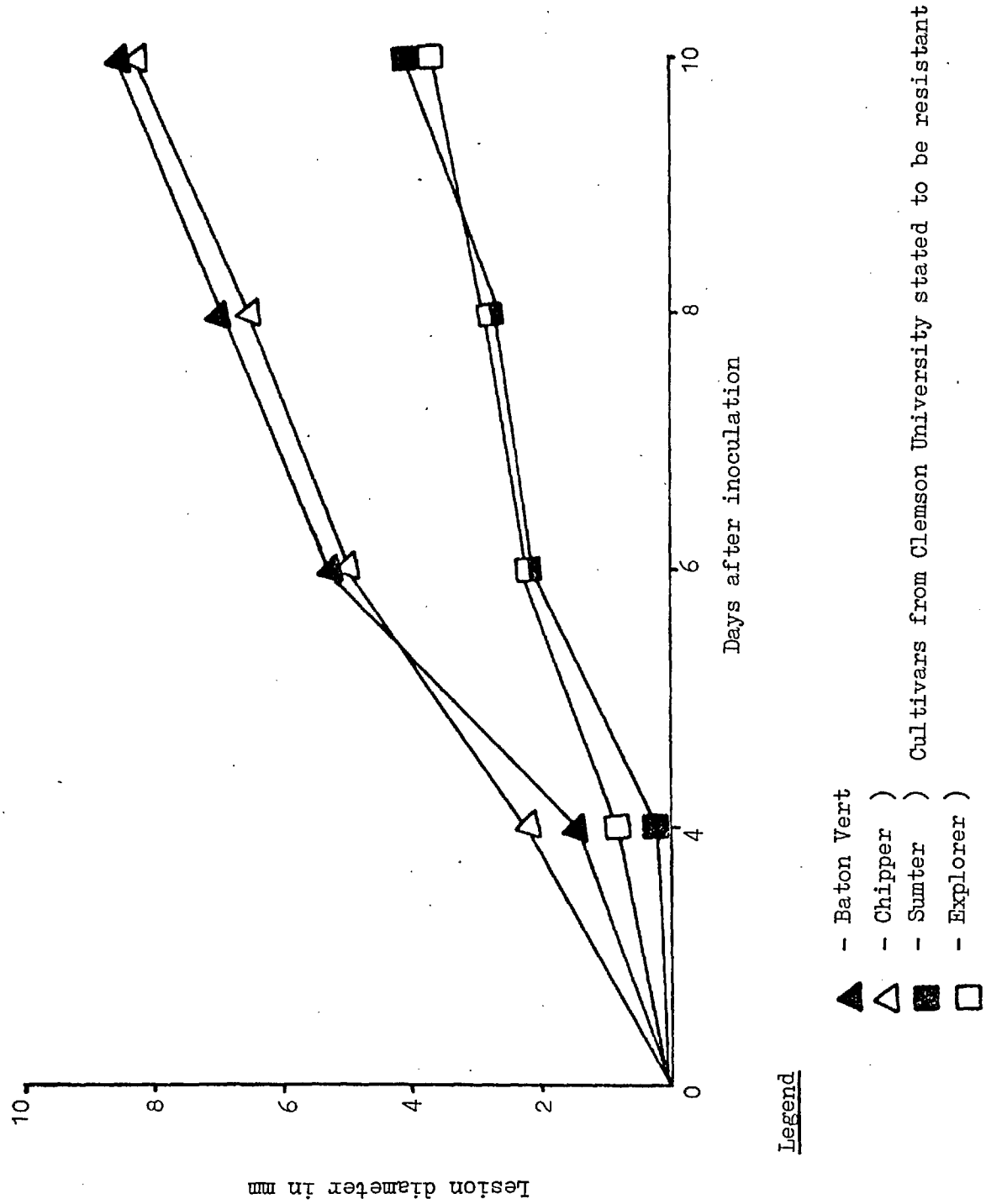
a() = shows rank of the cultivar according to increase of lesion size by the isolates

** = Rank differences 3 or few of cultivars when inoculated by the two isolates

8 cotyledons/isolate of each cultivar were inoculated

Table 4 shows that both isolates of C. lagenarium caused lesions of about the same size on the different cultivars. However, because the

Figure 1. Responses of detached cotyledons of cucumber cultivars inoculated with C. lagenarium



culture from Wye College sporulated more readily on V_8 agar, it was used for all the later research.

Three cv.obtained from Clemson University, U.S.A. and stated to be resistant were also tested together with Baton Vert against the Wye isolate. Lesion diameter in mm on detached cotyledons is given in Fig. 1.

Cv.Baton Vert was much more susceptible than cvs. Sumter and Explorer but cv. Chipper was also susceptible. Baton Vert and Sumter were selected as susceptible and resistant cultivars respectively for the subsequent research.

2.1 Histological observation

The host-parasite interactions was studied both on cucumber cotyledons and hypocotyls. Penetration by C. lagenarium and its subsequent development were compared on susceptible and resistant cvs.

Detached cotyledons of cucumber were prepared as described earlier. Hypocotyls of cucumbers were prepared from 15 d old cucumber seedlings. These were washed, dried, both ends sealed with vaseline and mounted on perspex holders. Both the detached cotyledons and hypocotyls were inoculated with 5 µl drops containing 1×10^5 spores/ml and observations were made on sections or epidermal strips.

Cotyledon disks were collected over a course of time and after being fixed and cleared, were mounted on glass slides and scanned under a light microscope. Strips were taken from hypocotyls and observed.

Germination occurred promptly and appressoria were formed within 4 - 8 h after inoculation. On both R and S cv. appressoria were formed equally abundantly within 24 h.

On cotyledons and hypocotyls, 48 h after inoculation, the penetration peg arising from an appressorium was commonly visible invading the epidermal cell and proceeded intracellularly to invade the mesophyll cells of cotyledons.

Although C. lagenarium penetrates the cotyledons in the same way and rate in both resistant and susceptible cv, hyphae were much less prevalent in resistant tissue than in susceptible.

The fungus penetrates much more quickly in hypocotyls than in cotyledons of cucumbers. The mode of penetration and the subsequent invasion of cells was the same as in French bean hypocotyls. Hypocotyls from resistant and susceptible cv. produced sunken lesions within 72 h after inoculation but susceptible hypocotyls collapsed within 4 - 5 d after inoculation whereas the resistant hypocotyls did not.

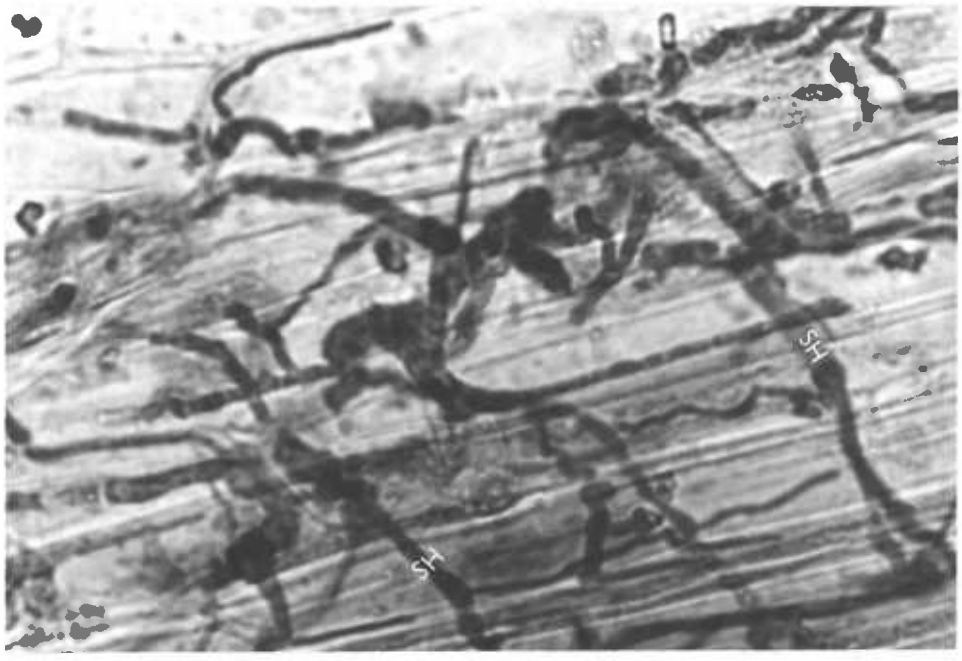


Plate 8. Branching of secondary hyphae (SH) of C. lagenarium on hypocotyls of Baton Vert, susceptible cv. of cucumber 72 h after inoculation x 600. The same type of branching was observed in hypocotyls of Sumter, resistant cucumber cv.

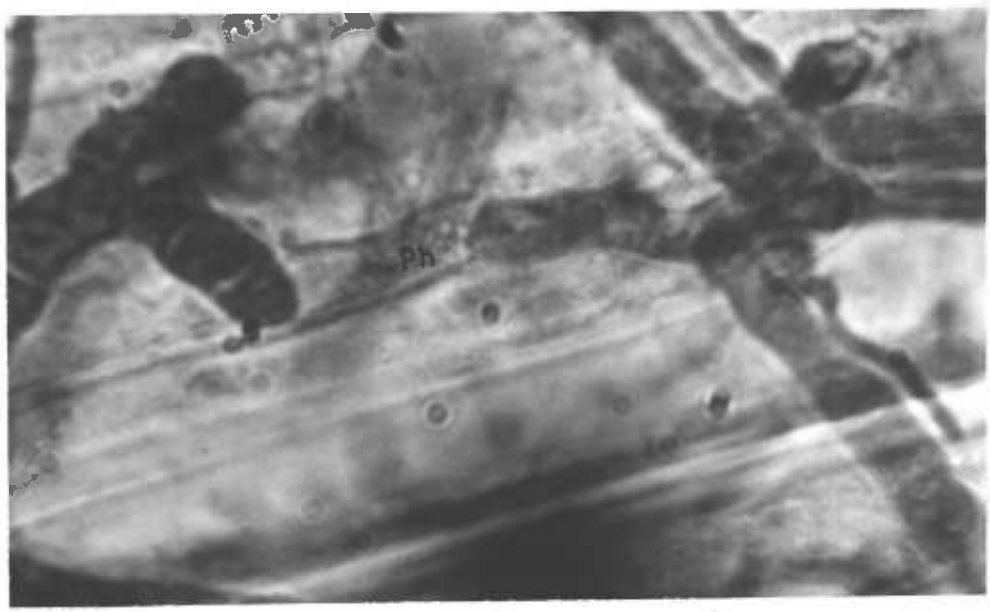


Plate 9. Primary hyphae (ph) formation by C. lagenarium on hypocotyls of Baton Vert, susceptible cv. of cucumber, 72 h after inoculation x 1500.

3. Coffee

C. coffeanum usually attacks berries of coffee as the causative agent of Coffee Berry Disease (CBD). Coffee trees reach the stage of producing berries 3 years from planting and to conduct a progeny test against C. coffeanum is therefore very slow if berries are used. Instead a new and quicker method of progeny test was under investigation at Coffee Research, Kenya. The method was to inoculate hypocotyls of young seedlings with spore suspension of C. coffeanum and to relate the results to susceptibility or resistance to CBD in the field.

Cv. SL34 and K7 susceptible and resistant respectively to C. coffeanum were grown and prepared as described in Materials and Methods. Hypocotyls of 5 - 6 weeks seedlings were sprayed with suspensions of spores of C. coffeanum (2×10^6/ml) from a laboratory spray gun. The spraying was repeated 48 h later and sprayed plants were incubated at 18° - 19°C.

Symptoms in the hypocotyls of susceptible cv. started to appear 7 d after inoculation as brown flecks and 14 d after lesions started to form. They gradually became larger and black in colour. Twenty one d after inoculation, there were clear differences between susceptible and resistant cultivars. In the susceptible cv. the hypocotyl was girdled but hypocotyls from the resistant cv. remained without any visible symptoms (Plate 10).

The results agreed with the field tests done on the berries and with tests of inoculation of the hypocotyls by the suppliers.

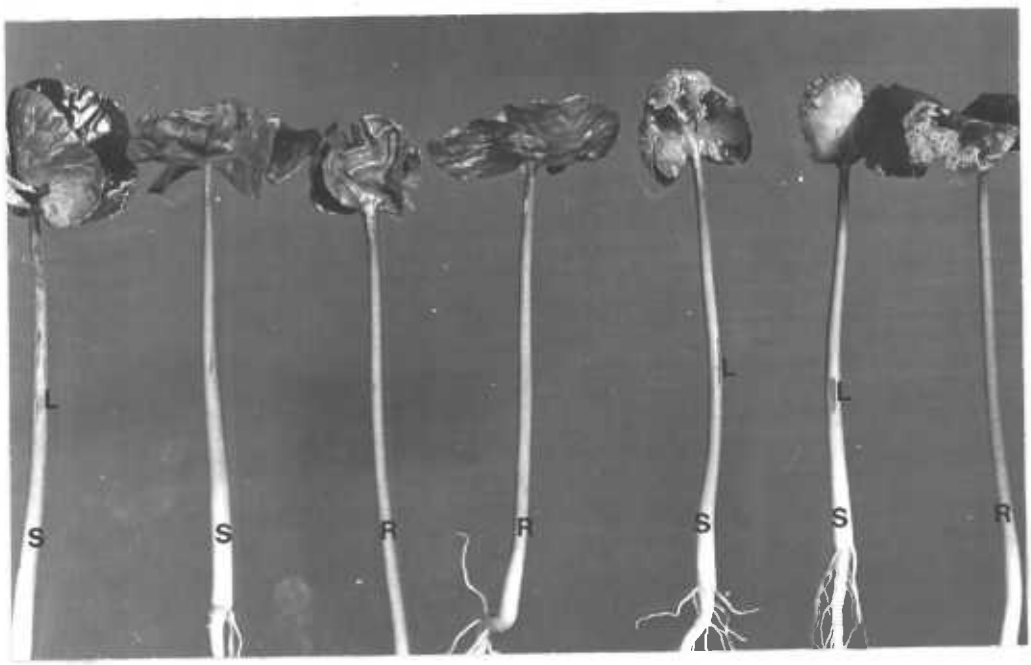


Plate 10. SL34 and K7 susceptible (S) and resistant (R) cultivars of coffee respectively inoculated with C. coffeanum. Lesion (L) formation 14 d after inoculation on S was observed and no symptoms on R cultivars of coffee.

B. The effect of carbohydrates on the germination of conidia of Colletotrichum spp.

Arabinose, xylose, fructose, galactose, glucose and sucrose were used in spore germination tests with C. lagenarium, C. coffeanum and C. lindemuthianum (races β and δ) to see if there were significant difference in germination, germ tube length and production of appressoria.

The spore germination test was carried out on glass slides. Germination and growth was stopped after 24 h. Percentage germination was measured by counting 150 spores from each drop and germ tube length was given as the average of 30 germ tubes in each drop.

Germination was the sum of conidia which produced appressoria and germ tube, and germ tubes without appressoria.

A Student t test (Steel and Torrie, 1960) was carried out to compare the germ tube length for control and treated conidia and an analysis of variance of the split plot design type was carried out to find out if different concentrations of the carbohydrates affect the germ tube lengths and if there was any interaction between the type of carbohydrate and concentrations.

1. Effect of carbohydrates on conidia of race β

Results are summarized in Tables 5 - 7.

Table 5. The effect of sugars on germination of conidia of C. lindemuthianum (race β)

Sugars	Concentrations (mg/ml)									
	0		1		0.5		0.1		0.05	
	GTL* μ	G** %	GTL μ	G %	GTL μ	G %	GTL μ	G %	GTL μ	G %
None	81.8 (± 8.8)[a]	81.3 43.3[b]+38[c]								
Arabinose			239.1 (±28.1)	42.9 7.6+35.3	213.8 (±34.8)	39.8 4.9+34.9	159.8 (±19.4)	35.8 3.1+32.7	123.9 (±11.9)	34.7 4+30.7
Xylose			240 (±35.8)	79.3 50+29.3	231 (±24.4)	61.6 27.6+34	196.7 (±20.2)	40.9 12.4+28.5	193.3 (±23)	72.7 41.8+30.9
Fructose			145.1 (±9.7)	76.9 38.9+38	140.7 (±15.1)	84.2 34.9+49.3	149 (±15.6)	84.2 53.8+30.4	154.5 (±18.6)	89 60.3+28.7
Galactose			299.1 (±22.1)	31.3 4+27.1	245.8 (±29.1)	32.4 3.3+29.1	251.2 (±25.2)	44.7 10+34.7	261.9 (±25.9)	31.3 3.5+27.7
Glucose			153.6 (±15.4)	72.7 20.9+51.8	165.2 (±15)	71.8 25.1+46.7	161.7 (±14.5)	74.7 33.8+40.9	141.6 (±11)	80.2 23.8+56.4
Sucrose			161.7 (±14.8)	94.2 19.1+75.1	173.2 (±18.1)	95.3 37.1+53.2	138.7 (±15.6)	95.3 11.6+83.7	135.3 (±17.6)	74.4 18.9+55.5

* GTL = germ tube length in μ with standard error (a)

** G = germination of conidia

b = germinated conidia with appressoria

c = germinated conidia without appressoria

Results are means of three replicates

Table 6. t test comparing germ tube length for control and conidia of C. lindemuthianum (race β) treated with carbohydrates

Carbohydrate	t $(\frac{d}{sd})$
Arabinose	33.65**
Xylose	7.02*
Fructose	24.27**
Galactose	19.95**
Glucose	15.66**
Sucrose	9.32*

** = Significant at 0.01 probability level

* = Significant at 0.05 probability level

Table 7. Analysis of variance of the effect of carbohydrates and concentrations on the germ tube length of C. lindemuthianum (race β)

Main effects and concentrations	df	SS	MS	F (observed)	Fp=0.01 (expected)
Carbohydrate	5	22307.3808	4461.47616	31.431**	5.06
Error (a)	12	1703.3521	141.946		
Concentration	3	3936.3806	1312.12687	11.398**	4.31
Interaction	15	4638.8553	309.2570	2.686**	2.52
Error (b)	36	4144.4366	115.1232		
Total	71				

** = Significant at 0.01 probability level

For conidia of C. lindemuthianum (race β) treated with the above carbohydrates, germ tube length was significantly increased compared to the controls (Table 6). The greatest increase of germ tube length was in conidia treated with galactose (1 mg/ml) when the increase was by 223% compared to the controls.

There was highly significant interaction between the carbohydrate and concentrations used i.e. the effect of concentration depended on the type of carbohydrate (Table 7).

The effects of carbohydrates on the conidial germination of race β can be summarized as follows.

Galactose decreased the total germination by 45 to 62% depending on the concentration. The greatest effect of galactose was in the decrease of appressoria production compared to the control. The decrease was between 77% and 99.9%.

Arabinose decreased the germination by 47 to 57% and appressoria production was decreased by 99.9%, with little or no effect on the germ tube production.

Glucose had little or no effect on the germination but it decreased appressoria production by 45% and 52% at the lowest and highest concentrations respectively.

Sucrose increased the germination by 16 to 17% and decreased the total appressoria production by 14 to 73%.

Fructose and xylose had little or no effect on germination and appressoria production.

This experiment was repeated with conidia of race δ.

2. Effect of carbohydrates on conidia of race δ

Results are summarized in Tables 8 - 10.

Table 8. The effect of sugars on germination of conidia of C. lindemuthianum (race δ)

Sugars	Concentrations (mg/ml)									
	0		1		0.5		0.1		0.05	
	GTL* μ	G** %	GTL μ	G %	GTL μ	G %	GTL μ	G %	GTL μ	G %
None	68.3 (±6.3)[a]	70.2[a] 48.6+24.6[c]								
Arabinose			65.8 (±7.4)	14 4 + 10	59.3 (±9.2)	14.4 4.9+9.5	75.8 (±6.3)	49.8 17.1+32.7	61 (±3.4)	15.6 3.3+12.3
Xylose			0	2 2+0	17 (±3.3)	16.2 10.7+5.5	7.8 (±4.6)	4.7 2.9+1.8	19.5 (±8.8)	10 2.5+7.5
Fructose			130.8 (±8.2)	88.9 56.2+32.7	120 (±8)	80.7 68+12.7	104.2 (±9.3)	77.1 69.1+8	106.7 (±8.5)	85.3 78.7+6.6
Galactose			56.5 (±6.8)	32.9 10+22.9	56 (±6.5)	53.6 21.8+31.8	36.5 (±3.1)	31.3 10.2+21.1	34.4 (±3.9)	21.8 8.7+13.1
Glucose			174.1 (±17)	89.8 7.8+82	169.1 (±13.8)	92.9 33.8+59.1	161.7 (±11)	86 48+38	160 (±12)	92,4 56.2+36.2
Sucrose			123 (±8.2)	97.3 1.3+96	151.7 (±9.2)	98.9 8.4+90.5	135.8 (±10.1)	94 46+48	152.5 (±8.2)	96.7 27.8+68.9

For *, **, a, b and c - see footnote under Table 5

Results are means of three replicates

Table 9. t test comparing germ tube length for control and conidia of C. lindemuthianum (race δ) treated with carbohydrates

Carbohydrate	t $(\frac{d}{sd})$
Arabinose	1.94^{NS}
Xylose	12.1**
Fructose	10.82**
Galactose	5.84*
Glucose	22.51**
Sucrose	14.82**

** = Significant at 0.01 probability level

* = Significant at 0.05 probability level

NS = Not significant

Table 10. Analysis of variance of the effect of carbohydrates and concentrations on the germ tube length of C. lindemuthianum (raceδ

Main effects and concentrations	df	SS	MS	F (observed)	F (expected)
Carbohydrate	5	33928.6375	6785.7275	390**	5.06(P = 0.01)
Error (a)	12	208.7879	17.399		
Concentration	3	143.0706	47.6902	3.30*	2.84(P = 0.05)
Interaction	15	805.2052	53.6803	3.715**	2.52(P = 0.01)
Error (b)	36	520.1567	14.449		
Total	71				

** = Significant at 0.01 probability level

* = Significant at 0.05 probability level

For conidia of C. lindemuthianum (race δ) treated with the above carbohydrates, compared to the control, germ tube length was significantly increased except for conidia treated with arabinose where the germ tube length was not significantly different from the control. The greatest increase of germ tube length was for conidia treated with glucose and the increase was by 155% at 1 mg/ml compared to the controls. For conidia of race δ treated with carbohydrates, there were highly significant and large differences for carbohydrates and concentrations on germ tube length (Table 10). There was a highly significant interaction between the carbohydrates and concentrations used, i.e. the effect of concentration depended on the type of carbohydrate.

The effects of carbohydrates on the conidial germination of race δ can be summarized as follows.

Arabinose did not have any significant effect on germ tube length. The decrease in the germination was between 30% and 80% depending on the concentration. The appressoria formation was decreased by 65 to 99% and germ tube production was decreased by 50 to 61% depending on the concentration.

Galactose decreased germination by 24 to 75% and germ tube length was decreased by 17 to 50% at lower concentrations.

Fructose increased the germ tube length by 53 to 92% and appressoria production by 16 to 62% depending on the concentration.

Xylose suppressed both germination and germ tube length production. The decrease in germination was between 72 and 97% and the decrease in germ tube length was between 90 and 100%.

Glucose increased the germ tube length by 134 to 155% and had little or no effect on the germination except at 1 mg/ml which decreased the appressoria production by 84%.

Sucrose increased germ tube length by 80 to 123% and total germination by 34 to 41%. The greatest effect of sucrose was on the decrease of appressoria by 99.9% at the highest concentrations.

This experiment was repeated with conidia of C. coffeanum.

3. Effect of carbohydrates on conidia of C. coffeanum

Results are summarized in Table 11.

The main effects of the carbohydrates was on the germination of conidia of C. coffeanum which can be summarized as follows.

All the carbohydrates at 0.05 mg/ml decreased the germination (mainly appressoria production) by 8 to 56%.

At 1 mg/ml all the carbohydrates increased germination by 9 to 31%.

Fructose, glucose and sucrose at 1 mg/ml increased germination type "d" (see footnote under Table 11) by 125, 159 and 61% respectively.

At 0.5 mg/ml, the carbohydrates had little or no effect on the germination of conidia of C. coffeanum.

At 0.1 mg/ml, xylose and sucrose increased germination by 6 and 4% respectively and the rest of the carbohydrates decreased germination by 13 to 39%.

Conidia of C. lagenarium which were treated with the above carbohydrates, did not have any significant difference in appressoria production compared to the control.

Table 11. The effect of sugars on germination of conidia of C. coffeanum

Sugars	Concentrations (mg/ml)									
	0		1		0.5		0.1		0.05	
	GTL* μ	G** %	GTL μ	G %	GTL μ	G %	GTL μ	G %	GTL μ	G %
None	3.8 (±2)[a]	66.9 60[b]+0.5[c]+ 6.4[d]								
Arabinose			0	74.9	0	76	0	40.7	0	35.6
Xylose			0	72.9	0	68.4	0	70.7	0	61.1
Fructose			0	87.3 72.9+14.4[d]	0	55.3	0	58	0	29.3
Galactose			0	75.8	0	77.8	0	48.9	0	40.2
Glucose			0	82.8 66.2+16.6[d]	0	40.9	0	46	0	55.8
Sucrose			0	80.7 70.4+10.3[d]	0	77.1 70.9+6.2[d]	0	69.6 67.3+2.3[d]	0	52.2 47.3+4.9[d]

For *, **, a, b, and c - see footnote under Table 5

d = Germinated conidia as percentage which produced appressoria from one end and germ tubes without appressoria from the other end

0 = no production of germ tube from germinated conidium of C. coffeanum i.e. an appressorium immediately attached to the conidium

C. Germination of conidia on host and non-host plants

C. lagenarium, C. coffeanum and C. lindemuthianum are specific for cucumber, coffee and bean in turn. It was, therefore, of interest to determine how conidia of these three species behaved on the surface of host and non-host plants.

Cucumber cotyledons from resistant and susceptible cv. and French bean hypocotyls were prepared as described in Materials and Methods. Suspensions (5×10^5/ml) of spores of C. lindemuthianum (race β), C. coffeanum and C. lagenarium were applied in 5 µl/drop and the cotyledons and hypocotyls were incubated at appropriate t^o.

Disks were cut from the inoculated sites on cotyledons (3 mm) using a cork borer and 10 mm long sections with free hand from French bean hypocotyls were collected every 4 h for 24 h and after 48 h. The cotyledon disks and hypocotyl sections were fixed, cleared and stained as described earlier.

Percentage germination and production of appressoria from three replicates were counted under the light microscope.

Results are given in Fig. 2 - 5.

C. lindemuthianum (race β) applied to resistant and susceptible cucumber cotyledons, produced appressoria within 4 h after inoculation (Fig. 2) and within 12 - 20 h, numbers reached their maxima. There was no significant difference in the appressoria production on resistant and susceptible cv.

Race β applied to French bean hypocotyls produced appressoria within 4 - 8 h after inoculation and reached maxima within 16 - 20 h (Fig. 5). There was no difference in the rate of production of appressoria on host and non-host plants.

Stereoscanning electron microscope of race β on susceptible cucumber cv. showed that the appressoria produced were resting on the surface but directly attached to the conidia (Plate 11), whereas they were

Figure 2. Germination of C. lindemuthianum (race β) on resistant and susceptible cucumber cotyledons

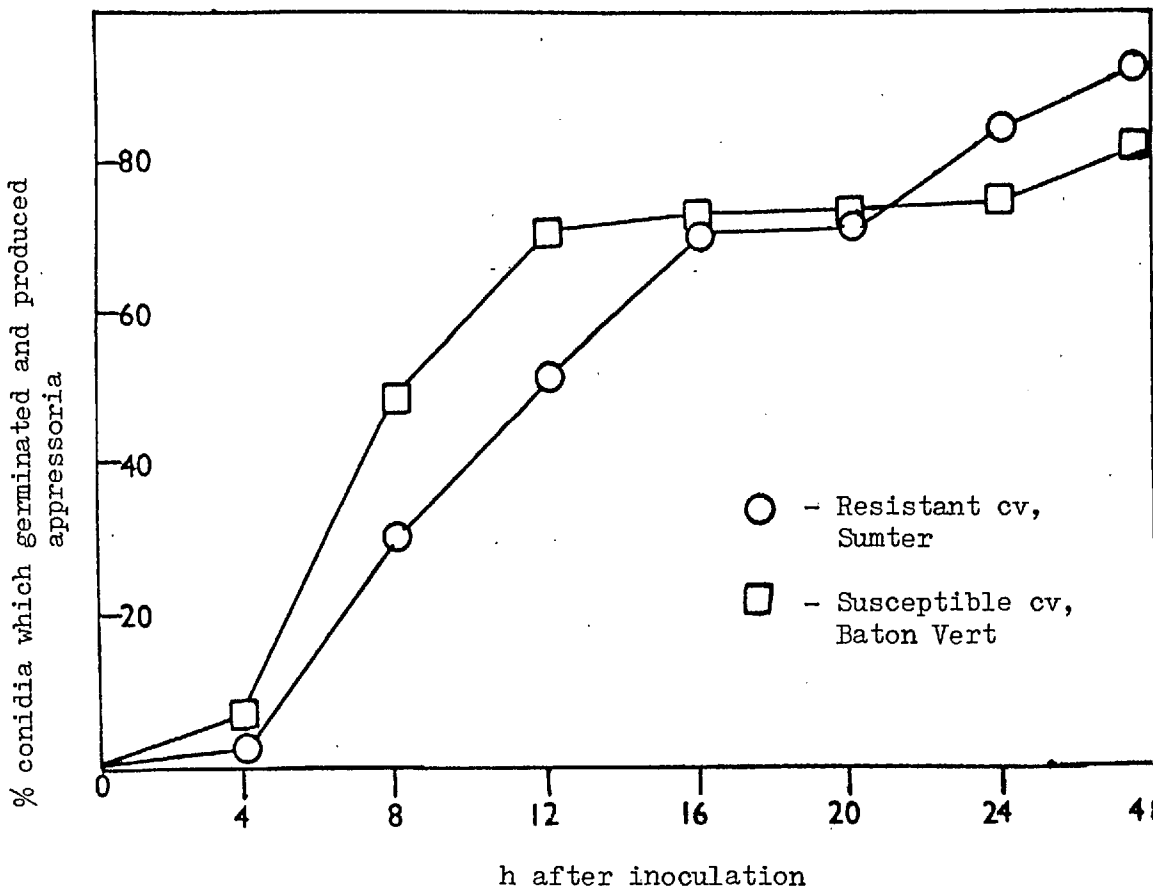


Figure 3. Germination of C. lagenarium on resistant and susceptible cucumber cotyledons

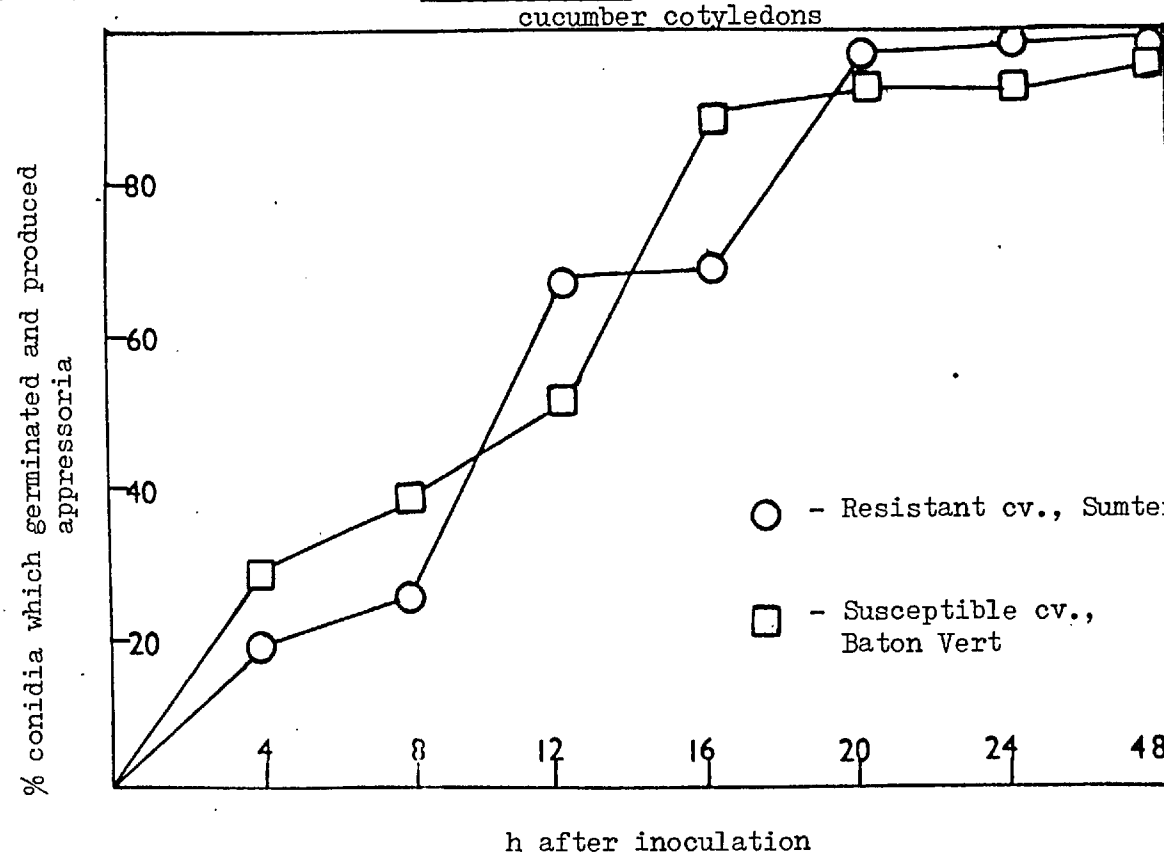


Figure 4. Germination of C. coffeanum on resistant and susceptible cucumber cotyledons

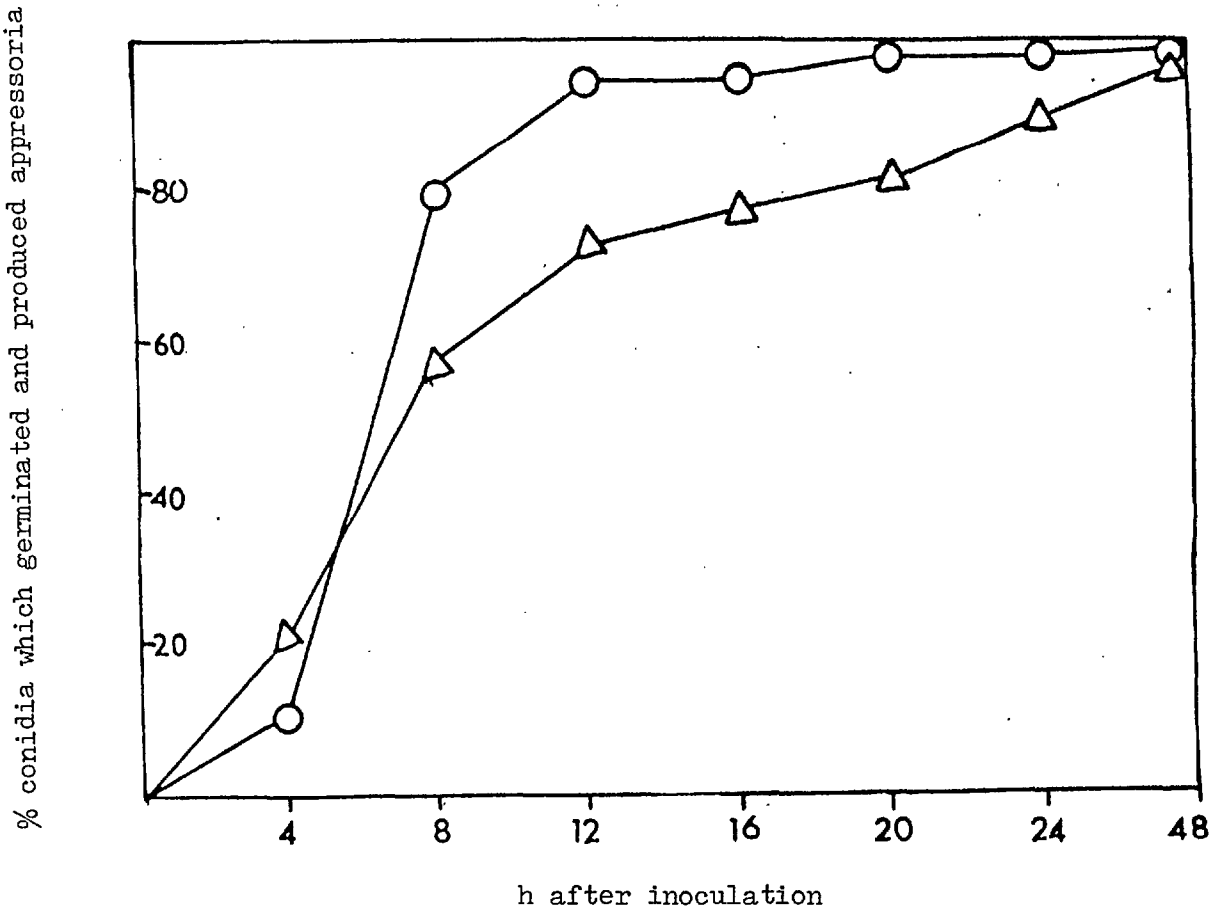
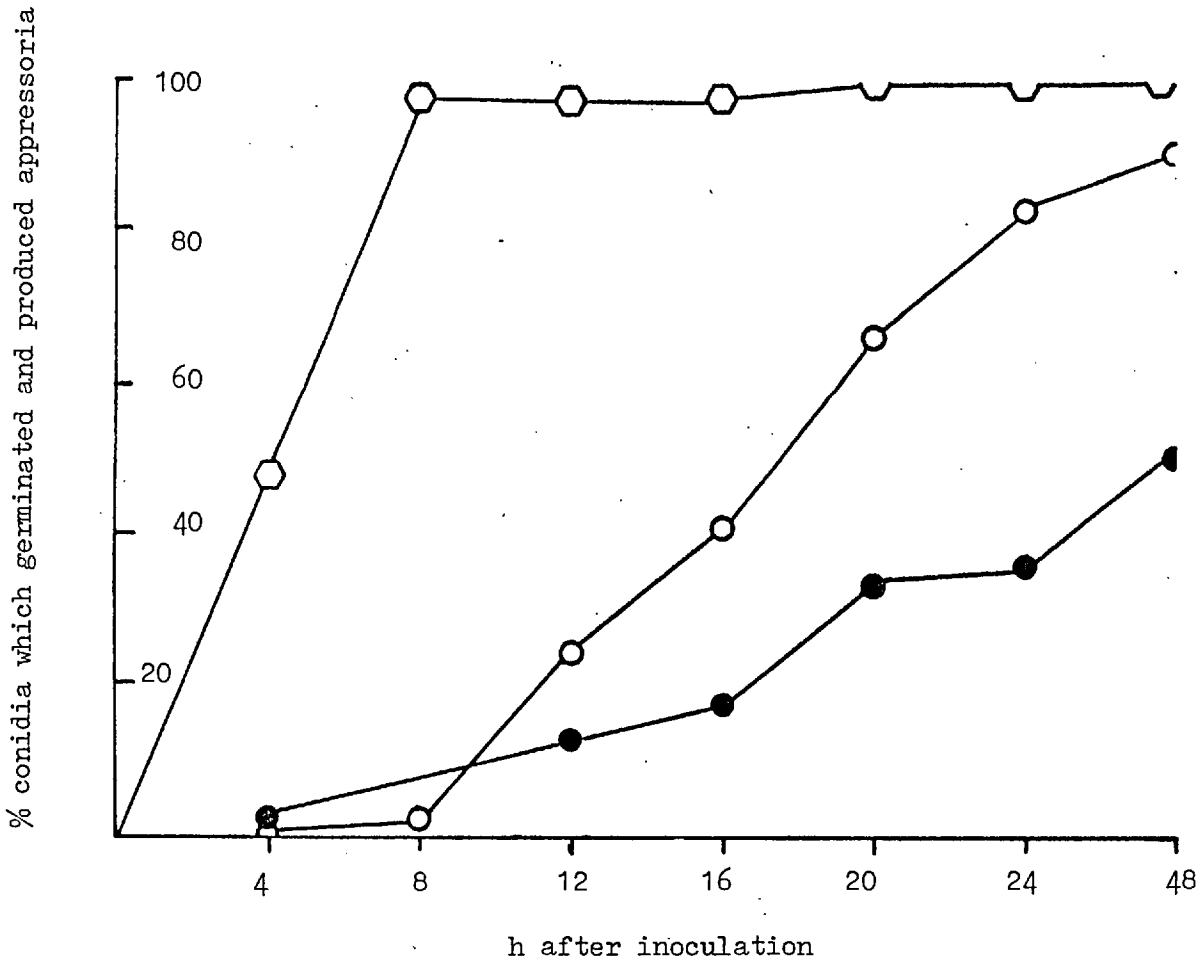


Figure 5. Germination of Colletotrichum spp. on French bean (Canadian Wonder CV) hypocotyl



Legend

- C. lagenarium

- C. lindemuthianum (race β)

- C. coffeanum

attached at the ends of germ tubes when germinated on a glass slide.

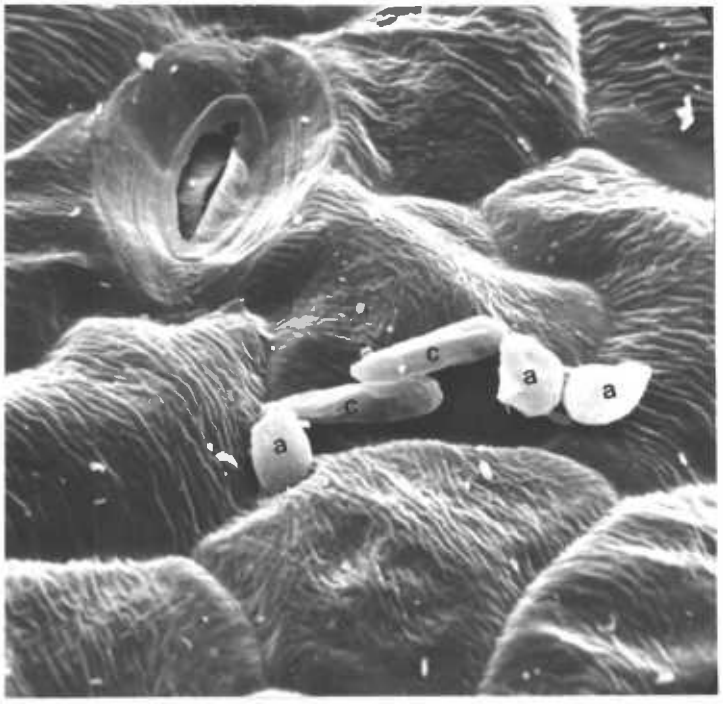


Plate 11. Stereoscan of C. lindemuthianum (race β) on Baton Vert cucumber cotyledon 48 h after inoculation x 2150

a = appressorium

c = conidium

C. coffeanum applied to resistant and susceptible cucumber cotyledons, produced appressoria 4 h after inoculation. 12 h after inoculation, C. coffeanum 70% of conidia produced appressoria on resistant cv. and 95% on susceptible cv. After 48 h, there was no difference on the production of appressoria on resistant and susceptible cucumber cv. (Fig. 4).

Only 48% of C. coffeanum conidia produced appressoria on bean hypocotyls compared to 95% on cucumber cotyledons 48 h after inoculation (Fig. 5). This low production of appressoria suggested the presence of diffusates which retarded the germination.

There was no significant difference in the production of appressoria by C. lagenarium on resistant and susceptible cucumber cotyledons (Fig. 3). 98% of germinated conidia produced appressoria 8 h after inoculation of bean hypocotyls compared to 3% with the pathogenic race β of C. lindemuthianum.

In summary, therefore :

1. The above Colletotrichum spp. started to produce appressoria within 4 h after inoculation both on host and non-host plants.

2. There was no significant difference in the total production of appressoria by the Colletotrichum spp. on resistant and susceptible cucumber cotyledons.

3. The production of appressoria by conidia of C. coffeanum on French bean hypocotyls was very low when compared to C. lagenarium and C. lindemuthianum (race β).

4. The results indicated that the inability of Colletotrichum spp. to parasitize non-host plants does not depend on the inability to germinate or lack of nutrition.

D. Germinating conidia on healthy plant extracts

From the previous data (Tables 2 - 5), all the Colletotrichum spp. produced appressoria on host and non-host plants but still were not able to parasitize the non-host plants. Attempts were made to find out if non-host plants contained substances which prevented non-pathogenic Colletotrichum

spp. from parasitizing them.

Water extracts of healthy plants were prepared as described in Materials and Methods. The extracts were concentrated (x 10) and serial dilutions were assayed against the Colletotrichum spp. on glass slides. 20 µl of the extract (three replicates) was applied to each glass slide and 1 µl (5×10^5 conidia/ml) of spore suspension was added. Germination and growth was stopped after 24 h.

Production of appressoria and germ tube were scored from 150 conidia and other characteristics were also recorded.

Results are given in Tables 12 - 14.

Cucumber extracts both from resistant and susceptible cultivars, inhibited the production of appressoria by C. lagenarium at all concentrations (Table 12). At higher concentrations, the extracts induced the production of germ tubes (without appressoria). Water extracts from resistant and susceptible cucumber cultivars had similar effects on the germination of conidia of C. lagenarium.

Water extracts from bean (susceptible to race β) leaves and hypocotyls did not increase production of appressoria by C. lagenarium. The concentrated extract of both leaves and hypocotyls caused all C. lagenarium conidia to produce appressoria but they were abnormal when compared to the controls in water. The diameter of the appressoria was 20 times more than normal and germ tubes were intertwined and hyaline in appearance.

The extracts from all the three cultivars of coffee leaves, inhibited production of appressoria by C. lagenarium at the highest concentrations. There was no significant difference in the production of appressoria by C. lagenarium by coffee leaves extracts.

Germination of conidia of C. coffeanum on water extract of healthy plants is given in Table 13. The concentrated extracts of coffee leaves, conidia of C. coffeanum produced germ tubes. The germ tubes were very long and intertwined. Compared to the controls in water and extracts from resistant and susceptible cultivars of cucumber, the extracts from coffee

Table 12. Germination of conidia of *C. lagenarium* on water extract of healthy plants

Source of Extracts	Dilution series of extracts									
	Control		10^{-1}		10^{-2}		10^{-3}		10^{-4}	
	APP*%	GT**%	APP %	GT %	APP %	GT %	APP %	GT %	APP %	GT %
Cucumber cotyledons	64.4	0								
S^a			8.2	68.7	7.6	25.7	11.3	0	8.2	0
R^b			2.9	87.8	11.1	15.3	8.7	0	4	0
French bean[c]	64.4	0								
Leaves			100	0	0	100	3.3	0	16.4	0
Hypocotyls			100	0	3.1	68.2	6.7	0	4	0
Coffee leaves	89.3	0								
S^d			0	100	40.7	59.3	96.2	0	95.1	0
I^e			0	100	0	100	95.4	0	90.7	0
R^f			0	100	82.4	13.3	95.7	0	100	0

* APP% = % conidia germinated with appressoria ** GT% = % conidia germinated without appressoria

S^a = Baton Vert cv. of cucumber susceptible to *C. lagenarium*. R^b = Sumter cv. of cucumber resistant to *C. lagenarium*

c = Canadian Wonder cv. of bean susceptible to *C. lindemuthianum* (race β)

d = F_8 cv. susceptible to *C. coffeanum* e = SN_5 cv. intermediate resistant to *C. coffeanum*

f = F_{24} cv. resistant to *C. coffeanum*

Table 13. Germination of conidia of C. coffeanum on water extract of healthy plants

Source of Extracts	Control		Dilution series of extracts							
			10^{-1}		10^{-2}		10^{-3}		10^{-4}	
	APP*%	GT**%	APP %	GT %	APP %	GT %	APP %	GT %	APP %	GT %
Cucumber cotyledons	55.8	22.7								
S[a]			42	58	69.6	30.4	58	15.8	65.8	25.8
R[b]			57.1	42.9	63.3	29.3	46.9	8.7	29.6	6.4
French bean[c]										
Leaves			8.5	91.6	g	-	g	-	g	-
Hypocotyls			11.3	88.7	g	-	g	-	60.9	12.2
Coffee leaves										
S[d]			0	100	0	100	3.1	56	35.3	19.3
I[e]			0	100	0	100	70.7		49.1	
R[f]			0	100	0	100	50.0		51.3	

For *, **, a, b, c, d, e and f - see footnote under Table 12

g = Each conidium produced an appressorium from one end and a germ tube without appressoria from the other end. Sometimes there was a production of a germ tube from an appressorium (Plate 16). Germination was 100%

Table 14. Germination of conidia of C. lindemuthianum (race β) on water extract of healthy plants

Source of Extracts	Dilution series of extracts									
	Control		10^{-1}		10^{-2}		10^{-3}		10^{-4}	
	APP*%	GT**%	APP %	GT %	APP %	GT %	APP %	GT %	APP %	GT %
Cucumber cotyledons	40.9	11.6								
S[a]			0	96	0	54.7	6	25.6	1.8	41.8
R[b]			0	73.6	0.9	44.2	9.3	24.9	22.7	21.1
French bean[c]										
Leaves			0	100	0	74.9	10.9	57.3	48.2	25.8
Hypocotyls			0	100	0	84.4	14.9	60.4	33.3	26.9
Coffee leaves										
S[d]			0	100	18	78.9	100	0	100	0
I[e]			0	100	15	77.3	100	0	100	0
R[f]			0	100	92	0	100	0	98.7	0

For *, **, a, b, c, d, e and f - see footnote under Table 12

leaves caused the germinating conidia to produce an appressorium from one end and a germ tube without appressorium from the other end and sometimes a germ tube from an appressorium (Plate 12).

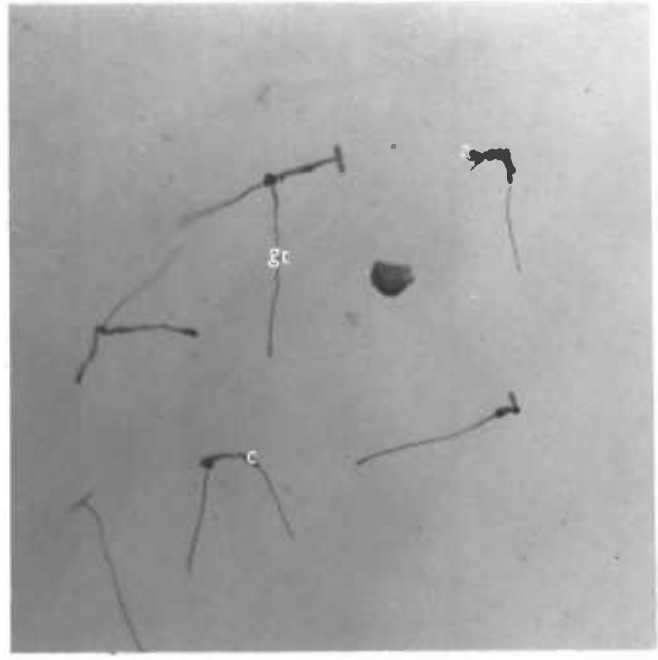


Plate 12. Production of appressoria (a) and germ tube (gt) from one conidium (c) and production of germ tube from an appressorium by C. coffeanum in water extract of coffee leaves x 150

None of the extract inhibited the germination of conidia of C. coffeanum except extracts from coffee leaves at highest concentrations.

There was no relation between resistance/susceptibility of the three cultivars of coffee and the effect of their extract on the germination of conidia of C. coffeanum.

Highest concentrations of bean leaves and hypocotyls, suppressed the production of appressoria from conidia of C. lindemuthianum (race β) while there was an induction of appressoria at low concentrations.

At all concentrations, extracts from resistant and susceptible cucumber cotyledon suppressed production of appressoria by conidia of C. lindemuthianum (race β).

Extracts from coffee leaves induced production of germ tubes from conidia of C. lindemuthianum (race β) at higher concentrations and appressoria at low concentrations.

In summary, therefore :

1. There was no relation between germination of conidia of C. lagenarium and resistance and susceptibility of cucumber plants.

2. There was no relation between germination of conidia of C. coffeanum and resistance, intermediate resistance and susceptibility of coffee plants.

3. The inability of Colletotrichum spp. to parasitize non-host plants was not due to the toxic compounds in the extracts of the plants.

Colletotrichum spp. were able to produce appressoria on non-host plants and there was little indication that water extracts of the non-host plants inhibited the Colletotrichum spp. from parasitizing these plants.

To investigate the possible role of epidermis against Colletotrichum spp., cucumber cotyledons were studied under SEM.

Epidermis was peeled from the lower surface of a susceptible cucumber cultivar. The area, where the epidermis was removed, was cut with a cork borer and placed in a moist chamber. The cotyledons were inoculated with suspension (5×10^5/ml) of spores of C. lindemuthianum (race β), C. coffeanum and C. lagenarium and incubated at appropriate t^o. The disks were prepared for SEM; results are shown in Plates 14 - 19.

C. lagenarium within 3 - 4 d after inoculation, parasitized the cells by producing infection hyphae and 5 d after inoculation, there was

disintegration of the cells with bacteria population which may later help in the disintegration of the cells (Plates 14, 15 and 16).

C. coffeanum and C. lindemuthianum (race β) germinated on the surface of cotyledons where the epidermis was peeled off but they failed to parasitize the cucumber cells (Plates 17, 18 and 19).

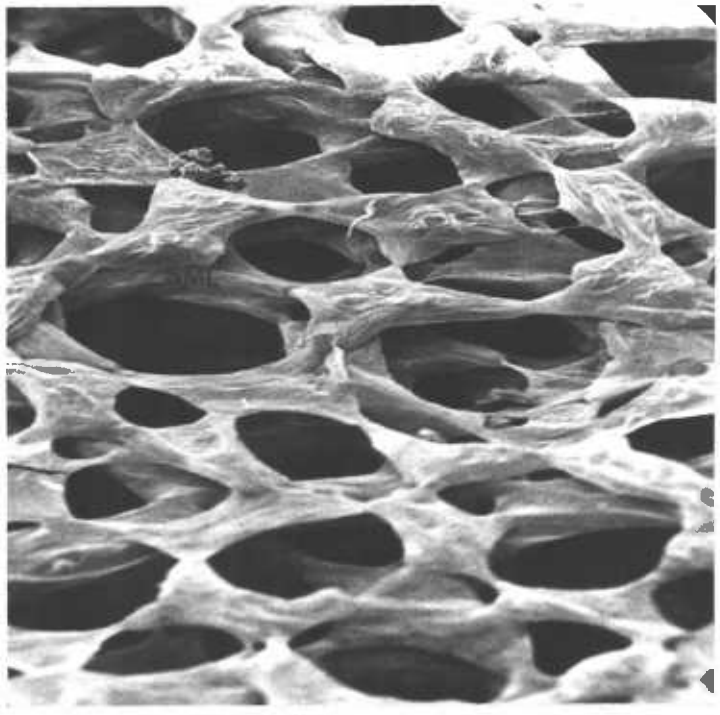
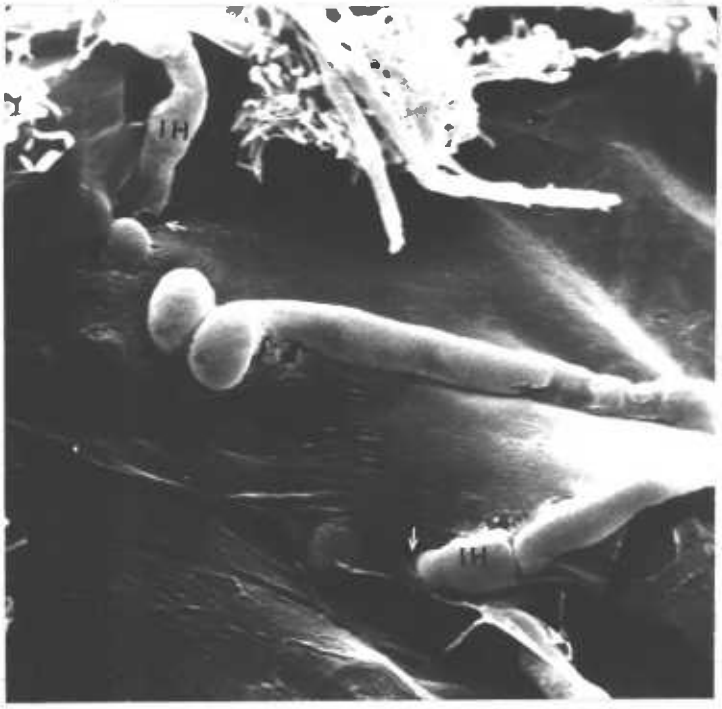


Plate 13. Lower surface of Baton Vert cucumber cotyledon where the epidermis is peeled off showing the spongy mesophyl layer (SML) x 2200

Plate 14. Penetration (arrowed) by infecting hyphae (IH) of C. lagenarium on Baton Vert cultivar of cucumber cotyledon where the epidermis is peeled off. 3 d after inoculation x 2100



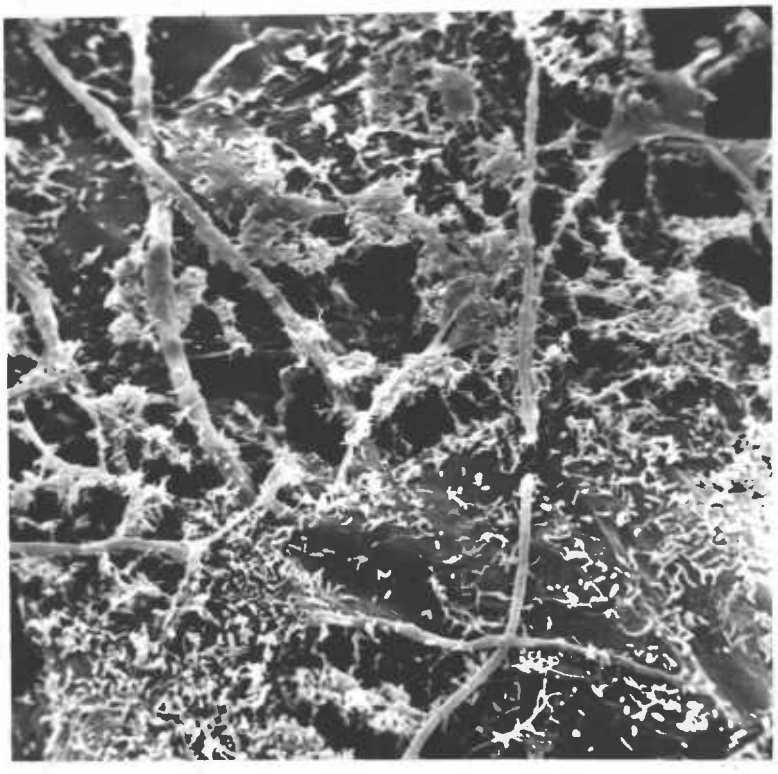


Plate 15. Disintegration of cells of Baton Vert cucumber cotyledon (epidermis peeled off) by C. lagenarium 5 d after inoculation x 1100

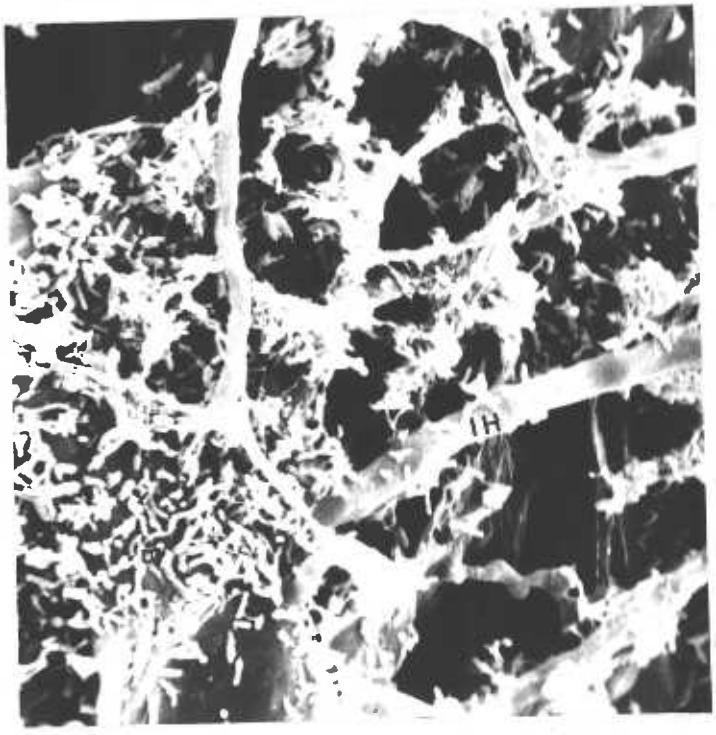


Plate 16. Magnification of the infecting hyphae (IH) of C. lagenarium on Baton Vert cucumber cotyledon (epidermis peeled off) 5 d after inoculation. Note disintegration of the cells and bacteria (B) on the surface x 2100

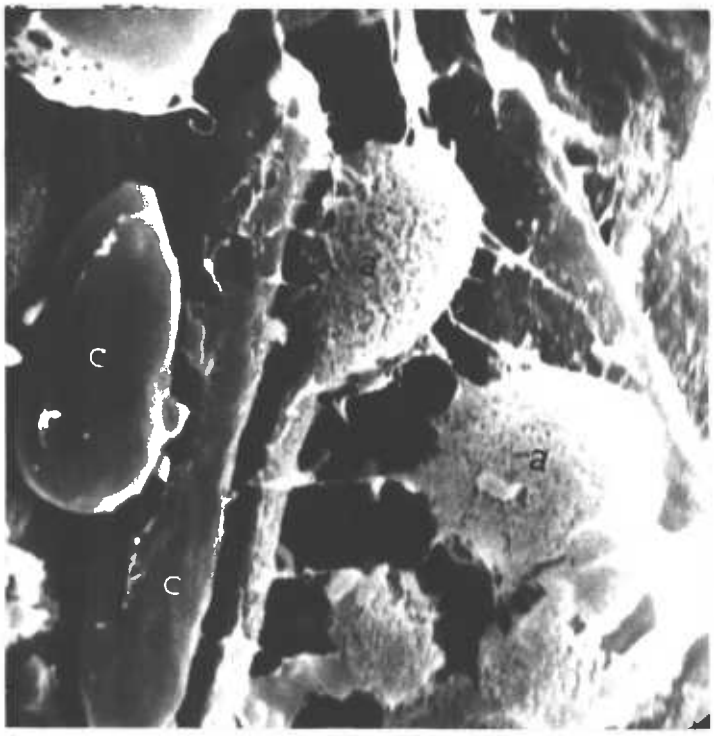


Plate 17. Production of appressoria (a) by C. coffeanum on lower surface of Baton Vert cucumber cotyledon where the epidermis is peeled off. 3 d after inoculation x 5300

c = conidium

gt = germ tube

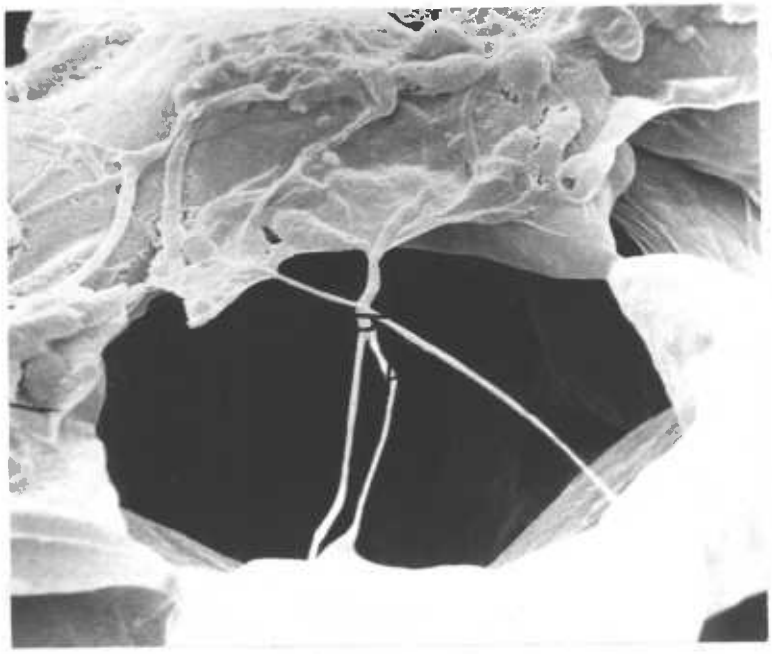


Plate 18. Hyphae (h) of C. coffeanum growing over the lower surface of Baton Vert cucumber cotyledon where the epidermis is peeled off. 5 d after inoculation, no sign of parasitizing the cells x 1100

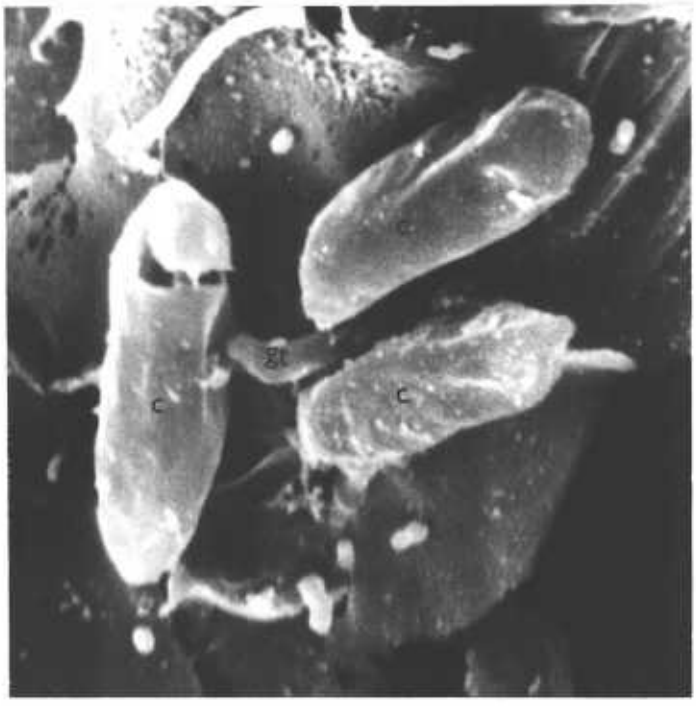


Plate 19. C. lindemuthianum (race β) on the lower surface of Baton Vert cucumber cotyledon where the epidermis is peeled off. 3 d after inoculation, the cells were intact and there was no sign of parasitism x 5300

gt = germ tube c = conidium

E. Induction of resistance in plants

1. Plant growth regulators (PGR)

Plant growth regulators such as indole acetic acid (IAA) and ethylene have been connected repeatedly by plant pathologists with resistance or susceptibility of plants to pathogens. Gibberellic acid (GA), indole acetic acid (IAA), ethylene as Ethrel, maleic hydrazide, kinetin and Cycocel were investigated for their ability to alter resistance in French bean hypocotyls inoculated with C. lindemuthianum and in cucumber cotyledons inoculated with C. lagenarium.

1.1 French bean

Canadian Wonder cultivar of French bean susceptible to C. lindemuthianum (race β) which was grown in vermiculite saturated with LANS was used throughout this work. PGR were prepared as solutions of 100, 50 and 10 mg/l in gdw. French bean plants grown for 4 d after germination in vermiculite were uprooted, roots were washed under running water and then placed for 72 h in solutions (50 ml) of PGR in 100 ml flasks. For a similar 24 h treatment, plants grown for 6 d in vermiculite were used. Hypocotyls prepared as described in Materials and Methods(M & M) were inoculated with drops (5 µl) of spore suspension (5×10^5/ml) of race β and incubated in boxes at $17^\circ - 19^\circ C$.

Similarly, solutions of PGR were applied in drops (5 µl) from an Agla syringe on to etiolated detached hypocotyls and with drops of gdw as controls. The sites where the PGR were applied, were marked with non water soluble ink. Drops of solutions of the PGR were left for 24 h or 72 h and then removed and inoculated with race β as above.

(i) Gibberellic acid (GA)

Bean plants treated with GA for 72 h at each concentration, had yellowish leaves and internodes between the cotyledonary node and the first true leaf node were longer by 114% compared to the controls (Control = 100% and GA = 214%).

Etiolated bean hypocotyls treated with drops of GA did not show any abnormality.

The effect of GA on disease is given in Tables 15 and 16. Results are means of 4 inoculated sites/hypocotyl/eight hypocotyls/ treatment.

In hypocotyls from plants treated for 72 h at 10 and 100 mg/l concentrations, numbers of expanding lesion were increased by 28.6% whereas 50 mg/l decreased the numbers of expanding lesions by 28% compared to the controls. But plants which were treated with GA for 24 h and inoculated, increased the number of expanding lesions by 57 to 72% depending on the concentrations. These increases recorded 12 d after inoculation were much more striking than 6 and 8 d after inoculation.

In hypocotyls which were treated with GA in 5 µl/drop for 72 h before inoculation, numbers of expanding lesions were decreased by 60 and 70% at 50 and 100 mg/l respectively and no expanding lesions developed at 10 mg/l. But in hypocotyls treated for 24 h, disease development was similar to that in the controls.

This experiment was repeated with essentially similar results.

Table 15. The effect of GA on disease of bean hypocotyls inoculated with C. lindemuthianum (race β)

GA mg/l	Treatment (h)*	Days after inoculation: 6			8			10			12		
		Lesion development											
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
100	24	8.3	8.3	66.7	0	0	100	0	0	100	0	0	100
	72	0	0	58.3	8.3	0	75	8.3	0	75	0	8.3	75
50	24	8.3	25	50	0	8.3	83.3	8.3	0	91.6	8.3	0	91.6
	72	8.3	8.3	25	8.3	0	41.7	0	8.3	41.7	0	8.3	41.7
10	24	25	0	66.7	75	0	66.7	8.3	0	91.6	8.3	0	91.6
	72	0	25	0	0	0	58.3	0	8.3	75	0	8.3	75
0	24	34.4	6.3	0	34.4	0	18.8	31.3	6.3	55.2	31.3	6.3	58.3

* Roots of intact plants dipped in GA and hypocotyls excised and inoculated

[a] Fl = Percentage inoculated sites with flecks

[b] LL = Percentage inoculated sites with limited lesions

[c] EL = Percentage inoculated sites with expanding lesions

Table 16. The effect of GA, applied in drops to excised etiolated bean hypocotyls, on disease caused by C. lindemuthianum (race β)

GA (mg/l)	Treatment (h)*	Expanding lesions[c]
100	24	100
	72	41.7
50	24	100
	72	33.3
10	24	100
	72	0
0		100[d]

* Sites on hypocotyls treated with 5 µl GA solution, then 24 h or 72 h later with 5 µl spore suspension

c See footnote of Table 15, lesions recorded 12 d after inoculation

d Excised etiolated bean hypocotyls are more susceptible than the non-etiolated hypocotyls and as a result there was 100% formation of expanding lesions and 58.3% (Table 15) in non-etiolated hypocotyls

(ii) Indole acetic acid (IAA)

Intact bean plants and excised etiolated bean hypocotyls were treated with solutions of IAA and inoculated with C. lindemuthianum (race β) as described earlier.

Intact plants treated with 100 and 50 mg/l for 72 h showed the following abnormalities compared to the controls.

- leaves were smaller and did not expand properly
- hypocotyls were thicker and shorter
- adventitious roots grew from the hypocotyl
- hypocotyls were distorted and did not grow straight

Plants treated for 24 h at all concentrations did not show any abnormalities.

IAA applied in drops to excised, etiolated hypocotyls for 72 h caused tissues at the sites to swell and become raised. After inoculation, the raised areas erupted and expanding lesions developed.

Results are given in Tables 17 and 18.

In hypocotyls from plants treated with 100 mg/l for 24 h and 72 h, number of expanding lesions formation was decreased by 14 and 43% respectively and for plants treated for 24 h at 10 mg/l number of expanding lesion formation was decreased by 43%. Treatment with 50 mg/l had little effect.

Hypocotyls treated with drops of IAA solution, the numbers of expanding lesion were decreased by 50 to 70% depending on concentration and time treatments but differences were not pronounced.

This experiment was repeated with essentially similar results.

Table 17. The effect of IAA on disease of bean hypocotyls inoculated with C. lindemuthianum (race β)

IAA (mg/l)	Treatment (h)*	Days after inoculation											
		6			8			10			12		
		Lesion development											
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
100	24	0	0	8.3	16.7	0	33.3	16.7	0	50	16.7	0	50
	72	0	8.3	8.3	0	8.3	33.3	0	8.3	33.3	0	8.3	33.3
50	24	8.3	0	0	0	0	50	0	0	58.3	0	0	58.3
	72	0	0	0	0	0	58.3	0	0	58.3	0	0	58.3
10	24	8.3	8.3	2.5	8.3	8.3	33.3	8.3	8.3	33.3	8.3	8.3	33.3
	72	8.3	0	0	0	0	58.3	0	0	58.3	0	0	58.3
0		34.4	6.3	0	34.4	0	18.8	31.3	6.3	55.2	31.3	6.3	58.3

For *, a, b, and c - see footnote under Table 15

Table 18. The effect of IAA, applied in drops to excised etiolated bean hypocotyls on disease caused by C. lindemuthianum (race β)

IAA (mg/l	Treatment (h)*	Expanding lesions [c]
100	24	41.7
	72	50.0
50	24	50.0
	72	50.0
10	24	33.3
	72	41.7
0		100[d]

For *, d - see footnote under Table 16

For c - see footnote under Table 15

(iii) Kinetin

Plants dipped in solutions of kinetin at 100 and 50 mg/l for 72 h were stunted, leaves did not fully expand and the hypocotyls were thicker than in controls.

Results are given in Tables 19 and 20.

Table 19. The effect of kinetin on disease of bean hypocotyls inoculated with C. lindemuthianum (race β)

Kinetin (mg/l	Treatment (h)*	Days after inoculation											
		6			8			10			12		
		Lesion development											
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
100	24	0	0	25	0	0	33.3	0	0	100	0	0	100
	72	12.5	12.5	37.5	0	0	87.5	0	0	87.5	0	0	100
50	24	0	0	12.5	0	0	100	0	0	100	0	0	100
	72	8.3	0	33.3	8.3	0	50	0	0	83.3	0	0	91.6
10	24	16.7	8.3	8.3	8.3	8.3	58.3	16.7	8.3	58.3	16.7	8.3	58.3
	72	0	8.3	41.7	16.7	0	66.4	25	0	66.4	33.3	0	66.4
0		34.4	6.3	0	34.4	0	18.8	31.3	6.3	55.2	31.3	6.3	58.3

For *, a, b and c - see footnote under Table 15

Table 20. The effect of kinetin, applied in drops to excised etiolated hypocotyls, on disease caused by C. lindemuthianum (race β)

Kinetin (mg/l)	Treatment (h)*	Expanding lesions [c]
100	24	50
	72	50
50	24	25
	72	91.6
10	24	50
	72	62.5
0		100[d]

For * and d - see footnote under Table 16

For c - see footnote under Table 15

Kinetin markedly increased the production of expanding lesions at 50 and 100 mg/l for 24 and 72 h. Treatment with 10 mg/l had little effect (Table 19).

In contrast, except at 50 mg/l for 72, kinetin markedly decreased numbers of expanding lesions when applied directly to sites inoculated later. Kinetin applied at 50 mg/l for 24 h, decreased the numbers of expanding lesions by 75% compared to the controls.

This experiment was repeated with essentially similar results.

(iv) Maleic hydrazide

Intact plants and hypocotyls treated with maleic hydrazide did not exhibit any abnormality compared to the controls. Results for disease development are given in Tables 21 and 22.

In hypocotyls from plants treated for 24 h at 10 and 50 mg/l concentrations, numbers of expanding lesions were increased by 29% and 43% respectively whereas 50 mg/l for 72 h decreased the numbers of expanding lesions by 14% compared to the controls. Numbers of lesions were increased by 57% by maleic hydrazide at 100 mg/l for 72 h.

When maleic hydrazide was applied directly to hypocotyls, the numbers of expanding lesions were reduced by 75 and 83% at 100 and 50 mg/l for 72 h.

This experiment was repeated with essentially similar results.

Table 21. The effect of maleic hydrazide on disease of bean hypocotyls inoculated with C. lindemuthianum (race β)

Maleic h. (mg/l)	Treatment (h)*	Days after inoculation											
		6			8			10			12		
		Lesion development											
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
100	24	25	16.7	33.3	33.3	0	58.3	33.3	0	58.3	33.3	0	58.3
	72	8.3	16.7	75	0	16.7	83.3	0	16.7	83.3	0	8.3	91.6
50	24	25	8.3	50	8.3	0	83.3	8.3	0	83.3	8.3	0	83.3
	72	25	0	33.3	25	0	50	25	0	50	25	0	50
10	24	41.7	8.3	25	25	8.3	66.7	25	0	75	25	0	75
	72	0	0	41.7	8.3	0	58.3	0	0	66.7	0	0	66.7
0		34.4	6.3	0	34.4	0	18.8	31.3	6.3	55.2	31.3	6.3	58.3

For *, a, b, and c - see footnote under Table 15

Table 22. The effect of maleic hydrazide, applied in drops to excised etiolated hypocotyls, in disease caused by C. lindemuthianum (race β)

Maleic hydrazide (mg/l)	Treatment (h)*	Expanding lesion [c]
100	24	91.6
	72	25.0
50	24	83.3
	72	16.7
10	24	66.7
	72	75.0
0		100[d]

For * and d - see footnote under Table 16

For c - see footnote under Table 15

(v) Cycocel

Intact plants and hypocotyls treated with Cycocel did not show any abnormalities.

The effects of Cycocel on disease development are given in Tables 23 and 24.

Table 23. The effect of Cycocel on disease of bean hypocotyls inoculated with C. lindemuthianum (race β)

Cycocel (mg/l)	Treatment (h)*	Days after inoculation											
		6			8			10			12		
		Lesion development											
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
100	24	0	0	0	25	8.3	16.7	25	0	25	25	8.3	25
	72	8.3	0	0	41.7	16.7	16.7	16.7	16.7	41.7	16.7	16.7	41.7
50	24	8.3	0	0	8.3	0	41.7	8.3	0	41.7	8.3	0	41.7
	72	8.3	0	0	8.3	0	16.7	8.3	0	16.7	8.3	0	16.7
10	24	0	0	0	0	0	25	0	0	25	0	0	25
	72	16.7	0	0	33.3	0	25	33.3	0	25	33.3	0	25
0		34.4	6.3	0	34.4	0	18.8	31.3	6.3	55.2	31.3	6.3	58.3

For *, a, b and c - see footnote under Table 15

Table 24. The effect of Cycocel, applied in drops to excised etiolated hypocotyls, on disease caused by C. lindemuthianum (race β)

Cycocel (mg/l)	Treatment (h)*	Expanding lesion [c]
100	24	75
	72	33.3
50	24	41.7
	72	33.3
10	24	41.7
	72	41.7
0		100[d]

For * and d - see footnote under Table 16

For c - see footnote under Table 15

In hypocotyls from plants treated for 24 and 72 h at 10 mg/l and at 100 mg/l for 24 h, numbers of expanding lesions were decreased by 57%. Treatment with 100 mg/l for 72 h and 50 mg/l for 24 h, numbers of expanding lesions were decreased by 28%. Cycocel at 50 mg/l for 72 h decreased the disease development by 71%.

Cycocel, applied in drops to excised etiolated hypocotyls, decreased numbers of expanding lesions by 25 to 66% compared to the controls. This experiment was repeated with essentially similar results.

(vi) Ethrel

Ethrel, which is stable in acid form but breaks down at pH 3.5 and above to form ethylene, is an effective generator of ethylene after absorption by plants.

Plants treated at 100 and 50 mg/l for 24 and 72 h, exhibited serious abnormalities. There was a collapse of the hypocotyls at the junction with the roots. Ethrel also depressed the development of leaves especially the leaf primordia. In plants treated for 24 h some cells grew excessively and the hypocotyls ruptured at a number of points. At 10, 5 and 1 mg/l plants did not exhibit any abnormalities. So these were the ones used with the results given in Tables 25 and 26.

In hypocotyls from plants treated for 24 and 72 h at 10 mg/l, numbers of expanding lesions were increased by 43 and 57% respectively. Plants which were treated for 72 h at 5 and 1 mg/l, the numbers of expanding lesions were increased by 43% but those treated for 24 h, the disease development was reduced by 7 and 50% respectively.

Ethrel, applied in drops to excised etiolated hypocotyls, decreased numbers of expanding lesions by 25 to 83% compared to the controls.

This experiment was repeated with essentially similar results.

Table 25. The effect of Ethrel, on disease of bean hypocotyls inoculated with C. lindemuthianum (race β)

Ethrel (mg/l)	Treatment (h)*	Days after inoculation											
		6			8			10			12		
		Lesion development											
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
10	24	25	0	58.4	8.3	8.3	62.5	8.3	8.3	83.3	12.5	8.3	83.3
	72	25	16.7	33.3	16.7	8.3	75	16.7	0	83.3	16.7	0	91.6
5	24	8.3	0	50	0	8.3	54.2	0	20.8	54.2	0	20.8	54.2
	72	16.7	8.3	50	16.7	0	75	8.3	0	83.3	8.3	0	83.3
1	24	50	25	16.7	41.7	25	29.2	41.6	25	29.2	41.6	25	29.2
	72	50	0	16.7	0	0	83.3	8.3	0	83.3	8.3	0	83.3
0		34.4	6.3	0	34.4	0	18.8	31.3	6.3	55.2	31.3	6.3	58.3

For *, a, b and c - see footnote under Table 15

Table 26. The effect of Ethrel, applied in drops to excised etiolated hypocotyls, on disease caused by C. lindemuthianum (race β)

Ethrel (mg/l)	Treatment (h)*	Expanding lesions [c]
10	24	58.3
	72	16.7
5	24	75
	72	41.7
1	24	100
	72	58.3
0		100[d]

For * and d - see footnote under Table 16

For c - see footnote under Table 15

1.1.1 Effects of PGR on formation of appressoria

The aqueous solutions of PGR tested for effects on the resistance of bean to C. lindemuthianum (race β) were used in tests in which spore germination and production of appressoria were scored after 24 h.

Three x 20 µl solution was applied to glass slides and 1 µl suspension of race β spores (5×10^5/ml) was added to the centre of each drop. Results scored after 24 h are given in Table 27.

Table 27. Effects of plant growth regulators on production of appressoria by conidia of C. lindemuthianum (race β)

PGR	Concentration (mg/l)			
	100	50	10	0
		Appressoria produced*		
Maleic hydrazide	82	119	128	46
IAA	94	206	171	
GA	15	26	143	
Cycocel	60	82	30	
Kinetin	174	158	152	
	10	5	1	0
		Appressoria produced*		
Ethrel	9	59	144	46.0

* Appressoria production by treated conidia as percentage of appressoria produced of untreated conidia

At the highest concentrations, all the PGR except kinetin reduced the production of appressoria by the germinating conidia with the greatest effect by Gibberellic acid and Ethrel. At the lowest concentrations, all the PGR except Cycocel increased production of appressoria.

There was little relation between the decrease of disease by PGR applied in drops to excised hypocotyls and appressoria produced.

The notable points about the results given in Tables 15 - 27 are as follows.

PGR applied to Roots

a) Disease, 12 d after inoculation, was greatly decreased in plants treated with Cycocel at all concentrations and times of treatments.

b) Ethrel applied through the roots at 1 mg/l for 24 h, decreased disease by 50%.

PGR applied to hypocotyls

c) Most of the PGR applied in drops to excised hypocotyls, increased resistance with greatest effect caused by Gibberellic acid at 10 mg/l for 72 h.

d) PGR had little effect on germination of conidia. Effects on production of appressoria were not related to those on disease. This implies that the PGR altered disease development through their effects on host tissues.

1.2 Cucumber

Resistant and susceptible cucumber cultivars were grown in John Innes No. 2 compost in plastic bags in the greenhouse at 24° - 25°C. Aqueous solutions of PGR, prepared as described earlier, were applied as follows.

1.2.1 Absorption through roots

Plants grown for 12 d, 8 cm high with a pair of cotyledons, were uprooted, roots were washed and placed in vials containing solutions of PGR for 72 h. Plants grown for 14 d, 10 cm high with a pair of cotyledons and with fully unexpanded first true leaf, roots

were treated similarly for 24 h. Roots of control plants were placed in gdw.

After treatment, cotyledons were removed and placed in plastic boxes and inoculated with 5 µl drops of a suspension of conidia of C. lagenarium (1×10^5/ml). The effects of the growth regulators were assessed from rates of lesion expansion in mm/d after incubating for different periods.

The following abnormalities developed after treatments.

- In resistant and susceptible plants placed in solutions of IAA and kinetin for 72 h at 100 and 50 mg/l and resistant plants placed in kinetin solution at 10 mg/l for 72 h, the cotyledons collapsed and remained flaccid.

- Ethrel, at 100, 50 mg/l for 24 and 72 h caused the same kind of damage as in bean plants, so 10, 5 and 1 mg/l were used instead.

Results as means of 32 lesions/4 plants/treatment are given in Tables 28 - 33.

Table 28. The effect of GA on expansion of lesions on cotyledons of cucumber inoculated with C. lagenarium

GA	Treatment	Days after inoculation 6		8		10	
		Expansion of lesions**					
(mg/l)	(h)*	S[a]	R[b]	S	R	S	R
100	24	0.17	0	0.25	0.45	0.22	0.43
	72	0.47	0	0.85	0.25	0.90	0.24
50	24	0.20	0	0.50	0.16	0.52	0.20
	72	0.31	0	0.77	0.53	0.80	0.53
10	24	0.27	0	0.40	0.06	0.43	0.10
	72	0.29	0	0.78	0.53	0.76	0.53
0		0.22	0	0.40	0.43	0.65	0.43

[a]S - Susceptible cultivar, Baton Vert

[b]R - Resistant cultivar, Sumter

** - Rate of lesion expansion in mm/d as $\frac{\text{lesion diameter(mm)}}{\text{d. after inoculation}}$

* - Roots of intact plants dipped in GA and cotyledons excised and inoculated

Results in Table 28 for GA show that in S plants treated with GA for 24 h, lesion expansion after 10 d was decreased by 66% at 100 mg/l and by 34% at 10 mg/l compared to the controls. Treatment for 72 h increased the lesion expansion by 17 to 40%. Treatment for 24 h at 10 mg/l, lesion expansion in R plants was decreased by c. 80%. GA at 100 mg/l for 24 h induced resistance in S plants and at 10 mg/l for 24 h, increased resistance of R plants.

Table 29. The effect of IAA on expression of lesions on cotyledons oc cucumber inoculated with C. lagenarium

IAA (mg/l)	Treatment (h)*	Days after inoculation: 6		8		10	
		Expansion of lesions**					
		S[a]	R[b]	S	R	S	R
100	24	1.0	0.25	1.0	0.36	0.94	0.35
	72	-	-	-	-	-	-
50	24	0.64	0.45	0.63	0.44	0.63	0.43
	72	-	-	-	-	-	-
10	24	0.67	0.61	0.71	0.56	0.71	0.53
	72	0.60	0.17	0.64	0.43	0.63	0.43
0		0.55	0.18	0.70	0.40	0.70	0.43

For a, b, * and ** - see footnote under Table 28

- Intact plants treated with IAA for 72 h cotyledons collapsed and were flaccid

Results given in Table 29 show that IAA applied at 100 mg/l for 24 h increased the lesion size by 34% in S plants whereas it decreased lesion size by c. 20% in R plants. IAA at 50 mg/l for 24 h decreased the lesion size by 10% in S plants and with no effect on R plants. IAA at 10 mg/l had little effect on lesion expansion on S plants but increased lesion expansion by 23% in R plants.

Kinetin at all concentrations was toxic to R cucumber plants which had been treated for 72 h. Kinetin was toxic to S plants at 100 and 50 mg/l for 72 h. R and S plants did not show any abnormalities treated at all concentrations for 24 h.

Table 30. The effect of kinetin on expansion of lesions on cotyledons of cucumber inoculated with C. lagenarium

Kinetin (mg/l)	Treatment (h)*	Days after inoculation: 6 — Expansion of lesions ** S[a]	6 R[b]	8 S	8 R	10 S	10 R
100	24	0.16	0	0.65	0.31	0.60	0.30
	72	-	-	-	-	-	-
50	24	0	0	0.23	0	0.23	0.10
	72	-	-	-	-	-	-
10	24	0.13	0	0.48	0.06	0.47	0.063
	72	0.67	-	0.60	-	0.60	-
0		0.55	0.18	0.70	0.40	0.70	0.43

For a, b, * and ** - see footnote under Table 28

- Intact plants treated with kinetin for 72 h, cotyledons collapsed and were flaccid

Results in Table 30 show that kinetin applied at 100, 50 and 10 mg/l for 24 h to R plants, lesion expansion after 10 d was decreased by 30, 77 and 85% respectively compared to the controls.

Treatments for 24 h at 100, 50 and 10 mg/l decreased the lesion expansion in S plants by 13, 67 and 33% respectively. Treatments at 10 mg/l for 72 h lesion expansion in S plants was decreased by 14% compared to the controls.

Therefore kinetin induced resistance in S plants and made R plants more resistant.

Table 31. The effect of maleic hydrazide on expansion of lesions on cotyledons of cucumber inoculated with C. lagenarium

Maleic hydrazide (mg/l)	Treatment (h)*	Days after inoculation: 6		8		10	
		Expansion of lesions**					
		S[a]	R[b]	S	R	S	R
100	24	0.55	0.09	0.73	0.68	0.71	0.68
	72	0.47	0.09	0.70	0.43	0.70	0.43
50	24	0.56	0.52	0.60	0.76	0.56	0.73
	72	0.45	0	0.78	0.25	0.76	0.21
10	24	0.73	0.01	0.70	0.38	0.70	0.38
	72	0.63	0.41	0.69	0.65	0.65	0.64
0		0.55	0.18	0.70	0.40	0.70	0.43

For a, b, * and ** - see footnote under Table 28

Maleic hydrazide at all concentrations and time treatments applied to S plants had little or no effect on lesion expansion compared to the controls.

The greatest effect of maleic hydrazide was on R cucumber plants. Maleic hydrazide at 100 and 50 mg/l for 24 h, increased lesion size by 58% and 70% respectively but it reduced lesion size by c. 50% at 50 mg/l for 72 h.

The effects of maleic hydrazide on lesion expansion was, therefore, much more pronounced for R than for S plants.

Table 32. The effect of Cycocel on expansion of lesions on cotyledons of cucumber inoculated with C. lagenarium

Cycocel (mg/l)	Treatment (h)*	Days after inoculation: 6		8		10	
		Expansion of lesions**					
		S[a]	R[b]	S	R	S	R
100	24	0.20	0	0.21	0	0.22	0
	72	0.38	-	0.36	-	0.34	-
50	24	0.05	0	0.12	0.22	0.18	0.24
	72	0.33	0	0.27	0.14	0.27	0.12
10	24	0.19	0	0.32	0.30	0.31	0.32
	72	0.33	0	0.39	0.13	0.40	0.16
0		0.22	0	0.40	0.43	0.65	0.43

For a, b, * and ** - see footnote under Table 28

\- The whole cotyledon yellowish in colour

Cycocel, applied at all concentrations and time treatments, greatly induced resistance in S cucumber plants and caused R plants to become more resistant compared to the controls.

In R cucumber plants treated with Cycocel at 100 mg/l for 24 h, small flecks developed at the site of inoculation and there was no lesion formation.

In both R and S plants, 100 and 50 mg/l for 24 and 72 h were highly effective in inducing resistance. Lesion size was reduced by 40 to 70% in S cotyledons and by 25 to 100% in R cotyledons.

Table 33. The effect of Ethrel in expansion of lesions on cotyledons of cucumber inoculated with C. lagenarium

Ethrel (mg/l)	Treatment (h)*	Days after inoculation: 6		8		10	
		Expansion of lesions **					
		S[a]	R[b]	S	R	S	R
10	24	0.33	0.83	0.58	0.83	0.62	0.92
	72	0.23	0.75	0.52	0.60	0.58	0.62
5	24	0.25	0.63	0.50	0.66	0.60	0.67
	72	0.27	0.42	0.60	0.52	0.62	0.60
1	24	0.20	0.40	0.35	0.69	0.41	0.68
	72	0.28	0	0.58	0.44	0.60	0.43
0		0.22	0	0.40	0.43	0.65	0.43

For a, b, * and ** - see footnote under Table 28

Ethrel greatly decreased the resistance of R plants to C. lagenarium when compared to the controls. At 10 mg/l applied for 24 h, Ethrel increased the lesion size in R plants by 114%. But Ethrel applied to S cucumber plants slightly reduced the lesion size with greatest effect at 1 mg/l for 24 h when the reduction was c. 40%.

a) Effects of PGR on formation of appressoria

Solutions of each of the PGR tested for effects on lesion expansion were bioassayed against C. lagenarium on glass slides as described earlier. Results are given in Table 34.

Table 34. Effects of plant growth regulators on the production of appressoria by conidia of C. lagenarium

PGR	Concentration (mg/l)			
	100	50	10	0
	Appressoria produced*			
Maleic hydrazide	114	125	95	81.0
IAA	0	121	100	
GA	127	136	121	
Cycocel	59	48	100	
Kinetin	114	115	96	
	10	5	1	0
	Appressoria produced*			
Ethrel	9	47	104	81.0

* Appressoria production by treated conidia as percentage of appressoria produced of untreated conidia

The greatest effect on the formation of appressoria was caused by Cycocel and Ethrel at 50 and 100 mg/l and by IAA at 100 mg/l where none were produced (pH of IAA at 100 mg/l = 5.2)

Summary

The notable effects of PGR, applied to roots are given in Tables 28 - 34, are as follows.

i) Cycocel at each concentration and time treatments caused S cultivar to become resistant and R cultivar to become more resistant.

ii) Ethrel induced slight resistance in the S cultivar at each time treatments with the greatest reduction in lesion size at 1 mg/l for 24 h. At 10 and 5 mg/l, resistance was almost eliminated.

iii) Kinetin induced resistance in S cultivar and R cultivar became more resistant at 50 and 10 mg/l for 24 h.

iv) Maleic hydrazide at all concentrations and time treatments had little or no effect on the S cultivar. But depending on the concentration and time treatments, maleic hydrazide made the R cultivar more resistant or more susceptible.

v) GA induced resistance in the S cultivar at 100 mg/l for 24 h and GA at 10 mg/l for 24 h increased resistance in the R cultivar.

vi) IAA had little or no effect on the induction of resistance in R and S cultivars.

vii) There was little relation between the effects of PGR on the production of appressoria by C. lagenarium on glass slides and alteration of resistance of R and S cultivars.

viii) These experiments were repeated with essentially similar results.

1.2.2 Continuous absorption of PGR by cotyledons

Cotyledons from 15 d old resistant and susceptible cucumber plants were prepared as described in M and M. Cut ends were placed in modified plastic vials containing solutions of PGR. Controls were placed in gdw. The cotyledons were inoculated 2 - 3 h after exposure to the PGR, and the effects of PGR while they were exposed to the solutions of the PGR were assessed as described earlier.

S cotyledons treated with kinetin at all concentrations collapsed. Other PGR did not have any obviously deleterious effects.

Results as means of 32 lesions/4 plants/treatment are given in Tables 35 - 40.

Table 35. The effect of GA on expansion of lesions on cotyledons of cucumber inoculated with C. lagenarium

GA (mg/l)	Days after inoculation					
	6		8		10	
	Expansion of lesions**					
	S[a]	R[b]	S	R	S	R
100	0.23	0.19	0.63	0.30	0.85	0.43
50	0.19	0	0.48	0.063	0.83	0.31
10	0.23	0.19	0.47	0.30	0.69	0.38
0	0.41	0.23	0.56	0.36	0.72	0.42

For a, b and ** - see footnote under Table 28

Results in Table 35 show that GA at 100 and 50 mg/l increased the lesion size in S cotyledons by 18% and 15% respectively, whereas 10 mg/l decreased the lesion size by 4%. GA applied to R cotyledons at 100, 50 and 10 mg/l, lesion expansion decreased by 2, 26 and 10% respectively.

Table 36. The effect of IAA on expansion of lesions on cotyledons of cucumber inoculated with C. lagenarium

IAA (mg/l)	Days after inoculation					
	6		8		10	
	Expansion of lesions**					
	S[a]	R[b]	S	R	S	R
100	0.33	0.50	0.53	0.51	0.83	0.51
50	0.73	0.50	1.00	0.55	1.14	0.50
10	0.22	0.17	0.41	0.19	0.66	0.20
0	0.41	0.23	0.56	0.36	0.72	0.42

For a, b and ** - see footnote under Table 28

Table 36 shows that IAA at 10 mg/l decreased lesion expansion by 8% in S cotyledons and by 52% in R cotyledons. IAA at 100 and 50 mg/l increased the lesion expansion in R and S plants when the increase in S plants was by 15% and 58% respectively, and by 26% and 19% respectively in R plants.

Table 37. The effect of kinetin on expansion of lesions on cotyledons of cucumber inoculated with C. lagenarium

Kinetin (mg/l)	Days after inoculation: 6		8		10	
	Expansion of lesions**					
	S[a]	R[b]	S	R	S	R
100	-	0.33	-	0.31	-	0.30
50	-	0.17	-	0.29	-	0.28
10	-	0.33	-	0.33	-	0.30
0	0.41	0.23	0.56	0.36	0.72	0.42

For a, b and ** - see footnote under Table 28

\- Hypocotyls collapsed and cotyledons became flaccid

In S cotyledons placed in solutions of kinetin at each concentration, the cut end collapsed within 4 - 5 d and the cotyledons became flaccid. In striking contrast, R cotyledons remained turgid throughout the experiment with all treatments which reduced lesion size by c. 30%. Also when the lesions expand normally, chlorotic zones develop at the periphery but for R cotyledons with continuous absorption of kinetin there was no chlorosis.

In R cotyledons treatment with kinetin suppressed production of the first true leaf.

Table 38. The effect of maleic hydrazide on expansion of lesions on cotyledons of cucumber inoculated with C. lagenarium

Maleic hydrazide (mg/l)	Days after inoculation 6		8		10	
	Expansion of lesions**					
	S[a]	R[b]	S	R	S	R
100	0.23	0.24	0.30	0.42	0.68	0.59
50	0.40	0.38	0.59	0.78	0.81	0.80
10	0.62	0.44	0.83	0.84	1.0	0.81
0	0.41	0.23	0.56	0.36	0.72	0.42

For a, b and ** - see footnote under Table 28

Table 38 shows that R cotyledons placed in solutions of maleic hydrazide at all concentrations, the lesion size was increased by 40 to 93%. Maleic hydrazide at 50 and 10 mg/l increased the lesion size in S cotyledons by 13% and 39% respectively whereas 100 mg/l decreased the lesion size by 8%.

Table 39. The effect of Cycocel on expansion of lesions on cotyledons of cucumber inoculated with C. lagenarium

Cycocel (mg/l)	Days after inoculation					
	6		8		10	
	Expansion of lesions**					
	S[a]	R[b]	S	R	S	R
100	0.35	0.10	0.45	0.33	0.89	0.66
50	0.19	0.19	0.34	0.38	0.60	0.65
10	0.13	0	0.30	0.23	0.45	0.48
0	0.41	0.23	0.56	0.36	0.72	0.42

For a, b and ** - see footnote under Table 28

Table 39 shows that R cotyledons placed in solutions of Cycocel at all concentrations, lesion size was increased by 14 to 57% whereas in S cotyledons Cycocel decreased the lesion size by 17% and 37% at 50 and 10 mg/l respectively. But Cycocel at 100 mg/l increased the lesion size in S cotyledons by 24%.

Table 40. The effect of Ethrel on expansion of lesions on cotyledons of cucumber inoculated with C. lagenarium

Ethrel (mg/l)	Days after inoculation					
	6		8		10	
	Expansion of lesions**					
	S[a]	R[b]	S	R	S	R
10	0.50	0.81	0.63	0.77	0.63	0.81
5	0.60	0.92	0.81	0.94	0.81	0.77
1	0.69	1.00	1.10	0.86	1.20	0.85
0	0.41	0.23	0.56	0.36	0.72	0.42

For a, b and ** - see footnote under Table 28

Results in Table 40 show that R cotyledons placed in solutions of Ethrel at all concentrations, the lesion size was increased dramatically when the increase was 93, 83 and 102% at 10, 5 and 1 mg/l respectively. Ethrel at 5 and 1 mg/l increased the lesions in S cotyledons by 13% and 67% respectively whereas 10 mg/l decreased the lesion size by 12%.

Summary

The notable effects of PGR, under continuous absorption by cotyledons are given in Tables 35 - 40, are as follows.

i) GA had little effect on lesion size in S cotyledons whereas in R cotyledons, it reduced lesion size by 26% at 50 mg/l.

ii) IAA at 10 mg/l increased resistance in R and S cotyledons and at 100 and 50 mg/l it increased the susceptibility of R and S cotyledons.

iii) Kinetin increased the resistance of R cotyledons at all concentrations.

iv) Maleic hydrazide increased the susceptibility of R cotyledons at all concentrations and at 50 and 10 mg/l in S cotyledons. 100 mg/l decreased the lesion size in S cotyledons by 6%.

v) Cycocel at all concentrations increased the lesion size in R cotyledons and in S cotyledons at 100 mg/l. But Cycocel at 50 and 10 mg/l decreased the lesion size in S cotyledons.

vi) Ethrel at all concentrations eliminated the resistance of R cotyledons and at 50 and 10 mg/l it increased the lesion size in S cotyledons.

vii) These experiments were repeated with essentially similar results.

1.2.3 Cotyledon disks

Disks of R and S cotyledon were prepared from 15 d old plants as described in M and M. Disks were placed on solutions of PGR or gdw for 24 h and 72 h. Some disks had the lower and other had the upper surface on the liquid. The disks were transferred to boxes lined with moist tissue paper and were inoculated with 5 µl drops of a suspension of conidia of C. lagenarium (1×10^5/ml). The effects of PGR were assessed as described earlier.

Disks treated with PGR, did not show any abnormalities compared with those described earlier.

The results, following inoculation, are given in Tables 41 - 46 and are means of 10 disks/position/treatment.

Table 41. Lesion size in cotyledon disks treated with GA and inoculated with C. lagenarium

		S[a]		R[b]	
GA	Treatment	Lesion size (mm)[c]			
(mg/l)	(h)***	L*	UP**	L	UP
100	24	119	160	50	60
	72	75	148	50	117
50	24	100	148	117	50
	72	57	233	117	83
10	24	57	141	117	33
	72	57	111	117	0
0		100	100	100	100
		[d](5.3)	(2.7)	(3.0)	(3.0)

For a and b - see footnote under Table 28

contd...../

c - Lesion size of treated cotyledon disks as percentage of lesion size of untreated cotyledon disks, 10 d after inoculation

*L - Lower surface placed on solutions and later the upper surface was inoculated

**UP - Upper surface placed on solutions and later the lower surface was inoculated

*** - Cotyledon disks placed on GA and inoculated

d() - Real figures of lesion size of control in mm

In S disks with upper surface placed on GA, lesion size was greatly increased at 100 and 50 mg/l for 24 and 72 h when the greatest increase of lesion size was by 133% at 50 mg/l for 72 h. GA at 10 mg/l for 24 and 72 h increased the lesion size by 41% and 11% respectively. But in S disks with lower surfaces placed on GA, lesion size was reduced by 43% at 50 mg/l for 72 h and by 43% at 10 mg/l for 24 and 72 h.

In R disks with the upper surface placed on GA, lesion size was reduced at all concentrations and time treatments except at 100 mg/l for 72 h when the lesion size was increased by 17%. The greatest effect of GA was at 10 mg/l for 72 h when there was 100% suppression of lesion production whereas the others reduced the lesion size by 17 to 67% compared to the controls. But in R disks with the lower surface placed on GA, lesion size was increased by 17% at 50 and 10 mg/l for 24 and 72 h and 100 mg/l for 24 and 72 h decreased the lesion size by 50%.

Table 42. Lesion size in cotyledon disks treated with IAA and inoculated with C. lagenarium

IAA (mg/l)	Treatment (h)***	S[a] Lesion size (mm)[c] L*	S[a] Lesion size (mm)[c] UP**	R[b] Lesion size (mm)[c] L	R[b] Lesion size (mm)[c] UP
100	24	30	76	50	76
	72	33	76	50	45
50	24	37	76	60	45
	72	20	50	90	70
10	24	27	76	100	104
	72	35	71	110	120
0		100	100	100	100
		[d](5.3)	(2.7)	(3.0)	(3.0)

For a and b - see footnote under Table 28

For c, d, *, ** and *** - see footnote under Table 41

Results in Table 42 show that S disks with upper and lower surfaces placed on IAA, lesion size was decreased by 24 to 50% and by 63 to 80% respectively at all concentration and time treatments. R disks with lower surface placed on IAA at 100 and 50 mg/l for 24 and 72 h, lesion size was decreased by 10 - 50% and at 10 mg/l for 72 h, lesion size was increased by 10%. R disks with upper surface placed on IAA, lesion size was decreased by 24 to 55% at 100 and 50 mg/l for 24 and 72 h, but 10 mg/l for 24 and 72 h increased the lesion size by 4% and 20% respectively.

Table 43. Lesion size in cotyledon disks treated with kinetin and inoculated with C. lagenarium

Kinetin (mg/l)	Treatment (h)***	S[a] Lesion size (mm)[c] L*	S[a] Lesion size (mm)[c] UP**	R[b] Lesion size (mm)[c] L	R[b] Lesion size (mm)[c] UP
100	24	9	7	0	0
	72	0	0	0	0
50	24	19	56	17	0
	72	0	0	13	0
10	24	40	93	33	40
	72	36	56	67	47
0		100	100	100	100
		[d](5.3)	(2.7)	(3.0)	(3.0)

For a and b - see footnote under Table 28

For c, d, *, ** and *** - see footnote under Table 41

Results in Table 43 show that S disks with lower surface placed on kinetin at 100 and 50 mg/l for 24 and 72 h, lesion size was decreased by c. 80 to 100% and at 10 mg/l for 24 and 72 h, lesion size decreased by 60% and 64% respectively. S disks with upper surface on kinetin at 100 and 50 mg/l for 72 h, lesion production was suppressed by 100% and kinetin at 50 mg/l for 24 h and 10 mg/l for 72 h, lesion size was decreased by 44%. Kinetin at 100 and 10 mg/l for 24 h, lesion size was decreased by 93% and 7% respectively.

R disks with upper surface placed on kinetin at 100 and 50 mg/l for 24 and 72 h, lesion production was suppressed by 100% whereas at 10 mg/l for 24 and 72 h, lesion size was decreased by 60% and 53% respectively.

R disks with lower surface placed on kinetin at 100 mg/l for 24 and 72 h, lesion production was suppressed by 100% and at 50 mg/l for 24 and 72 h, lesion size was decreased by c. 85%. Kinetin at 10 mg/l for 24 and 72 h, lesion size was decreased by 67% and 33% respectively.

Table 44. Lesion size in cotyledon disks treated with maleic hydrazide and inoculated with C. lagenarium

Maleic hydrazide (mg/l)	Treatment (h)***	S[a] Lesion size (mm)[c] L*	S[a] Lesion size (mm)[c] UP**	R[b] Lesion size (mm)[c] L	R[b] Lesion size (mm)[c] UP
100	24	30	76	152	160
	72	53	104	100	50
50	24	30	88	168	85
	72	65	109	85	40
10	24	30	43	90	60
	72	52	109	150	90
0		100	100	100	100
		[d](5.3)	(2.7)	(3.0)	(3.0)

For a and b - see footnote under Table 28

For c, d, *, ** and *** - see footnote under Table 41

In S disks with lower surface placed on maleic hydrazide at all concentrations for 24 and 72 h the lesion size was decreased by 70% and by 35 to 48% respectively, but S disks with upper surface placed on maleic hydrazide at all concentrations for 72 h, the lesion size was increased by 4 to 9% whereas at 100, 50 and 10 mg/l for 24 h, the lesion size was decreased by 24, 12 and 57% respectively.

In R disks with lower surface placed on maleic hydrazide at 100 and 50 mg/l for 24 h and at 10 mg/l for 72 h, the lesion size was increased by 52, 68 and 50% respectively. Maleic hydrazide at 50 mg/l for 72 h and at 10 mg/l for 24 h, the lesion size was decreased by 15% and 10% respectively. In R disks with upper surface on maleic hydrazide at 100 and 50 mg/l for 72 h and at 10 mg/l for 24 h, the decrease in lesion size was between 40 and 60% whereas 50 mg/l for 24 h and 10 mg/l for 72 h, the lesion size was decreased by 15% and 10% respectively. Maleic hydrazide at 100 mg/l for 24 h, increased the lesion size by 60%.

Table 45. Lesion size in cotyledon disks treated with Cycocel and inoculated with C. lagenarium

Cycocel (mg/l)	Treatment (h)***	S[a]		R[b]	
		Lesion size (mm)[c]			
		L*	UP**	L	UP
100	24	76	85	83	133
	72	76	37	183	167
50	24	89	100	133	67
	72	100	48	100	83
10	24	89	137	150	67
	72	81	137	83	100
0		100	100	100	100
		[d](5.3)	(2.7)	(3.0)	(3.0)

For a and b - see footnote under Table 28

For c, d, *, ** and *** - see footnote under Table 41

For S disks with lower surface placed on Cycocel at 100 mg/l for 24 and 72 h, lesion size was decreased by 24% and at 50 and 10 mg/l for 24 h, lesion size was decreased by 11%. Cycocel at 10 mg/l for 72 h, the decrease in lesion size was by 19%. In S disks with upper surface on Cycocel at 100 and 50 mg/l for 72 h, lesion size was decreased by 63% and 52% respectively whereas 100 mg/l for 24 h, decreased the lesion size by 17%. But in S disks with upper surface on Cycocel at 10 mg/l for 24 and 72 h, lesion size was increased by 37%.

In R disks with lower surface placed on Cycocel at 100 mg/l for 72 h, 50 and 10 mg/l for 24 h, lesion size was increased by 83, 33 and 50% respectively whereas Cycocel at 100 mg/l for 24 h and at 10 mg/l for 72 h, decreased the lesion size by 17%. In R disks with upper surface placed on Cycocel at 100 mg/l for 24 and 72 h, lesion size was increased by 33% and 67% respectively whereas Cycocel at 50 and 10 mg/l for 24 h, decreased the lesion size by 33% and at 50 mg/l for 72 h, by 17%.

Table 46. Lesion size in cotyledon disks treated with Ethrel and inoculated with C. lagenarium

Ethrel (mg/l)	Treatment (h)***	S[a] Lesion size (mm)[c] L*	S[a] Lesion size (mm)[c] UP**	R[b] Lesion size (mm)[c] L	R[b] Lesion size (mm)[c] UP
10	24	126	159	223	100
	72	145	185	337	100
5	24	126	100	277	167
	72	72	159	250	150
1	24	126	370	333	167
	72	94	122	250	50
0		100	100	100	100
		[d](5.3)	(2.7)	(3.0)	(3.0)

For a and b - see footnote under Table 28

For c, d, *, ** and *** - see footnote under Table 41

Results in Table 46 show that in S disks with lower surface placed on Ethrel at 10, 5 and 1 mg/l for 24 h, lesion size was increased by 26% and Ethrel at 10 mg/l for 72 h, increased the lesion size by 45%. Ethrel at 5 and 1 mg/l for 72 h, decreased the lesion size by 28% and 6% respectively. In S disks with the upper surface on Ethrel at 10 mg/l for 24 h and at 5 mg/l for 72 h, the lesion size was increased by 59% and at 10 and 1 mg/l for 72 h, the lesion size increased by 85% and 22% respectively. The greatest increase in lesion size was in S disks with upper surface

placed on Ethrel at 1 mg/l for 24 h when the increase was by 270%.

In R disks with lower surface on Ethrel at 5 and 1 mg/l for 72 h, the lesion size was increased by 150% and at 10 and 5 mg/l for 24 h, the lesion size was increased by 123% and 177% respectively. Ethrel at 10 mg/l for 72 h and at 1 mg/l for 24 h, increased the lesion size by 237% and 233% respectively. In R disks with upper surface on Ethrel at 5 and 1 mg/l for 24 h, lesion size was increased by 67% and Ethrel at 5 mg/l for 72 h, increased the lesion size by 50% whereas at 1 mg/l for 72 h, it decreased the lesion size by 50%. Ethrel at 10 mg/l for 24 and 72 h did not have any effect on the lesion size when compared to the controls.

Summary

The notable effects of PGR, on cotyledon disks with upper and lower surface on PGR are given in Tables 41 - 46, are as follows.

i) GA increased susceptibility in S disks with upper surface placed on GA and except at 100 mg/l for 24 and 72 h, GA also increased the susceptibility of R disks with lower surface placed on GA. The greatest effect of GA was on R disks with upper surface placed when it totally suppressed lesion formation. Other combinations had some effect on the reduction of lesion size in R and S disks.

ii) IAA generally decreased the lesion size in S disks and except at 10 mg/l for 24 and 72 h, IAA decreased the lesion size in R disks.

iii) Kinetin reduced dramatically the lesion size both in R and S disks.

iv) Depending on concentration and time treatments and on position of placing disks of R and S on maleic hydrazide, lesion size was both increased and reduced.

v) In S disks with lower and upper surfaces placed on Cycocel at all concentrations and time treatments, lesion size was either reduced, or increased or with no effect compared to the controls. In R disks with lower surface placed on Cycocel at all concentrations set on different time treatments, lesion size was either greatly increased or slightly reduced but in R disks with upper surface placed on Cycocel at 50 and 10 mg/l for 24 and 72 h, lesion size was slightly reduced but at 100 mg/l for 24 and 72 h, lesion size was increased by 33% and 67% respectively.

vi) Ethrel generally increased the lesion size dramatically in R and S disks.

vii) These experiments were repeated with essentially similar results.

2. *Colletotrichum* spp. as inducers of resistance

Several workers have shown that avirulent races of pathogenic fungi can induce resistance in a host when they are applied before pathogenic races. In this work non-pathogenic *Colletotrichum* spp. were used to induce resistance in bean hypocotyls and cucumber cotyledons.

2.1 Effects of concentrations of spores and interval between inoculation in inducing resistance to *Colletotrichum* spp. in bean hypocotyls

Etiolated bean hypocotyls were grown and prepared as described in Materials and Methods. Conidia from cultures of *C. coffeanum* and *C. lagenarium* grown on V_8 agar for 9 - 10 d and used at final concentrations of 200, 500 and 2000/µl. Five µl drops of suspensions of *C. lagenarium* and *C. coffeanum* spores were applied to hypocotyls; distilled water was applied to controls. Sites, where non-pathogenic conidia were applied, were marked with a non-water soluble ink. Drops were left for 24 or 72 h at 24° - 25°C. and then removed with absorbent paper. The sites were then inoculated with drops (5 µl) of a suspension (5 x 10^5/ml) of *C. lindemuthianum* (race β) spores and incubated at 17° - 19°C.

The effects of non-pathogenic *Colletotrichum* spp. on disease expression was scored on the scale described earlier.

Results as means of 32 sites/8 hypocotyls/treatment are given in Tables 47 - 48.

Results in Tables 47 and 48 show that after pre-inoculation with high concentrations of *C. lagenarium* and *C. coffeanum* conidia, the numbers of expanding lesions were decreased by 100% compared to the controls. Suspensions of *C. lagenarium* spores (5 x 10^5 and 2 x 10^5/ml) for 24 h decreased the numbers of expanding lesions by 72%. Treatment with a

Table 47. The effect of C. lagenarium (non-pathogenic) on disease of etiolated bean hypocotyls inoculated with C. lindemuthianum (race β)

Spores/µl*	Treatment (h)	Days after inoculation: 6			8			10			12		
		Lesion development											
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
2000	24	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0
1000	24	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0
500	24	0	0	0	8.3	0	8.3	0	0	16.7	0	0	25
	72	0	0	0	0	0	0	0	0	0	0	0	0
200	24	0	0	0	8.3	0	16.7	8.3	0	25	8.3	0	25
	72	0	0	0	0	0	0	8.3	0	0	8.3	0	0
0		25	0	75	12.0	0	88	12	0	88	12	0	88

[a] Fl = % inoculated sites with flecks

[b] LL = % inoculated sites with limited lesions

[c] EL = % inoculated sites with expanding lesions

* = Excised etiolated hypocotyls treated with conidia of C. lagenarium and inoculated later with race β of C. lindemuthianum

O = indicates no macroscopically visible symptoms

Table 48. The effect of C. coffeanum (non-pathogenic) on disease of etiolated bean hypocotyls inoculated with C. lindemuthianum (race β)

Spores/µl*	Treatment (h)	Days after inoculation: 6			8			10			12		
		Lesion development											
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
2000	24	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0
1000	24	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0
500	24	0	0	0	0	0	0	0	0	0	0	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0
200	24	33.3	0	8.3	33.3	0	8.3	33.3	0	8.3	33.3	0	8.3
	72	0	0	0	0	0	0	0	0	0	0	0	0
0		25	0	75	12	0	88	12	0	88	12	0	88

For a, b, c and 0 - see footnote under Table 47

* - Excised etiolated hypocotyls treated with conidia of C. coffeanum and inoculated later with race β of C. lindemuthianum

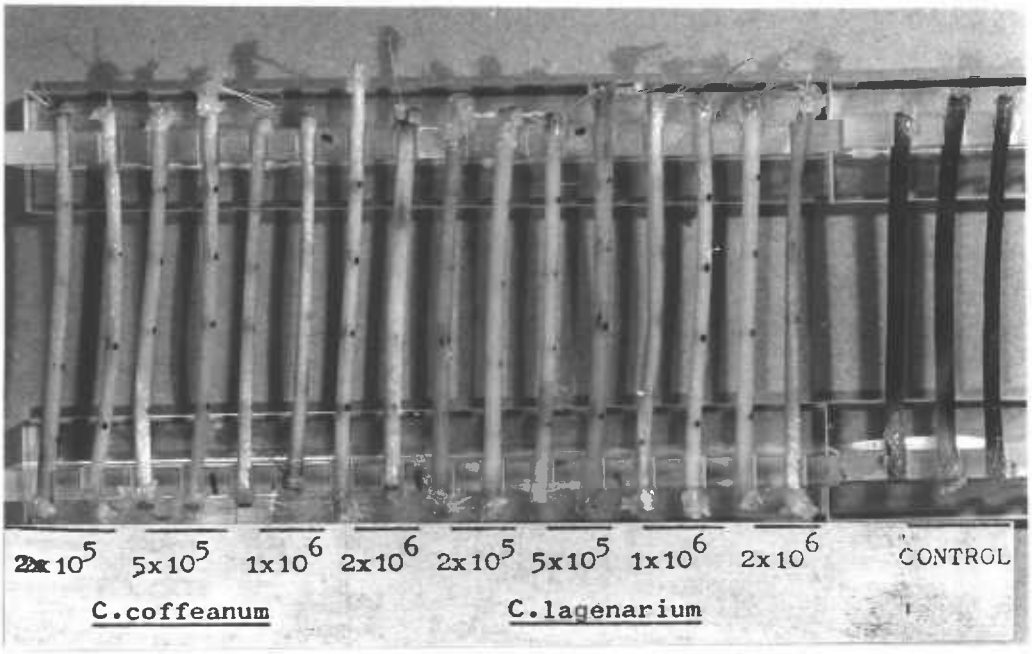


Plate 20. Inoculated sites on etiolated hypocotyls of Canadian Wonder cultivar of French bean, after being protected for 24 or 72 h with different concentrations of suspensions of C. coffeanum and C. lagenarium conidia, and controls, 12 d after inoculation

suspension of C. coffeanum spores (2 x 10^5/ml) for 24 h, decreased numbers of expanding lesion by 91%.

Therefore, these non-pathogenic Colletotrichum spp. suppressed the production of expanding lesions with little difference between treatments for 24 and 72 h.

The above procedure was used on non-etiolated bean hypocotyls with some modification. Etiolated and non-etiolated bean hypocotyls were used with Colletotrichum spp. grown on V_8 agar for 10 d. Spores suspensions were adjusted to a concentration of 500 spores/µl. The effects of first applying the protectant then C. lindemuthianum and first applying C. lindemuthianum then the protectant were studied. This was done for periods of 24 and 72 h. In the second approach, a 5 µl/drop of suspension of C. lindemuthianum (race β)(5 x 10^5/ml) was applied to 4 sites/hypocotyl and incubated at 17° - 19°C; 24 or 72 h later, the inoculum was removed and the sites were inoculated with drops of a suspension (5 x 10^5/ml) of C. lagenarium or C. coffeanum spores and were incubated at 17° - 19°C.

Results as means of 32 sites/8 hypocotyls/treatment are given in Tables 49 - 51.

In non-etiolated hypocotyls first inoculated with non-pathogenic Colletotrichum spp. and later with C. lindemuthianum (race β) numbers of expanding lesion were decreased by 86 to 100% (Table 49).

Results in Table 50 show that in hypocotyls which were first inoculated with race β and 24 or 72 h later with non-pathogenic Colletotrichum spp., there was no difference in the numbers of expanding lesions compared to the controls but in non-etiolated hypocotyls (Table 51), first inoculated with race β and 72 h later with a suspension (5 x 10^5/ml) of C. coffeanum spores, numbers of expanding lesion were decreased by 37%. Similarly when a suspension (5 x 10^5/ml) of C. lagenarium spores were added 24 and 72 h later, the numbers of expanding lesion were decreased by 37% and 13% respectively. Interestingly when suspension of race β (5 x 10^5/ml) spores

Table 49. The effect of non-pathogenic Colletotrichum spp. on disease of bean hypocotyls inoculated with C. lindemuthianum (race β)

Non-pathogenic* Colletotrichum spp.	Treatment* (h)	Days after inoculation: 6 — Lesion development			8			10			12		
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
C. lagenarium	24	0	0	0	16.7	0	0	16.7	0	0	16.7	0	0
	72	0	0	0	0	0	0	0	0	0	0	0	0
C. coffeanum	24	0	0	0	33.3	0	8.3	33.3	0	8.3	33.3	0	8.3
	72	0	0	0	0	0	0	0	0	0	0	0	0
Control (race β)		25	8.3	16.7	16.7	0	58.3	16.7	0	58.3	16.7	0	58.3

For 0, a, b and c - see footnote under Table 47.

* - Excised non-etiolated bean hypocotyls treated with conidia of non-pathogenic Colletotrichum spp. and inoculated later with race β of C. lindemuthianum

Table 50. The effect of disease development on etiolated bean hypocotyls inoculated first with race β of C. lindemuthianum (pathogenic) and later with C. lagenarium or C. coffeanum

Inoculation* sequence	Treatment* (h)	Days after inoculation: 6			8			10			12		
		Lesion development											
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
race β/ C. lagenarium	24	25	0	58.3	0	0	100	0	0	100	0	0	100
	72	8.3	0	91.7	0	0	100	0	0	100	0	0	100
race β/ C. coffeanum	24	33.3	0	66.7	0	0	100	0	0	100	0	0	100
	72	16.7	0	83.3	0	0	100	0	0	100	0	0	100
race β/race β	24	19	0	81	0	0	100	0	0	100	0	0	100
	72	0	25	75	0	0	100	0	0	100	0	0	100
race β/ sterile gdw	24	50	0	50	0	0	100	0	0	100	0	0	100
	72	0	0	100	0	0	100	0	0	100	0	0	100
Control (race β)		19	0	81	0	0	100	0	0	100	0	0	100

For a, b and c - see footnote under Table 47

* - Bean hypocotyls inoculated with C. lindemuthianum race β, and 24 or 72 h later inoculated with non-pathogenic Colletotrichum spp. and sterile water in controls

Table 51. The effect of disease development on non-etiolated bean hypocotyls inoculated first with race β (pathogenic) and later with C. lagenarium or C. coffeanum added

Inoculation* sequence	Treatment* (h)	Days after inoculation: 6			8			10			12		
		Lesion development											
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
race β/ C. lagenarium	24	25	16.7	33.3	33.3	16.7	33.3	33.3	16.7	33.3	33.3	16.7	41.7
	72	41.7	0	58.3	41.7	0	58.3	41.7	0	58.3	41.7	0	58.3
race β/ C. coffeanum	24	8.3	0	50	8.3	0	66.7	8.3	0	66.7	8.3	0	66.7
	72	25	0	25	25	0	25	25	0	41.7	25	0	41.7
race β/race β	24	12.5	0	37.5	50	0	37.5	50	0	37.5	50	0	37.5
	72	50	8.3	41.7	41.7	16.7	41.7	41.7	8.3	50	41.7	0	58.3
race β/ sterile gdw	24	41.7	0	50	41.7	0	58.3	41.7	0	58.3	33.3	0	66.7
	72	41.7	0	58.3	33.3	8.3	58.3	25	0	66.7	25	0	66.7
Control (race β)		25	8.3	16.7	16.7	0	58.3	16.7	0	58.3	16.7	0	66.7

For a, b and c - see footnote under Table 47

* - see footnote under Table 50

added 24 and 72 h later, the numbers of expanding lesion were decreased by 44% and 13% respectively (Table 51).

Summary

The notable points about the results given in Tables 47 - 51 are as follows :

i) The non-pathogenic Colletotrichum spp. when used as protectants on etiolated and non-etiolated hypocotyls, reduced the numbers of expanding lesion by 75 to 100% compared to the controls.

ii) There were no significant differences for the time intervals for applying the protectants and C. lindemuthianum (race β) and for concentrations of spore suspension of the protectants.

iii) When suspensions of Colletotrichum spp. spores added 24 or 72 h later to sites already inoculated with race β, numbers of expanding lesion in non-etiolated hypocotyls were reduced by 13 to 37%. In etiolated hypocotyls there was no difference in the numbers of expanding lesion compared to the controls.

These experiments were repeated with essentially similar results.

2.2 Mixed inocula of pathogenic and non-pathogenic Colletotrichum spp. on bean hypocotyls

Etiolated and non-etiolated bean hypocotyls were prepared as described earlier and Colletotrichum spp. were grown on V_8 agar for 10 d for the preparation of spore suspensions at final concentrations of 200, 500 and 1000 spores/µl. Each of these concentration of race β was mixed with equal volumes of suspension of spores of the non-pathogenic Colletotrichum spp. A 5 µl/drop of the mixed suspension was applied to each of 4 sites/

hypocotyl and incubated at 17 - 19°C.

The effects of mixed inocula of the pathogenic and non-pathogenic Colletotrichum spp. on disease on 32 sites/8 hypocotyls/treatment are given in Tables 52 - 53.

Results in Table 52 show that when 1000 spores/µl of race β were mixed with equal volumes of 500 and 200 spores/µl of C. lagenarium, the numbers of expanding lesion in etiolated hypocotyls were decreased by 50% and 0% respectively. In non-etiolated hypocotyls, 500 spores/µl suppressed formation of expanding lesions by 100% whereas 200 spores/µl increased the numbers of expanding lesions by 17%. When 500 spores/µl of race β were mixed with equal volumes of 1000 spores/µl of C. lagenarium, the numbers of expanding lesion were reduced by 42% and 50% in etiolated and non-etiolated hypocotyls respectively. When race β and C. lagenarium were mixed in equal volumes, both at 500 spores/µl, the numbers of expanding lesion were decreased by 33% in non-etiolated hypocotyls but there was no effect in etiolated hypocotyls compared to the controls. For race β at 200 spores/µl mixed with equal volumes of 1000/µl of C. lagenarium, the numbers of expanding lesion were decreased by 17% and 86% in etiolated and non-etiolated hypocotyls respectively.

Results in Table 53 show that a mixture of race β and C. coffeanum spores at the above combination of concentration did not affect disease development in etiolated hypocotyls compared to the controls, whereas in non-etiolated hypocotyls, the numbers of expanding lesion were reduced by 16 to 75% depending on the combination of concentrations.

Summary

Notable points about the results given in Tables 52 and 53 are as follows :

i) When suspensions of spores of race β and C. lagenarium were mixed in different concentrations for inocula, the numbers of expanding

Table 52. The effect of mixed inocula of C. lindemuthianum (race β) and C. lagenarium on disease of etiolated and non-etiolated bean hypocotyls

Conidia/ml		Days after inoculation: 6			8			10			12		
Race β/C.lagenarium	Hypocotyl*	Lesion development											
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
1×10^{6}**/5×10^{5}	Et	33.3	16.7	16.7	25	8.3	33.3	16.7	0	50	16.7	0	50
	Non-Et	18.8	0	0	25	18.8	0	63	25	0	63	25	0
$1 \times 10^{6}/2 \times 10^{5}$	Et	8.3	0	91.7	0	0	100	0	0	100	0	0	100
	Non-Et	31.3	0	18.8	18.8	6.3	75	12.5	0	87.5	12.5	0	87.5
$5 \times 10^{5}/1 \times 10^{6}$	Et	16.7	0	41.7	8.3	0	58.3	8.3	0	58.3	8.3	0	58.3
	Non-Et	6.3	0	6.3	0	6.3	18.8	18.8	6.3	37.5	25	6.3	37.5
$5 \times 10^{5}/5 \times 10^{5}$	Et	8.3	8.3	75	0	0	100	0	0	100	0	0	100
	Non-Et	37.5	12.5	0	31.3	0	37.5	37.5	0	43.8	31.3	0	50
$2 \times 10^{5}/1 \times 10^{6}$	Et	50	8.3	41.7	16.7	0	83.3	16.7	0	83.3	16.7	0	83.3
	Non-Et	0	0	0	18.8	6.3	12.5	18.8	6.3	12.5	18.8	6.3	12.5
race β 1×10^{6}	Et	0	0	100	0	0	100	0	0	100	0	0	100
	Non-Et	6.3	12.5	12.5	6.3	6.3	68.8	6.3	6.3	68.8	6.3	0	75
5×10^{5}	Et	0	0	100	0	0	100	0	0	100	0	0	100
	Non-Et	0	0	0	6.3	6.3	75	6.3	6.3	75	6.3	6.3	75

contd...../

Table 52 contd...../

2×10^5	Et	0	0	100	0	0	100	0	0	100	0	0	100
	Non-Et	12.5	3.1	37.5	6.3	3.1	87.5	6.3	3.1	87.5	6.3	3.1	87.5

For a, b, c - see footnote under Table 47

* - Etiolated hypocotyls (Et) from plants grown in the dark at 24° - 25°C. and non-etiolated hypocotyls (Non-Et) from plants grown in 12 h light/d at 24° - 25°C.

** - 1 ml of spore suspension of race β (1×10^6/ml) mixed with 1 ml of spore suspension of C. lagenarium (5×10^5/ml)

Table 53. The effect of mixed inoculum of C. lindemuthianum (race β) and C. coffeanum on disease of etiolated* and non-etiolated bean hypocotyls

Conidia/ml race β/C.coffeanum	Non-etiolated hypocotyls	Days after inoculation: 6			8			10			12		
		Lesion development											
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
1 x 10^6/5 x 10^5		0	0	0	0	0	18.8	0	0	18.8	6.3	6.3	18.8
1 x 10^6/2 x 10^5		0	12.5	6.3	6.3	18.8	37.5	6.3	18.8	37.5	0	18.8	43.8
5 x 10^5/1 x 10^6		0	23.5	0	0	31.3	37.5	0	12.5	63	0	0	63
5 x 10^5/5 x 10^5		6.3	6.3	0	6.3	12.5	37.5	12.5	12.5	37.5	12.5	12.5	37.5
2 x 10^5/1 x 10^6		0	0	6.3	0	0	25	6.3	0	25	6.3	0	25
race β 1 x 10^6		6.3	12.5	12.5	6.3	6.3	68.8	6.3	6.3	68.8	6.3	0	75
5 x 10^5		0	0	0	6.3	6.3	75	6.3	6.3	75	6.3	6.3	75
2 x 10^5		12.5	3.1	37.5	6.3	3.1	87.5	6.3	3.1	87.5	6.3	3.1	87.5

For ** - see footnote to Table 52

For a, b and c - see footnote to Table 47

* - Mixed inocula of race β and C. coffeanum in all the above combinations, 6 d after inoculation caused 100% production of expanding lesions in etiolated hypocotyls. Results are given only for non-etiolated hypocotyls

lesion in etiolated and non-etiolated hypocotyls were decreased by 17 to 50% and by 33 to 100% respectively.

ii) When suspensions of spores of race β and C. coffeanum were mixed in different concentrations for inocula the numbers of expanding lesion in non-etiolated hypocotyls were reduced by 16 to 75% whereas there were no effects on etiolated hypocotyls compared to the controls.

2.3 Effect of non-pathogenic Colletotrichum spp. on cucumber cotyledons

Cotyledons from susceptible cucumber plants growing in John Innes (No. 2) compost in plastic trays for 15 d in the glasshouse at 24° - 25°C., were prepared as described in Materials and Methods. C. lindemuthianum (race β) and C. coffeanum, both non-pathogenic to cucumber plants, were grown for 10 d on V_8 agar and C. lagenarium was grown for 7 - 8 d. Suspension of spores of the non-pathogenic Colletotrichum spp. were adjusted to a final concentration of 500 spores/µl and C. lagenarium spores were adjusted to a concentration of 100 spores/µl.

Cotyledons were inoculated with a 5 µl drop of suspension of spores of race β and C. coffeanum (5×10^5/ml) on 4 sites/cotyledon and incubated at 17° - 19°C. (race β) or at 24 - 25°C. (C. coffeanum); 24 and 72 h later, a 5 µl drop of suspension of spores of C. lagenarium (1×10^5/ml) was added to each pre-inoculated site and cotyledons were incubated at 24 - 25°C. Controls were pre-treated with water.

The effects of the non-pathogenic Colletotrichum spp. were assessed from the diameters (mm) of the lesions that developed.

Results as means of 64 sites/8 pairs of cotyledons/treatment are given in Table 54.

Table 54. Lesion size in cucumber cotyledons pre-treated with non-pathogenic Colletotrichum spp. and inoculated later with C. lagenarium

Non-pathogenic Colletotrichum spp.	Treatment (h)*	Days after inoculation 6	8	10
		Lesion size as % of Control		
C. coffeanum	24	68	58	70
	72	73	62	83
C. lindemuthianum (race β)	24	25	34	55
	72	95	91	85
Control		100	100	100
		**(4.0)	(7.4)	(10.1)

* - Cotyledons first inoculated with the non-pathogenic Colletotrichum spp. and later inoculated with C. lagenarium

**() - Diameters of lesions in controls pre-treated with water

In cotyledons first inoculated with C. coffeanum and 24 and 72 h later with C. lagenarium, the lesion sizes were decreased by 30% and 17% respectively and in cotyledons first inoculated with race β and 24 and 72 h later with C. lagenarium, the lesion sizes were decreased by 45% and 15% respectively.

For cotyledons first inoculated with the non-pathogenic Colletotrichum spp. and 24 h later with C. lagenarium the decrease in lesion size was more pronounced than in cotyledons inoculated 72 h later.

The above procedure with some modifications was used in susceptible cucumber cotyledons. Cotyledons prepared as described in Materials and Methods were first inoculated with 5 µl (drop) of a suspension of spores of

C. lagenarium (1×10^5/ml) and incubated at 24° - 25°C.; 24 and 72 h later each site was inoculated with 5 µl (drop) of a suspension of spores of race β or C. coffeanum (both 5×10^5/ml) and incubated at 17° - 19°C. Controls first inoculated with sterile gdw were later inoculated with C. lagenarium and incubated at 17° - 19°C.

In the second approach cotyledons were first inoculated with 5 µl (drop) of a suspension of spores of C. lagenarium (1×10^5/ml) and 72 h later, with spores of suspensions of race β or C. coffeanum and incubated at 25°C.

Results as means of 64 sites/8 pairs of cotyledons/treatment are given in Tables 55 - 56.

Table 55. Lesion size in cucumber cotyledons inoculated with C. lagenarium (pathogenic) and later with non-pathogenic Colletotrichum spp.at 17° - 19°C.

Post-treatment	Treatment (h)*	Days after inoculation		
		6	8	10
		Lesion size		
C. coffeanum	24	80	112	106
	72	122	119	116
C. lindemuthianum (race β)	24	52	82	111
	72	130	146	143
C. lagenarium	24	81	91	115
	72	62	83	112
Control (water)		100	100	100
		**(4.6)	(7.9)	(10.1)

* - Cotyledons first inoculated with C. lagenarium and later inoculated with non-pathogenic Colletotrichum spp.

**() - Lesion size (mm) controls

Table 56. Lesion size in cucumber cotyledons inoculated with C. lagenarium and 72 h later with non-pathogenic Colletotrichum spp. at 24° - 25°C.

Post-treatment	Days after inoculation		
	6	8	10
	Lesion size as % of Control		
C. coffeanum	102	103	103
C. lindemuthianum (race β)	90	95	95
C. lagenarium	101	100	100
Control (water)	100 **(8.5)	100 (10.6)	100 (13.2)

For ** - see footnote under Table 55

In susceptible cucumber cotyledons inoculated with a suspension of spores of C. lagenarium (1 x 10^5/ml) and incubated at 17 - 19°C., lesions developed without producing chlorotic zones around the periphery of lesions whereas those incubated at 24° - 25°C., developed chlorotic areas around the lesions.

Results in Table 55 show that cotyledons first inoculated with C. lagenarium and 24 h later, the same sites inoculated with suspensions of spores of C. coffeanum (5 x 10^5/ml), race β (5 x 10^5/ml) and C. lagenarium (1 x 10^5/ml) and incubated at 17° - 19°C., the lesion sizes were increased by 6, 11 and 15% respectively and those inoculated 72 h later, the lesion sizes were increased by 16, 43 and 12% respectively. But results in Table 56 show that cotyledons first inoculated with C. lagenarium and 72 h later, the same sites inoculated with suspension of spores of the above

Table 57. Lesion size in cucumber cotyledons treated with mixed inocula of *C. lagenarium* and *C. coffeanum* spores

C. lagenarium/*C. coffeanum* (conidia/ml)	Days after inoculation 6	8	10
	Lesion size as % of control		
1×10^6**/5×10^5	105	122	125
$1 \times 10^6/2 \times 10^5$	110	115	112
$5 \times 10^5/1 \times 10^6$	139	126	102
$5 \times 10^5/5 \times 10^5$	120	109	87
$2 \times 10^5/1 \times 10^6$	125	100	93
Control (*C. lagenarium*)			
1×10^6	100	100	100
	*(5.3)	(7.0)	(8.5)
5×10^5	100	100	100
	*(4.88)	(6.83)	(10.71)
2×10^5	100	100	100
	*(5.0)	(7.96)	(9.95)

** - 1 ml of a suspension of spores of *C. lagenarium* (1×10^6/ml) mixed with 1 ml of a suspension of spores of *C. coffeanum* (5×10^5/ml)

* - Lesion size (mm) controls

Table 58. Lesion size in cucumber cotyledons treated with mixed inocula of *C. lagenarium* and race β of *C. lindemuthianum* spores

C. lagenarium/race β	Days after inoculation		
	6	8	10
	Lesion size as % of Control		
1×10^6/5×10^5	22	29	34
1×10^6/2×10^5	120	105	127
5×10^5/1×10^6	130	105	84
5×10^5/5×10^5	45	54	44
2×10^5/1×10^6	110	100	106
Control (*C. lagenarium*)			
1×10^6	100	100	100
	*(5.3)	(7.0)	(8.5)
5×10^5	100	100	100
	*(4.88)	(6.83)	(10.7)
2×10^5	100	100	100
	* (5)	(7.96)	(9.95)

For * and ** - see footnote under Table 57

Colletotrichum spp. and incubated at 24° - 25°C, there was little effect on the lesion size compared to the controls.

Therefore, the effect of non-pathogenic Colletotrichum spp. on cucumber cotyledons inoculated with C. lagenarium depended on incubation temperature.

2.4 Mixed inocula of pathogenic and non-pathogenic Colletotrichum spp. on cucumber cotyledons

Susceptible cucumber cotyledons were prepared as described earlier. C. coffeanum and race β were grown on V_8 agar for 10 d and C. lagenarium for 7 - 8 d and suspensions of spores at final concentrations of 200, 500 and 1000 spores/µl were prepared. Each of the concentrations of C. lagenarium spores was mixed in equal volumes with suspensions of the non-pathogenic Colletotrichum spp. spores. A 5 µl/drop of the mixed suspension was applied to 4 sites/cotyledon which then were incubated at 24° - 25°C.

Results as means of 64 sites/8 pairs of cotyledon/treatment are given in Tables 57 - 58.

Results in Table 57 show that when 1000 spores/µl of C. lagenarium were mixed in equal volumes with 500 and 200 spores/µl of C. coffeanum and cotyledons inoculated with the mixed inoculum, lesion sizes were increased by 25% and 12% respectively, but when both of them were mixed at 500 spores/µl the lesion sizes were reduced by 13%. The other combinations had little effect on lesion sizes compared to the controls.

Results in Table 58 show that when 1000 and 500 spores/µl of C. lagenarium were mixed in equal volumes with 500 spores/µl of race β, and cotyledons inoculated with the mixed inoculum, lesion sizes were decreased by 66% and 56% respectively whereas when 1000 spores/µl of C. lagenarium were mixed with 200 spores/µl, lesion sizes were increased by 27%. When 500 spores/µl of C. lagenarium were mixed with 1000 spores/µl

of race β, the decrease in lesion size was 16%.

Therefore, race β was more effective in reducing lesion sizes than was C. coffeanum in mixtures with C. lagenarium.

These experiments were repeated with essentially similar results.

2.5 Alternately inoculated cucumber cotyledons

Susceptible cucumber cotyledons were prepared as described earlier. One cotyledon from each cucumber plant was sprayed with a suspension of spores of C. lagenarium, C. coffeanum and race β (all at 5×10^5/ml). Controls were sprayed with sterile gdw. 72 and 96 h later the untreated, second cotyledons were inoculated with 5 µl (drop) suspension of spores of C. lagenarium (1×10^5/ml) to see if they had become systemically protected against C. lagenarium. Cotyledons were incubated at 24° - 25°C.

Results as means of 40 sites/10 cotyledons/treatment are given in Table 59.

Results in Table 59 show that when one cotyledon was sprayed with spores of Colletotrichum spp. and 72 h later the other cotyledon was inoculated with spores of C. lagenarium, lesion size was decreased by 21 to 25%.

When cotyledons were sprayed with race β and C. coffeanum and 96 h later, the other cotyledons were inoculated with C. lagenarium, lesion size was decreased by 58% and 36% respectively; C. lagenarium decreased lesion size by only 9%.

Table 59. Lesion size in one cucumber cotyledon after the other cotyledon had been sprayed with Colletotrichum spp.

Pre-treatment	Treatment (h)*	Days after inoculation		
		6	8	10
		Lesion size as % of Control		
C. coffeanum	72	65	79	79
	96	38	64	64
C. lindemuthianum (race β)	72	78	79	79
	96	38	42	42
C. lagenarium	72	74	75	75
	96	54	91	91
Control (water)	72	100	100	100
		**(5.75)	(6.1)	(7.63)
	96	100	100	100
		**(3.0)	(4.3)	(5.38)

* - One of the pair of cotyledons of cucumber plants was sprayed with spores of Colletotrichum spp. and after 72 or 96 h the other cotyledon was inoculated with spores of C. lagenarium

** - Lesion sizes (mm) controls

2.6 *Colletotrichum* spp. on true leaves of cucumber

Seedlings of susceptible cucumber plants were transplanted to compost in pots (20 cm diameter). Fully expanded first true leaves (20 - 23 d after planting seed) were sprayed to run-off with a suspension of spores of *C. lagenarium* (1×10^8/ml). Controls were sprayed with gdw. The plants were covered with polythene bags for 48 h and left on benches in the glasshouse at 22° - 24°C. 7 - 8 d later, 20 sites on the second leaf were inoculated with a 5 µl (drop) of suspension of spores of *C. lagenarium* (1×10^5/ml) and plants covered again with polythene bags for 48 h.

Results were scored on the second, challenged true leaves by measuring lesion diameters and numbers of lesions developed/20 inoculated sites/10 leaves.

10 d after inoculation, the lesion sizes in the control leaves were 5 - 7 mm (diameter) and 90 - 100% of the inoculated sites produced lesions whereas in the second challenged leaves the lesion sizes were 1 - 1.5 mm (diameter) and 85 - 100% of the inoculated sites did not produce lesions.

Plate 21. The first leaves of Baton Vert cucumber cultivar sprayed with gdw and 7 - 8 d later the second leaves were inoculated with a suspension of spores of *C. lagenarium* (1×10^5/ml). All the inoculated sites produced lesions 10 d after inoculation. Photograph 10 d after inoculation.

Plate 22. The first leaves of Baton Vert cucumber cultivar were inoculated with a suspension of spores of C. lagenarium (1×10^8/ml) and 7 - 8 d later the second leaves were inoculated with 5 µl (drop) of suspension of spores of C. lagenarium (1×10^5). 10 d after inoculation there were no lesions on the second leaves

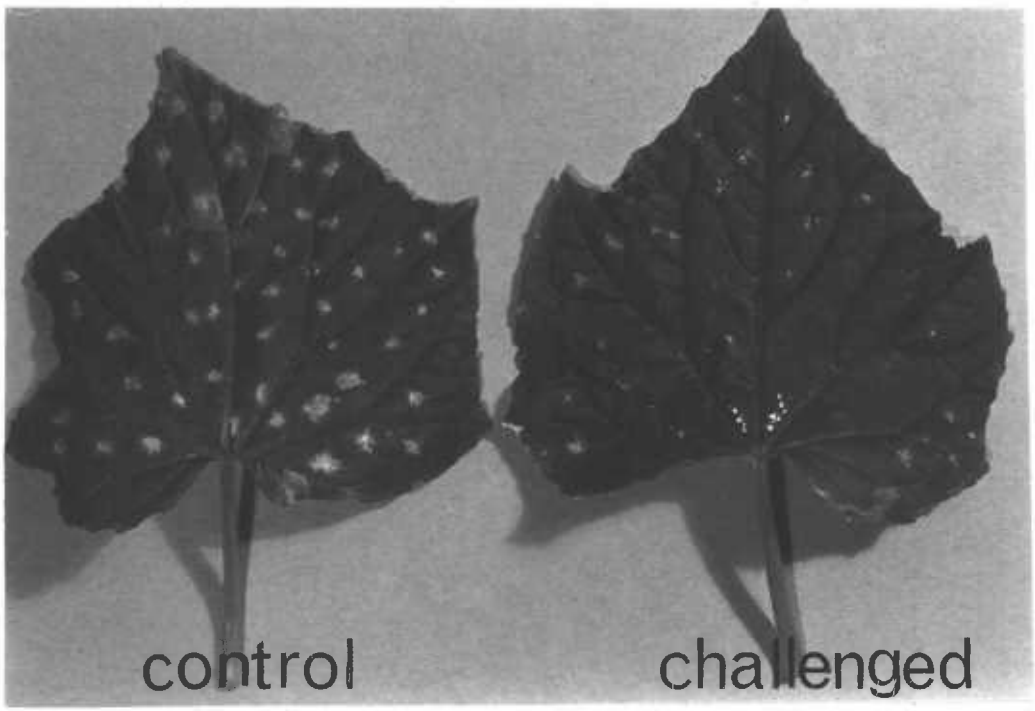


Plate 23. The first leaves of Baton Vert cucumber cultivar were inoculated with a suspension of spores of C. lagenarium (1×10^8/ml) and 4 - 5 d later, the second leaves were inoculated with 5 µl (drop) of suspension of spores of C. lagenarium (1×10^5). 10 d after inoculation minute lesions were produced compared to the controls

In a second series of experiments, seedlings of a susceptible cucumber cultivar were transplanted to compost in pots (20 cm diameter). 15 d after planting seed, the cotyledons were inoculated with a suspension of spores of C. lagenarium (1×10^8/ml) or with gdw. The plants were covered with polythene bags for 48 h. 10 d after inoculation, 20 sites on the first leaf above the inoculated cotyledons, were inoculated with a 5 µl (drop) of a suspension of spores of C. lagenarium (1×10^5/ml) and plants were covered with polythene bags for 48 h.

Results 10 d after inoculation showed the lesions in the controls were 6 - 7 mm (diameter) and 100% of the inoculated sites produced lesions whereas in the challenged first leaves the lesion sizes were 2 mm (diameter) and 90 - 100% of the inoculated sites did not produce lesions (Plate 24).

Plate 24. Cotyledons (arrowed) of Baton Vert cucumber cultivar first inoculated with a suspension of spores of C. lagenarium (1×10^8/ml) and 10 d later the first leaves (CH) were inoculated with a suspension of spores of C. lagenarium (1×10^5/ml). Limited lesions or no lesions developed on challenged (CH) true leaves. Normal lesions developed at each inoculated site in the controls.

In a third series of experiments, seedlings of a susceptible cucumber cultivar were prepared as described earlier. The second true leaves were inoculated with a suspension of spores of C. lagenarium (1×10^8/ml) and plants were covered with polythene bags for 48 h; 10 d later, the first true leaves below the inoculated leaves were inoculated and results were assessed as described earlier.

The lesion size in the first leaves, challenged after inoculating the second leaf, was 5 - 7 mm (diameter) very similar to the controls and all the inoculated sites produced lesions (Plate 25).

Plate 25. Second leaves (arrowed) of Baton Vert cucumber cultivar inoculat with a suspension of spores of C. lagenarium (1×10^8/ml) and 10 d later, the first leaves (CH) inoculated with suspension of spores of C. lagenarium (1×10^5/ml). The lesion sizes were similar to the controls

Summary

In leaves of a susceptible cucumber cultivar challenged after inoculating the first leaves or cotyledons below it with suspension of spores of C. lagenarium (1×10^8/ml), lesion sizes were reduced by 80 - 100% and the numbers of inoculated sites which produced lesions were reduced by 90 - 100%. But in first leaves challenged after inoculating the second leaves, there was no significant difference on lesion size and in numbers of inoculated sites which produced lesions compared to the controls.

When C. coffeanum and C. lindemuthianum (race β) were applied to the first leaves and 10 d later the second leaves were inoculated with a suspension of spores of C. lagenarium (1×10^5/ml), lesions developed as in the controls.

3. Fungal material in induction of resistance

Attempts were made to induce resistance in bean hypocotyls against C. lindemuthianum (race β) and in cucumber cotyledons against C. lagenarium by treating parts of the plants with materials prepared from the Colletotrichum spp.

3.1 Diffusates

A suspension of spores of C. coffeanum, C. lagenarium and race β (5×10^5/ml) were applied to cotyledons of resistant and susceptible cucumber plants from an Agla syringe and incubated for 48 - 60 h. Cotyledons treated with race β were incubated in the dark at 17° - 19°C. and the rest were incubated at 24° - 25°C. The inocula (diffusate) were collected with a Pasteur pipette, centrifuged (850 x g for 15 min) and reduced to ½ of the original volume at 4 - 5°C.

A 5 µl (drop) of the inocula (diffusates) was applied to 4 sites/ cotyledon to cucumber plants. Sites were marked with non water soluble ink. 24 and 72 h later each site on bean hypocotyl was inoculated with a suspension of spores of C. lindemuthianum (race β) (5×10^5/ml) and each site on cucumber cotyledon was inoculated with a suspension of spores of C. lagenarium (1×10^5/ml).

Results as means of 40 sites/10 hypocotyls/treatment and as means of 64 sites/8 pairs of cotyledon/treatment are given in Tables 60 - 61.

Table 60. The effect of diffusates of Colletotrichum spp. on disease of etiolated bean hypocotyls inoculated with C. lindemuthianum (race β)

Diffusate	Time of treatment (h)*	Expanding lesions[c]	
		Source of diffusate	
		S[a]	R[b]
C. lagenarium	24	83.3	83.3
	72	25.0	41.7
C. coffeanum	24	75	91.7
	72	33.3	25.0
C. lindemuthianum (race β)	24	83.3	75
	72	75.0	25
0		100	100

S[a] - Susceptible cultivar of cucumber, Baton Vert

R[b] - Resistant cultivar of cucumber, Sumter

c - Percentage inoculated sites with expanding lesions 12 d after inoculation

* - Etiolated hypocotyls treated with diffusates and later inoculated with race β of C. lindemuthianum

Table 61. Lesion size in cucumber cotyledons treated with diffusates of *Colletotrichum* spp. and inoculated with *C. lagenarium*

Diffusate	Time of treatment (h)*	Lesion size as % of Control	
		Source of diffusates	
		S[a]	R[b]
C. lagenarium	24	95	100
	72	85	75
C. coffeanum	24	110	105
	72	90	85
C. lindemuthianum	24	100	100
(race β)	72	90	72
0		100	100
		**(6.2)	(5.8)

For a, b and * - see footnote under Table 60

** - Lesion size (mm) controls 10 d after inoculation

Results in Table 60 show that for hypocotyls treated with diffusates of *C. lagenarium* and *C. coffeanum*, collected from S cucumber cultivar for 24 h, the numbers of expanding lesion were decreased by 17% and 25% respectively whereas in hypocotyls treated with these diffusates for 72 h, the numbers of expanding lesion were decreased by 75% and 67% respectively.

In hypocotyls treated with the diffusates of race β, collected from S cucumber cultivar, for 24 and 72 h, the numbers of expanding lesion were decreased by 17% and 25% respectively.

In hypocotyls treated with diffusates of Colletotrichum spp., collected from R cucumber cultivar, for 72 h, the numbers of expanding lesion were decreased by 58 - 75% and in hypocotyls treated with diffusates of C. lagenarium and race β for 24 h, the numbers of expanding lesions were decreased by 17% and 25%. Diffusates from C. coffeanum after 24h had only slight effects on lesion production compared to the controls.

Results in Table 61 show that treatment of cucumber cotyledons with diffusates of Colletotrichum spp., collected from S cucumber cultivar, for 24 and 72 h, had little or no effect on lesion size compared to the controls.

But in cucumber cotyledons treated with diffusates of Colletotrichum spp., collected from R cucumber cotyledons for 72 h, the decrease in lesion size was between 15% and 28% whereas in cotyledons treated for 24 h, there was little or no effect on lesion size compared to the controls.

In a second series of experiments, a suspension of spores of Colletotrichum spp. (5×10^5/ml) was incubated as above in sterile Petri dishes for 48 - 60 h. Some 90% of spores produced appressoria. These and the spores were removed by centrifugation and the volume of the supernatant was reduced to $\frac{1}{2}$ of its original volume. Bean hypocotyls and S cucumber cotyledons were then treated as above. These diffusates had little effect on the numbers of expanding lesion in bean hypocotyls and on size of lesions in cucumber cotyledons compared to the controls.

Summary

Notable points from the results given in Tables 60 and 61 are as follows :

i) In bean hypocotyls treated with diffusates of the non-pathogenic Colletotrichum spp., collected from S cucumber cotyledons, the decrease in the numbers of expanding lesion was more pronounced than

when diffusates from pathogenic species were used. Diffusates of Colletotrichum spp., collected from R cucumber cotyledons, had greatly reduced numbers of expanding lesion in hypocotyls inoculated with race B.

ii) In S cucumber cotyledons treatment with the diffusates of Colletotrichum spp., collected from S cucumber cotyledons, had little effect in reducing the lesion sizes but diffusates of Colletotrichum spp. collected from R cucumber cotyledons, greatly reduced the lesion sizes in S cucumber cotyledons.

iii) Diffusates tested in the production of appressoria by conidia of race β and C. lagenarium, appressoria production was increased under all sources of spore diffusates of Colletotrichum spp.

These experiments were repeated with essentially similar results.

3.2 Culture extracts

Colletotrichum spp. were grown on V_8 agar in 250 ml flasks for 7 - 8 d at 22° - 24°C; 20 ml of sterile gdw was added, shaken for 2 - 3 min and then the flasks were left at 22° - 24°C. for 48h. The liquid was removed, filtered and centrifuged. The final preparation which is referred to as culture diffusate was reduced to $^1/10$ of the original volume and sterilized by passage through a membrane filter (0.22 μ). Etiolated bean hypocotyls and S cucumber cotyledons were prepared as described in Materials and Methods.

A 5 μl/drop of the culture diffusate was applied to 4 sites/bean hypocotyl and to 4 sites/cucumber cotyledon; 24 and 72 h later each site was inoculated with a suspension of spores of race β (5×10^5/ml) and with a suspension of spores of C. lagenarium (1×10^5/ml) respectively.

In a second series of experiments, the whole of R and S cucumber cotyledons, attached to plants, were injected with serial dilutions of culture diffusates of Colletotrichum spp. The controls were injected with sterile gdw. The infiltrated areas were allowed to become normal in appearance and 3 - 5 mm disks were cut from them. Loss of electrolytes from the disks was estimated after washing rapidly by monitoring changes with time in the conductivity of bathing solutions; 10 disks replicate were suspended in 10 ml distilled water and results as means of 4 replicates are given in Fig. 6.

Results as means of 40 sites/10 hypocotyls/treatment and 64 sites/16 cotyledons/treatment are given in Tables 62 and 63.

In hypocotyls treated with the diffusates of cultures of C. lagenarium, C. coffeanum and race β and 72 h later inoculated with a suspension of spores of race β (5×10^5/ml), the numbers of expanding lesion were decreased by 50, 50 and 37% respectively whereas in hypocotyls treated with the diffusates of cultures of the non-pathogenic Colletotrichum spp. and 24 h later inoculated with race β, the numbers of expanding lesion were decreased by 33 to 44% compared to the controls.

In susceptible cucumber cotyledons treated with diffusates of cultures of race β and C. coffeanum and 24 h later inoculated with a suspension of spores of C. lagenarium (1×10^5/ml), the increase in lesion size was 9% and 8% respectively, whereas diffusate of cultures of C. lagenarium increased lesion size by 33%. But in susceptible cotyledons treated with the diffusates of cultures of Colletotrichum spp. and 72 h later inoculated with C. lagenarium, there was little or no effect on lesion size compared to the controls.

In resistant cucumber cotyledons treated with diffusates of cultures of C. lagenarium and C. coffeanum and later inoculated with suspension of spores of C. lagenarium (1×10^5/ml), the decrease in lesion size was 34 - 45% and 54 - 55% respectively whereas diffusates of race β cultures increased the lesion size in R cotyledon by 10 - 20%.

Table 62. The effect of culture diffusates on disease of etiolated bean hypocotyls inoculated with *C. lindemuthianum* (race β)

Source of culture diffusate	Treatment (h)*	Days after inoculation: 6			8			10			12		
		Lesion development											
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
C. lagenarium	24	50	0	50	43.7	0	56.3	37.5	0	62.5	37.5	0	62.5
	72	50	25	25	50	6.3	43.7	50	0	50	50	0	50
C. coffeanum	24	43.7	6.3	50	37.4	6.3	56.3	37.4	6.3	56.3	37.4	6.3	56.3
	72	50	6.3	43.7	50	6.3	43.7	50	0	50	50	0	50
C. lindemuthianum (race β)	24	0	12.5	87.5	0	6.3	93.7	0	6.3	93.7	0	6.3	93.7
	72	0	56.3	43.7	0	42.8	56.3	0	42.8	56.3	0	37.5	62.5
V_8 + water	24	56.3	0	43.7	37.5	0	62.5	31.3	0	68.7	0	0	100
	72	25	25	50	6.2	0	93.8	6.2	0	93.8	0	0	100
Control		18.8	0	81.2	0	0	100	0	0	100	0	0	100

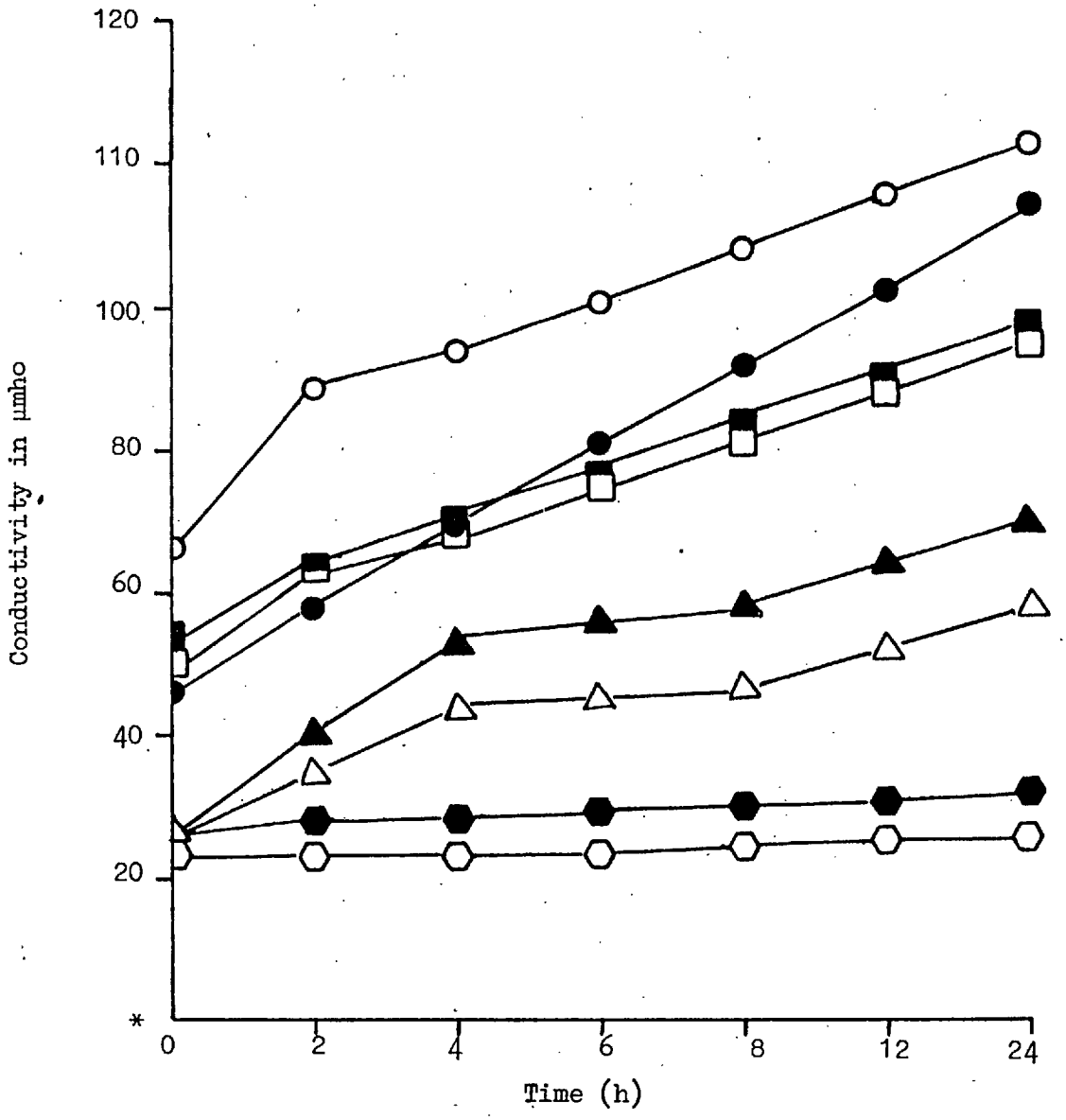
Fl[a] – % inoculated sites with flecks

LL[b] – % inoculated sites with limited lesions

EL[c] – % inoculated sites with expanding lesions

* – Excised etiolated hypocotyls treated with diffusates of cultures of *Colletotrichum* spp. and later inoculated with race β of *C. lindemuthianum*

Figure 6. Conductivity of solutions around cucumber cotyledon disks infiltrated with diffusates** of cultures of Colletotrichum spp.



Baton Vert (unshaded) and Sumter (shaded) cucumber cotyledons susceptible and resistant respectively, infiltrated with diffusates of cultures of race β (○—●), C. lagenarium (△—▲), C. coffeanum (□—■) and sterile gdw (⬡—⬢)

* The time the disks cut from the cotyledons

** Dilution = 10^{-2} at 24-25°C

Table 63. Lesion size in cotyledons treated with culture diffusates and inoculated with C. lagenarium

Source of culture diffusate	Treatment (h)*	Lesion size (mm) S[a]	Lesion size (mm) R[b]
C. lagenarium	24	133	66
	72	100	55
C. coffeanum	24	108	46
	72	90	45
C. lindemuthianum (race β)	24	109	120
	72	95	110
V_8 + water	24	100	100
	72	100	100
Control (water)		100	100
		**(5.38)	(3.03)

For a, b and * - see footnote under Table 60

** - Lesion size (mm) of controls 10 d after inoculation

Results in Fig. 6 show that for R and S cucumber cotyledons infiltrated with diffusates of cultures of non-pathogenic Colletotrichum spp., release of electrolytes was much higher than from cotyledons infiltrated with diffusates of cultures of C. lagenarium. The loss of electrolytes was higher in R than in S cotyledons. Loss of electrolytes in S cotyledons, infiltrated with diffusates of cultures of race β, was higher by 32% than in R cotyledons. There was no significant difference in loss of electrolytes between S and R cotyledons infiltrated with diffusates of cultures of C. lagenarium.

Summary

Notable points from the results given in Tables 62 and 63 and Fig. 6 are as follows :

i) In bean hypocotyls treated with the diffusates of cultures of non-pathogenic Colletotrichum spp., numbers of expanding lesion were reduced greatly whereas with culture diffusates from the pathogenic, race β, there was no significant difference from the controls because all inoculated sites produced lesions (limited and expanding lesions).

ii) Diffusates of cultures of C. lagenarium and C. coffeanum reduced the lesion size in R cotyledons but there were only slight effect with S cotyledons.

iii) Cotyledons of R and S cucumber plants, infiltrated with diffusates of cultures of non-pathogenic Colletotrichum spp., released more electrolytes than did cotyledons infiltrated with the diffusates of cultures of pathogenic, C. lagenarium. Also, R cotyledons

released more electrolytes than S cotyledons when infiltrated with the diffusates of cultures of C. lagenarium and C. coffeanum.

3.3 Mycelial cell walls

C. lindemuthianum (race β), C. lagenarium and C. coffeanum were grown in liquid culture for 8 d and the cell wall fractions were prepared as described in Materials and Methods.

Elicitor was extracted from cell walls by suspending 1 g of dry walls in 100 ml of water and autoclaving for 20 min at 121°C. The autoclaved suspension was then clarified by centrifugation and concentrated to $^{1}/_{10}$ of original volume under reduced pressure.

Etiolated bean hypocotyls and cucumber cotyledons were prepared as described in Materials and Methods.

Serial dilutions of elicitors were prepared and a 5 µl (drop) was applied to 4 sites/hypocotyl and to 4 sites/cotyledon. 24 h later, each site on hypocotyls was inoculated with a suspension of race β spores (5×10^5/ml) and cucumber cotyledons were inoculated with 5 µl/drop of a suspension of C. lagenarium spores (1×10^5/ml).

In a second series of experiments seeds of S cucumber were soaked in dilutions of elicitors of Colletotrichum spp. for 24 h and the controls were soaked in sterile gdw. Soaked seeds after being air dried were planted in John Innes (No. 2) compost in the glasshouse for 15 d. Cotyledons were prepared as described in Materials and Methods and were inoculated with 5 µl/drop suspension of C. lagenarium spores (1×10^5/ml) and incubated at 24° - 25°C.

The effects of elicitors on disease expression of bean hypocotyls and on lesion size of cucumber cotyledons were scored as described in earlier sections.

Results as means of 40 sites/10 hypocotyls/treatment and as means of 64 sites/8 pairs of cotyledon/treatment are given in Tables 64, 65 and 66.

Table 64. Effect of mycelial cell wall extracts on disease of bean hypocotyls inoculated with C. lindemuthianum (race β)

Source of mycelial cell wall extracts	Serial dilutions (10^{-x})*	Days after inoculation: 6			8			10			12		
		Lesion development											
		Fl[a]	LL[b]	EL[c]	Fl	LL	EL	Fl	LL	EL	Fl	LL	EL
C. lagenarium	1	91.7	0	0	91.7	0	0	91.7	0	0	91.7	0	0
	2	33.3	0	0	33.3	0	0	33.3	0	0	33.3	0	0
	3	8.3	0	8.3	8.3	0	16.7	8.3	0	25	8.3	0	25
	4	8.3	0	0	25	0	33.3	25	8.3	33.3	25	8.3	33.3
C. coffeanum	1	91.7	0	0	91.7	0	0	91.7	0	0	91.7	0	0
	2	16.7	0	0	0	0	16.7	0	0	16.7	0	0	16.7
	3	2.5	0	0	8.3	0	41.7	0	0	50	0	0	50
	4	33.3	0	33.3	8.3	8.3	50	8.3	0	75	8.3	0	75
C. lindemuthianum (race β)	1	100	0	0	100	0	0	100	0	0	100	0	0
	2	8.3	0	0	0	0	16.7	8.3	0	16.7	8.3	0	16.7
	3	41.7	0	0	0	16.7	50	0	16.7	50	8.3	16.7	50
	4	8.3	0	25	8.3	0	25	8.3	16.7	58.3	8.3	16.7	58.3
0		18.8	0	75	0	0	100	0	0	100	0	0	100

For a, b and c - see footnote under Table 62

* 10^{-1} - Extract of 1 g dried wall material in 10 ml (see text for details)

Table 65. Lesion size in cucumber cotyledons treated with mycelial cell wall extracts and inoculated with C. lagenarium

Source of mycelial cell wall extracts	Serial dilutions (10^{-x})	Days after inoculation 6	8	10
		Lesion size as % of control		
C. lagenarium	1	29	48	53
	2	35	55	39
	3	53	58	45
	4	59	63	50
C. coffeanum	1	56	87	78
	2	17	23	47
	3	35	36	45
	4	30	40	42
C. lindemuthianum (race β)	1	46	58	83
	2	86	64	96
	3	52	97	101
	4	60	100	110
0		100 *(2.9)	100 (3.88)	100 (5.61)

* - Lesion sizes (mm) controls

Table 66. Lesion size in cucumber cotyledons from seeds soaked in mycelial cell wall extracts, inoculated with C. lagenarium

Source of mycelial cell wall extracts	Serial dilutions (10^{-x})	Days after inoculation		
		6	8	10
		Lesion size as % of Control		
C. lagenarium	1	65	82	90
	2	58	68	72
	3	25	39	44
	4	27	40	42
C. coffeanum	1	57	66	80
	2	32	39	45
	3	40	46	50
	4	34	38	42
C. lindemuthianum (race β)	1	50	58	74
	2	44	48	61
	3	39	50	53
	4	41	46	50
0		100 *(3.54)	100 (5.04)	100 (6.58)

For * - see footnote under Table 65

In hypocotyls treated with elicitors of the above Colletotrichum spp. at 10^{-1} dilution, all sites produced visible flecks but no expanding lesions. In hypocotyls treated with elicitors at 10^{-2} dilution, decrease in numbers of expanding lesion was between 83% and 100%. Hypocotyls treated with elicitors of C. lagenarium, C. coffeanum and race β at 10^{-3} dilution the decrease in numbers of expanding lesion were 75, 50 and 50% respectively and at 10^{-4}, the decrease in numbers of expanding lesion were 67, 25 and 42% respectively.

In cucumber cotyledons treated with elicitors of C. lagenarium and C. coffeanum at 10^{-2} - 10^{-4}, the lesion sizes decreased by 50% to 61% and by 53% to 58% respectively whereas dilution at 10^{-1} decreased the lesion size by 47% and 22% respectively.

In cotyledons treated with elicitors of race β at 10^{-1} and 10^{-2} dilution the lesion size decreased by 17% and 4% respectively and the rest of the dilutions had little effect on lesion size compared with the controls.

When susceptible cucumber seeds soaked in elicitors of Colletotrichum spp. and 15 d later cotyledons were inoculated with C. lagenarium, lesion size was decreased by 28% to 58% at 10^{-2} - 10^{-4} serial dilutions whereas at 10^{-1} the decrease in lesion size was between 10% and 26% compared to the controls.

3.3.1 Carbohydrate and protein determination in elicitors of Colletotrichum spp.

The above results showed that there was no significant difference between the effects of the elicitors of Colletotrichum spp. when tested on bean hypocotyls and on cucumber cotyledons. To see if there was any difference in their total carbohydrate and protein contents, elicitors were analysed by the Anthrone and Folin-Ciacalteu reagent methods respectively as described in Materials and Methods.

Standard curves for carbohydrate determination were prepared from

glucose and glycogen solutions (10 mg/100 ml) and standard curves for protein determination was prepared from solutions of crystalline bovine serum albumin. Absorbances for carbohydrate and protein solutions were measured at 620 nm and 750 nm respectively, against blank reagents. Results as means of 5 replicates/elicitor are given in Table 67.

Table 67. Total carbohydrates and proteins in mycelial cell wall extracts of Colletotrichum spp.

Mycelial cell wall extract from	Total carbohydrates (mg/gram of cell wall)*	Total protein (mg/gram of cell wall)**
C. lagenarium	20	19.3
C. coffeanum	19.8	22
C. lindemuthianum (race β)	20.4	17.7

* - Total carbohydrates as mg of equivalence of glucose/gram of cell wall

** - Total proteins are expressed as mg of equivalence of bovine serum albumin/gram of cell wall

Results in Table 67 show that there was no significant difference in the total carbohydrate and protein content of elicitors of Colletotrichum spp.

Summary

Notable points from the results given in Tables 64 - 67 are as follows :

i) In bean hypocotyls treated with a concentrated preparation of elicitors of Colletotrichum spp., numbers of expanding lesions were decreased by 100% and as the concentration of the elicitors decreased the numbers of expanding lesion increased.

ii) In cucumber cotyledons treated with solutions of elicitors of C. lagenarium and C. coffeanum lesion sizes decreased greatly whereas elicitors from race β had little or no effect on lesion size compared to the controls.

iii) When seeds of cucumber soaked in solutions of elicitors of Colletotrichum spp. and cotyledons were inoculated with C. lagenarium, lesion sizes were reduced greatly.

These experiments were repeated with essentially similar results.

4. Lesion diffusates

When bean hypocotyls and cucumber cotyledons are infected with their pathogens, exudates are found on the lesions especially when plants are kept in humid conditions. The lesion exudates or diffusates were examined for their capacity to induce resistance.

Etiolated bean hypocotyls and cucumber cotyledons were prepared and inoculated with suspensions of spores of C. lindemuthianum (race β) (5×10^5/ml) and C. lagenarium (1×10^5/ml) respectively.

Inoculated hypocotyls were incubated at 17° - 19°C. in c. 100% RH and exudates were collected in a Pasteur pipette soon after they appeared. The exudates were combined and were made free of spores or contaminant bacteria by passing through a membrane filter (0.22 μ).

In R and S cucumber cotyledons, lesions started to appear 4 - 5 d after inoculation. Four inoculated cotyledons were detached and placed in a sterile Petri dish with 20 ml of sterile gdw. Uninoculated cucumber cotyledons were used as controls. The inoculated side of the cotyledon was placed on the water and they were left for another 72 h at 24° - 25°C. The diffusate was prepared as described in Materials and Methods.

Bean hypocotyls and cucumber cotyledons were treated with 5 μl (drop) dialysed and undialysed lesion diffusates and later inoculated with suspension of race β spores (5×10^5/ml) and C. lagenarium (1×10^5/ml) respectively.

Twelve days after inoculation, the dialysed fraction of R and S cucumber lesions had reduced the numbers of expanding lesion by 13%; undialysed fraction had little effect.

Lesion diffusates from bean hypocotyls had little effect on lesion size of cucumber cotyledons inoculated with C. lagenarium.

The effects of lesion diffusates from bean hypocotyls and cucumber cotyledons on disease of bean hypocotyls and on lesion size of cotyledons as means of 32 sites/8 hypocotyls/treatment and 64 sites/8 pairs of cotyledons/

treatment respectively are given in Tables 68 and 69.

Table 68. The effect of lesion diffusate from bean hypocotyls on disease of bean hypocotyls inoculated with C. lindemuthianum (race β)

Treatment (h)*	Expanding lesions**
24	33.3
72	0
0	100

* Excised etiolated hypocotyls treated with lesion diffusates and later inoculated with race β

** Percentage of inoculated sites with expanding lesions 12 d after inoculation

Table 69. Lesion size in cucumber cotyledons treated with lesion diffusates collected from inoculated cucumber cotyledons and inoculated with C. lagenarium

Source of lesion diffusate	Treatment (h)*	Lesion size (mm)** S[d]	Lesion size (mm)** R[b]
S[a]	24	112	62
	72	110	55
R[b]	24	100	101
	72	90	100
0		100	100
		[c](5.8)	[c](3.03)

cont'd ...

a Susceptible cucumber cultivar, Baton Vert

b Resistant cucumber cultivar, Sumter

c Lesion size (mm) of controls

* Cucumber cotyledons treated with lesion diffusates and later inoculated with C. lagenarium

** Lesion size (mm) of treated cotyledons as percentage of lesion size (mm) of untreated cotyledons 10 d after inoculation

Results in Table 68 show that in hypocotyls treated with lesion diffusates for 24 and 72 h from bean hypocotyls, numbers of expanding lesion were decreased by 67% and 100% respectively.

In S cucumber cotyledons treated with lesion diffusates from R and S cotyledons, lesion size was not significantly different from controls. But R cotyledons treated with lesion diffusates from S cotyledons for 24 and 72 h, lesion size was decreased by 38% and 45% respectively whereas in R cotyledons treated with lesion diffusates from R cotyledons, lesion size was not significantly different from controls.

4.1 Effects of cucumber lesion diffusates on production of appressoria

Lesion diffusates from cucumber cotyledons tested for effects on induction of resistance of bean and cucumber to race β and C. lagenarium respectively were used in further tests in which production of appressoria was scored after 24 h.

Three x 20 µl of dialysed and undialysed lesion diffusates were applied to glass slides and 1 µl suspension of race β and C. lagenarium spores (both 5×10^5/ml) were added to the centre of each drop. Results scored after 24 h are given as means of 3 replicates and count of 150 spores/replicate in Table 70.

Table 70. Effects of lesion diffusates on production of appressoria by conidia of C. lagenarium and C. lindemuthianum (race β)

Source of lesion diffusate	Colletotrichum spp.	Appressoria production * dialysed	undialysed	0
S[a]	C. lagenarium	83	78	100(100)**
	C. lindemuthianum (race β)	8	151	100(53.5)**
R[b]	C. lagenarium	90	94	
	C. lindemuthianum (race β)	39	93	

For a and b - see footnote under Table 69

* Appressoria production by treated conidia as percentage of appressoria produced by untreated conidia

** Actual figures for the controls

Results in Table 70 show that dialysed lesion diffusates from S and R cotyledons, decreased appressoria production by race β by 92% and 61% respectively, and decreased appressoria production by C. lagenarium by 17% and 10% respectively. Undialysed lesion diffusates from S and R cotyledons, decreased appressoria production by C. lagenarium by 22% and 6% respectively, whereas undialysed lesion diffusate from S cotyledons increased appressoria production by race β by 51%. But undialysed lesion diffusate from R cotyledons, decreased appressoria production by race β by 7%.

5. Temperature

Etiolated bean hypocotyls were prepared as described in Materials and Methods and placed in boxes in cabinets at 37°, 45° and 50°C for

various periods. The boxes were taken out from the cabinet and left for 1 - 2 h at room temperature. Hypocotyls were inoculated with suspension of pathogenic and non-pathogenic Colletotrichum spp. spores (5×10^5/ml) and incubated at 24 - 25°C. (C. lagenarium and C. coffeanum) and at 17° - 19°C. (race β).

In a second series of experiments, after the hypocotyls had been treated with heat, boxes were incubated under room temperature for 24 or 72 h and later inoculated with suspension of race β spores (5×10^5/ml).

The effects of heat on disease development of bean hypocotyls were scored as described in previous sections.

Results as means of 40 sites/10 hypocotyls/treatment are given 12 d after inoculation in Table 71.

Resistant hypocotyls treated at 37° and 45°C. for a period of time and later inoculated with C. lagenarium and C. coffeanum remained resistant and susceptible hypocotyls remained susceptible when inoculated with race β.

Hypocotyls treated at 50°C. for longer periods (70 - 90 min) appeared flaccid soon after being removed from the cabinet which suggested that heating had caused severe damage whereas those heated for a period of 5 - 60 min did not show any abnormalities.

In hypocotyls treated with heat at 50°C. for 5 - 60 min and 24 h later inoculated with race β, there was no difference in numbers of expanding lesion compared to the controls but in hypocotyls treated for 60 and 45 min and inoculated 72 h later, numbers of expanding lesion were decreased by 83% and 50% respectively. In hypocotyls treated with heat (50°C.) for 5 - 30 min and 72 h later inoculated with race β, the numbers of expanding lesion were decreased by 17 to 25% compared to the controls.

Table 71. The effect of heat (50°C.) on disease development in bean hypocotyls inoculated with C. lindemuthianum (race β)

Duration of treatment (min)	Treatment (h)*	Fl[a]	Expanding lesion[b]
60	0	0	100
	24	0	100
	72	0	16.7
45	0	0	100
	24	0	100
	72	8.3	50
30	0	0	100
	24	0	100
	72	16.7	75
15	0	0	100
	24	0	100
	72	0	75
5	0	0	100
	24	0	100
	72	8.3	83.3
0		0	100

a Percentage inoculated sites with flecks 12 d after inoculation

b Percentage inoculated sites with expanding lesions 12 d after inoculation

* Hypocotyls treated with heat (50°C.) for a period of time and later inoculated with race β

Summary

(i) Resistant hypocotyls treated with heat at 37°, 45° and 50°C. and inoculated with non-pathogenic Colletotrichum spp. remained resistant.

(ii) Susceptible hypocotyls treated with heat at 37° and 45°C. for various periods remained susceptible to race β.

(iii) In hypocotyls treated with heat (50°C.) and 72 h later inoculated with race β, disease development was suppressed by 17 to 83% depending on the duration of treatment.

These experiments were repeated with similar results.

6. Non-ionizing radiation (UV)

The following experiments were investigated whether UV can induce resistance in susceptible plants or cause non-pathogenic Colletotrichum spp. to penetrate bean hypocotyls and cucumber cotyledons and hypocotyls.

Etiolated bean hypocotyls of resistant and susceptible cultivars and cucumber cotyledons and hypocotyls were prepared as described in Materials and Methods.

6.1 French bean

Hypocotyls were irradiated for various periods with UV light from a "Philips TUV 30 Watt" germicidal single tube with an output of 8 watts at 2537 Å. After irradiation hypocotyls were inoculated with Colletotrichum spp. and incubated at appropriate t°.

Hypocotyls of R and S cultivars of bean irradiated with UV for 15 min to 4 h, and inoculated with C. coffeanum, C. lagenarium and race β developed flecks within 24 h and 48 h later brown lesions appeared at all inoculated sites.

The non-irradiated S and R hypocotyls inoculated with C. coffeanum and C. lagenarium, and R hypocotyls inoculated with race β, conidia produced appressoria and within 48 - 72 h the cells reacted in a hypersensitive way by granulation of the cytoplasm of epidermal cells (Plate 27). But in irradiated etiolated and non-etiolated hypocotyls of R and S bean cultivars, all the Colletotrichum spp. penetrated the cells below the germinated conidia by producing primary hyphae and subsequently invading the cells intracellularly by producing secondary hyphae (Plates 29 - 32).

48 - 72 h after inoculation, C. lagenarium and race β produced very dark brown lesions and C. coffeanum produced light brown lesions. The Colletotrichum spp. invaded the xylem parenchyma cells within 72 h and caused browning of the cell walls. Irradiated but not inoculated hypocotyls remained healthy in appearance during the experiments.

In a second series of experiments, a portion of the hypocotyl was protected from UV by covering with 2 layers of alluminium foil while the rest was irradiated. After later inoculation with Colletotrichum spp. lesions developed only in areas exposed to UV radiation (Plates 36 and 37).

In a third series of experiments, susceptible etiolated bean hypocotyls after irradiation with UV for 1 h, were incubated either in the dark or light at 25°C. 24 or 72 h later hypocotyls were inoculated with pathogenic and non-pathogenic Colletotrichum spp.

Results as means of 40 sites/10 hypocotyls/treatment are given in Table 72.

Table 72. Effects of UV irradiation on disease development in susceptible Canadian Wonder etiolated bean hypocotyls inoculated with *C. lindemuthianum* (race β)

Duration of UV irradiation (min)	Time before inoculation (h)*	Lesion development	
		Fl[a]	EL[b]
60	0	0	100
	24	33.3	66.7
	72	0	0
45	0	0	100
	24	58.3	41.7
	72	0	0
30	0	0	100
	24	100	0
	72	0	0
15	0	0	100
	24	100	0
	72	0	0
0	-	0	100

a - Percentage inoculated sites with flecks 12 d after inoculation

b - Percentage inoculated sites with expanding lesions 12 d after inoculation

* - Etiolated hypocotyls of Canadian Wonder irradiated with UV and later inoculated with race β

Plate 26. UV irradiated hypocotyls of Canadian Wonder hypocotyls showing normal cytoplasm (CY) x 600

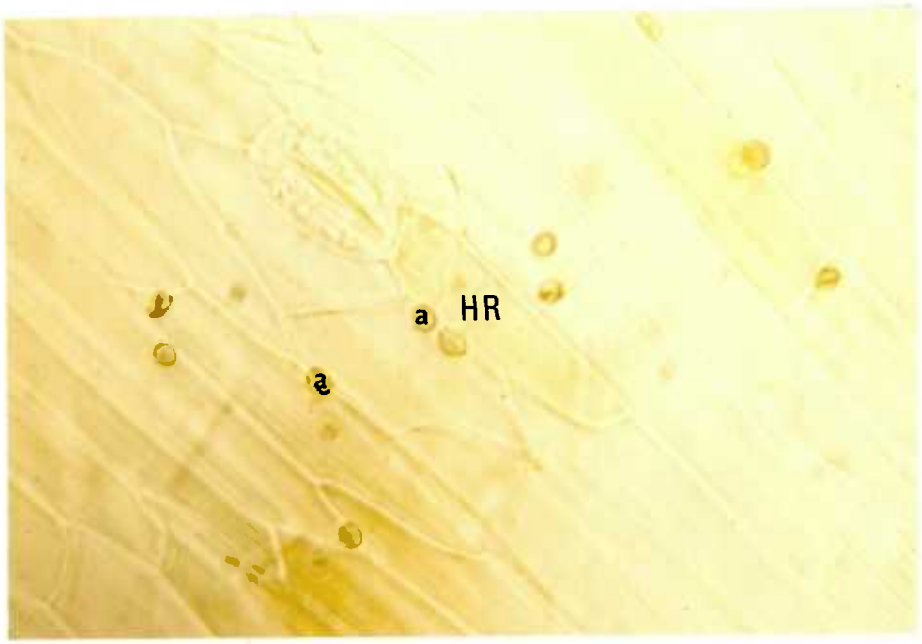


Plate 27. Hypersensitive reaction (HR) in cells of hypocotyls of Kievit cultivar below appressoria (a) of C. lagenarium, C. coffeanum and race β, 48 h after inoculation. x 600

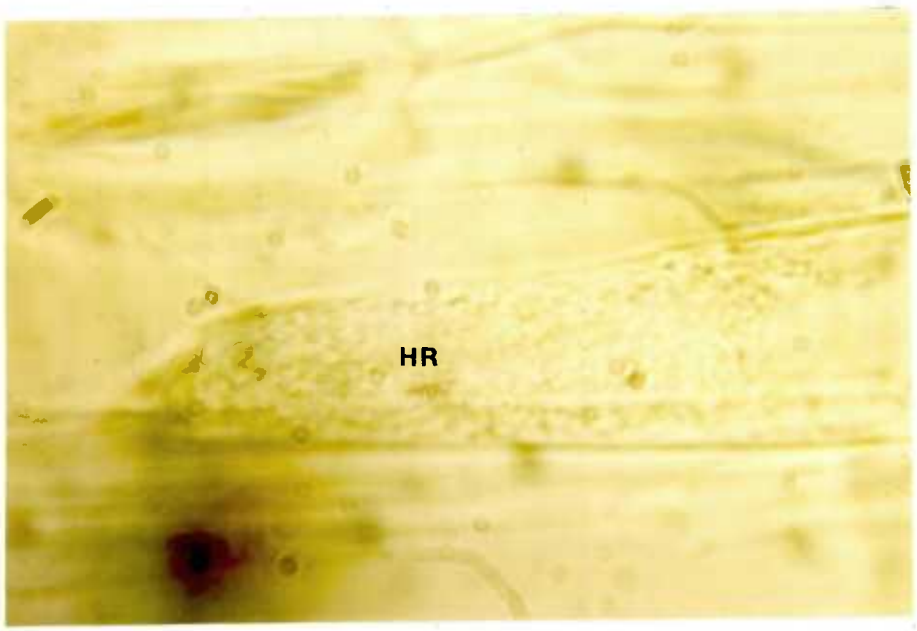


Plate 28. Magnification x 1500 of the hypersensitive reaction (HR) on cells of hypocotyls of Kievit cultivar caused by C. lagenarium, C. coffeanum and race β, 48 h after inoculation.

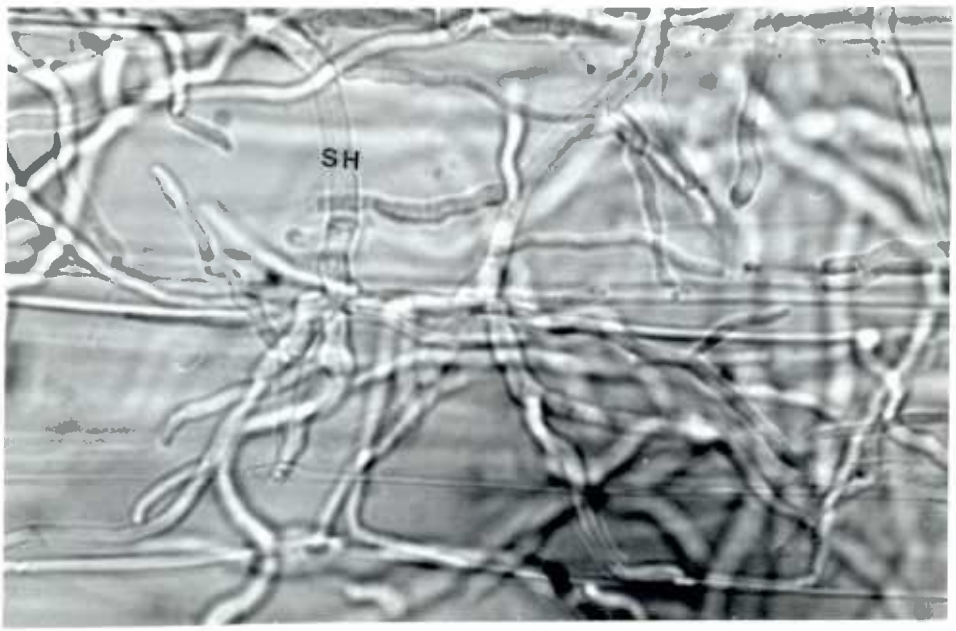


Plate 29. Intracellular penetration by secondary hyphae (SH) of C. lagenarium on eticlated Canadian Wonder hypocotyls irradiated with UV for 1 h. 72 h after inoculation x 600

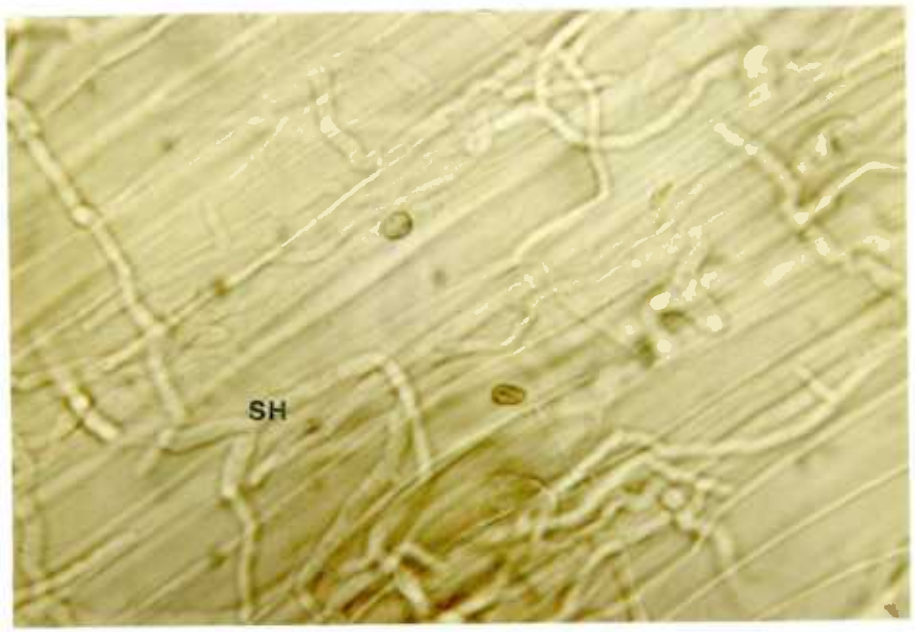


Plate 30. Intracellular penetration by secondary hyphae (SH) of C. lagenarium in cells of etiolated Kievit hypocotyls irradiated with UV for 1 h. 72 h after inoculation x 600

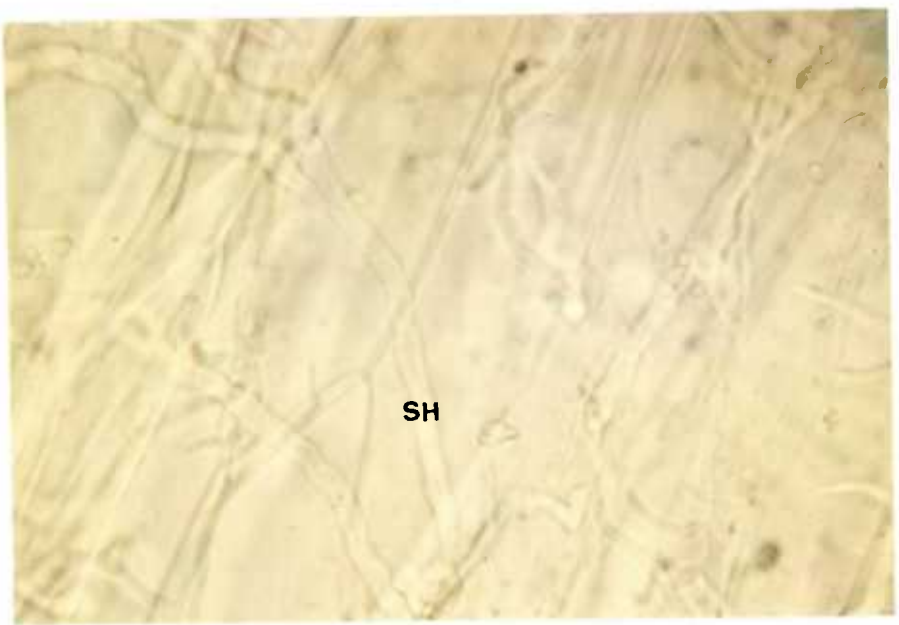


Plate 31. Intracellular penetration of cells of etiolated Kievit hypocotyls, irradiated with UV for 1 h, by secondary hyphae of C. coffeanum. 72 h after inoculation x 600

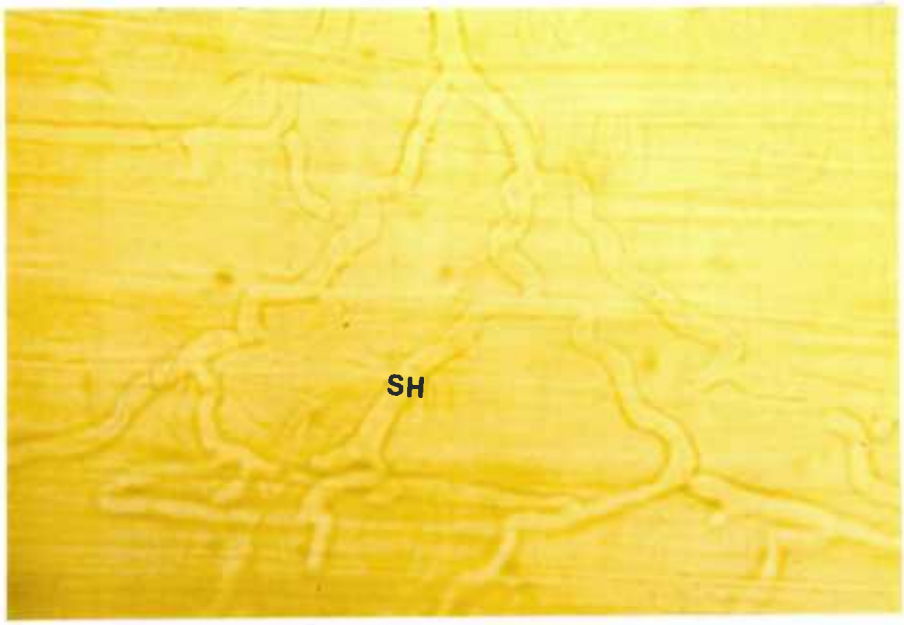


Plate 32. Intracellular penetration of cells of etiolated Canadian Wonder hypocotyls, irradiated with UV for 1 h, by secondary hyphae of C. coffeanum. 72 h after inoculation x 600

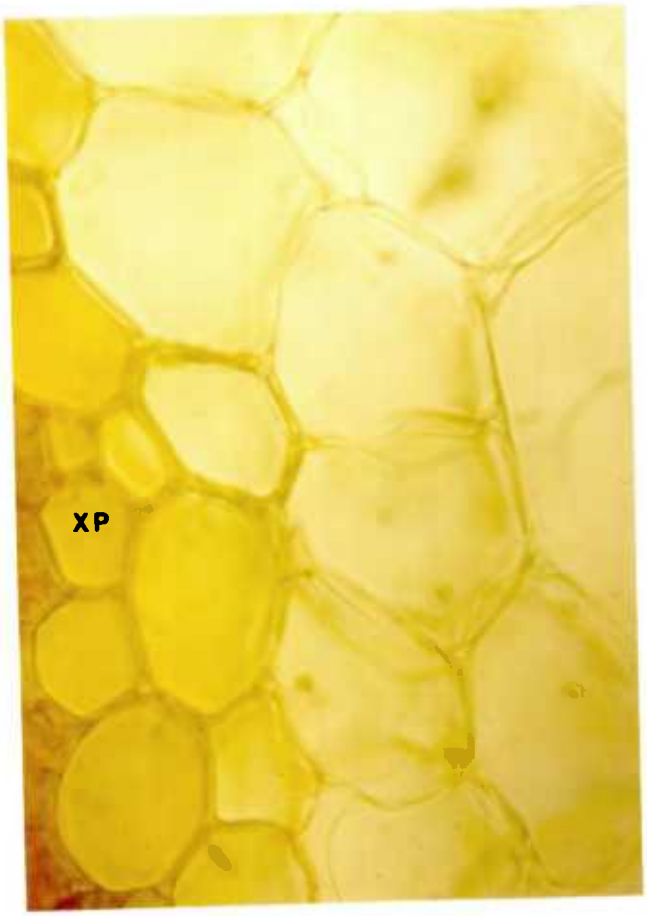


Plate 33. Browning of xylem parenchyma (XP) cells of Kievit hypocotyls, irradiated with UV for 1 h by race β, 96 h after inoculation x 1500

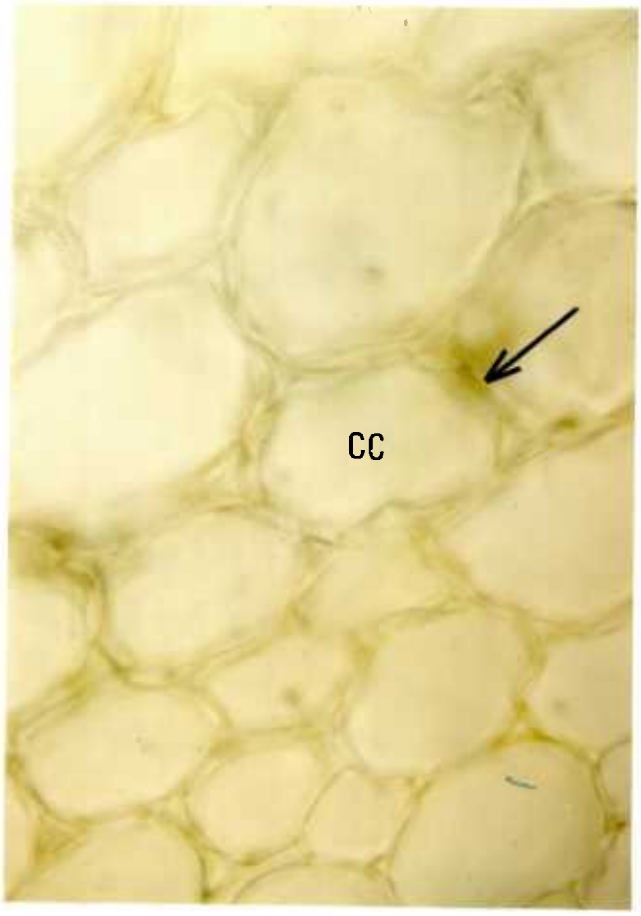


Plate 34. Browning of cell wall (arrowed) of cortical cells (CC) in etiolated Canadian Wonder and Kievit hypocotyls, irradiated with UV for 1 h, by C. coffeanum and C. lagenarium. 72 h after inoculation x 1500

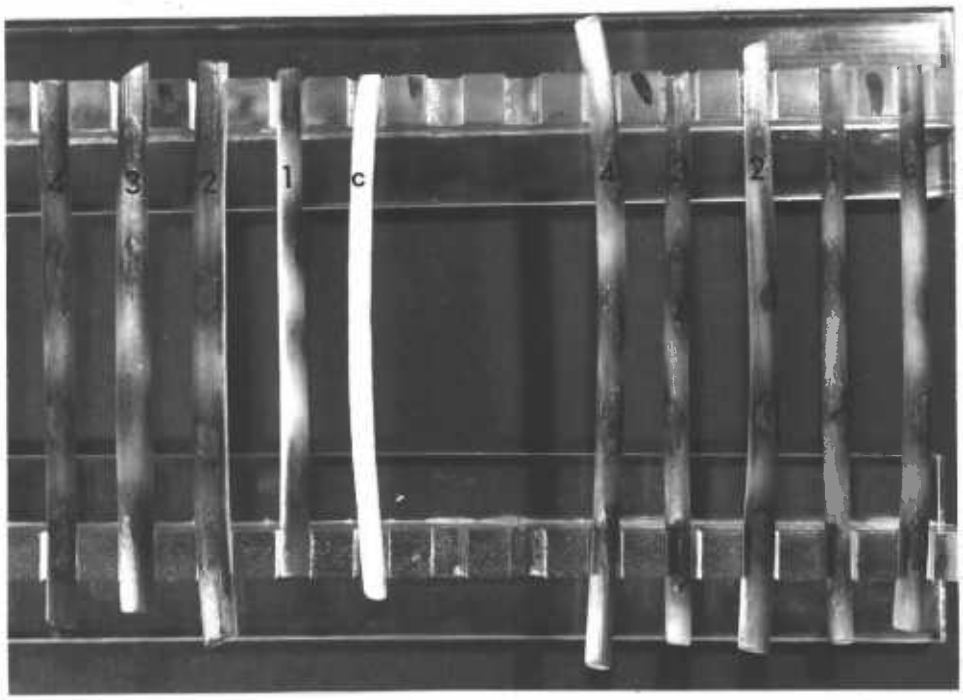


Plate 35. Etiolated susceptible Canadian Wonder (right) and resistant Kievit (left) hypocotyls irradiated with UV for 1, 2, 3 and 4 h and then inoculated with race β. Photographed 4 d after inoculation. C = control not irradiated but inoculated with race β.

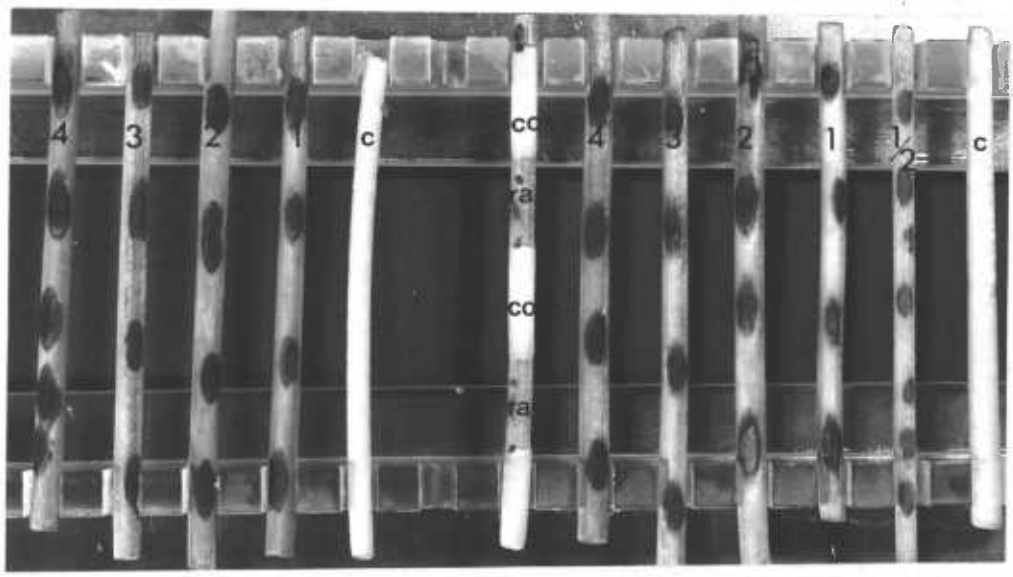


Plate 36. Etioliated susceptible Canadian Wonder (right) and resistant Kievit (left) hypocotyls irradiated with UV for 0.5, 1, 2, 3 and 4 h and then inoculated with C. lagenarium. Photographed 4 d after inoculation. C = control not irradiated hypocotyls inoculated with C. lagenarium. CO = covered portion hypocotyl and ra = irradiated portion of hypocotyl inoculated with C. lagenarium. Note lesion formed up to the border of irradiation.

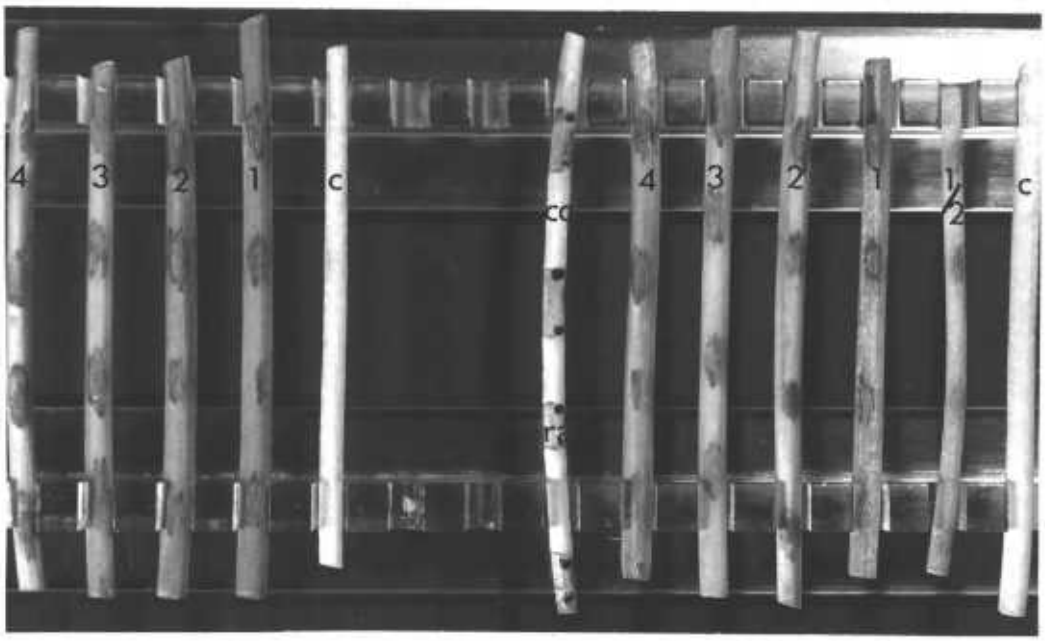


Plate 37. Etioliated susceptible Canadian Wonder (right) and Kievit (left) hypocotyls irradiated with UV for 0.5, 1, 2, 3 and 4 h and then inoculated with C. coffeanum. Photographed 4 d after inoculation. Controls (C) not irradiated were inoculated with C. coffeanum. For CO and ra see footnote under Plate 35.

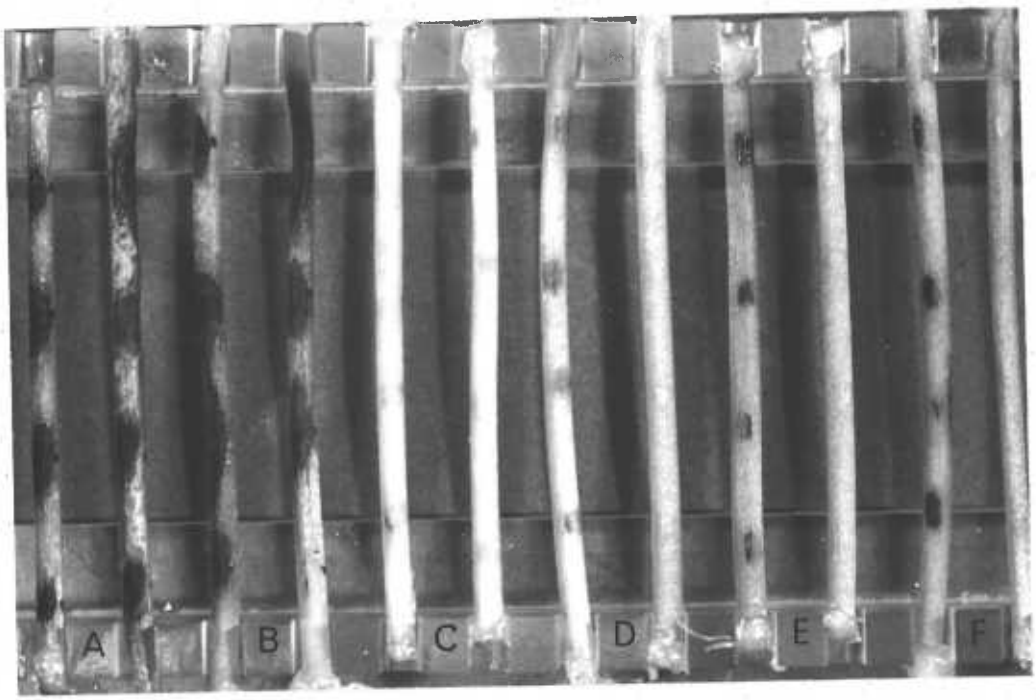


Plate 38. Etiolated hypocotyls of susceptible Canadian Wonder cultivar irradiated with UV for 0 - 60 min and inoculated at 0, 24 and 72 h later with race β. Photographed 12 d after inoculation

A = control L = left R = right

B = irradiated for 15 - 60 min and inoculated at 0 time

C = irradiated for 15 min and inoculated 24 h (L) and 72 h (R) later

D = irradiated for 30 min and inoculated 24 h (L) and 72 h (R) later

E = irradiated for 45 min and inoculated 24 h (L) and 72 h (R) later

F = irradiated for 60 min and inoculated 24 h (L) and 72 h (R) later

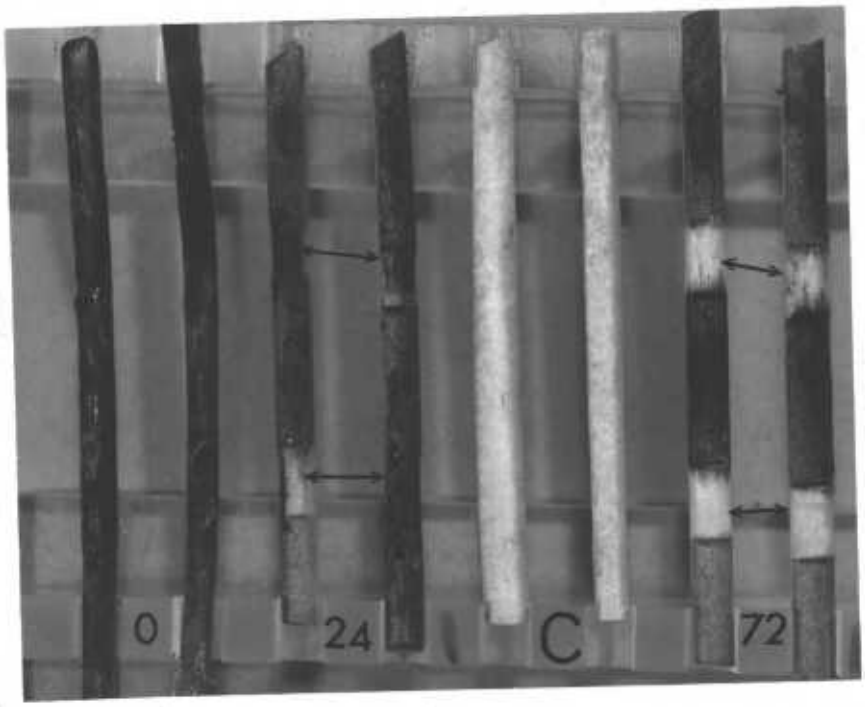


Plate 39. Etiolated hypocotyls of susceptible Canadian Wonder cultivar irradiated with UV for 1 h except arrowed areas. Hypocotyls were later inoculated with race β 0, 24 and 72 h later.

Lesions developed in untreated (arrowed) hypocotyls inoculated 24 h later but no lesions developed in untreated (arrowed) hypocotyls inoculated 72 h later.

C = UV irradiated hypocotyls of Canadian Wonder but not inoculated

Hypocotyls irradiated with UV for 1 h and inoculated at 0 time with non-pathogenic Colletotrichum spp. developed lesions at each site but hypocotyls inoculated 24 h later developed flecks only which were atypical of resistant reaction.

In hypocotyls irradiated with UV for 45 and 60 min and 24 h later inoculated with race β, the numbers of expanding lesion were decreased by 58% and 33% respectively, whereas in hypocotyls irradiated for 15 and 30 min all lesions were confined to flecks.

In hypocotyls irradiated with UV for 15 - 60 min and 72 h later inoculated with race β no expanding lesion developed (Plate 38).

Summary

Notable points from Section 6.1 are as follows :

i) C. lagenarium and C. coffeanum, non-pathogenic to French bean, caused lesions in etiolated hypocotyls of beans irradiated with UV for 1 h and then inoculated.

ii) In hypocotyls irradiated with UV for 1 h and 24 h later inoculated with C. lagenarium or C. coffeanum, no lesions developed.

iii) Etiolated hypocotyls of Kievit cultivar, resistant to race β, irradiated with UV for 1 h and inoculated with race β at 0 time, produced expanding lesions but did not produce any symptoms when inoculated 24 h after irradiation.

iv) In etiolated hypocotyls of Canadian Wonder irradiated with UV for 15 - 60 min and inoculated with race β 24 and 72 h later, expanding lesions were either suppressed or greatly decreased in number depending on the duration of exposure.

These experiments were repeated with similar results.

6.2 Cucumber

Cucumber cotyledons and hypocotyls from R and S cultivars were prepared as described earlier. Cotyledons and hypocotyls were irradiated with UV for 1 h and then inoculated with pathogenic and non-pathogenic Colletotrichum spp.

In hypocotyls of R and S cucumber hypocotyls irradiated for 1 h and inoculated with C. coffeanum at 0 time, penetration occurred within 40 - 48 h after inoculation, whereas race β failed to penetrate. But penetration by C. coffeanum was limited to 2 - 3 cells.

Cotyledons irradiated with UV for 1 h and inoculated with Colletotrichum spp. turned yellowish in colour within 24 h and it was not possible to differentiate the effect of UV from the chlorosis caused by C. lagenarium so these experiments were not continued.

F. Biochemical aspects of disease resistance

One form of disease resistance in plants is associated with the rapid death of cells in response to infection which is described as hypersensitivity. In some diseases, tissues killed in response to infection contain sufficient of substances called phytoalexins, toxic to the pathogen to prevent its growth. Attempts were made to investigate the role of such substances in resistance of bean and cucumber hypocotyls and cotyledons.

1.1 Cotyledons

Seeds of Canadian Wonder susceptible to C. lindemuthianum (race β) were germinated as described in Materials and Methods. Healthy germinated seeds were selected and testas and embryos were removed. A portion (c. 0.5 - 0.7 mm) of the epidermis was removed with a razor blade and cotyledons were placed in a box lined with moist tissue paper. Cotyledons were sprayed with suspensions (5×10^5/ml) of spores of race β,

C. lagenarium and C. coffeanum. Controls were sprayed with sterile gdw. Boxes were incubated at appropriate temperatures. Brown areas which developed were removed with a razor blade and were comminuted in ethanol in a Sorval omnimixer. The final extract was made ready for preparative TLC as described earlier. The TLC plates were run first in chloroform : methanol (25 : 1) and later purified by running in a solvent of benzene : ethylacetate : methane (25 : 8 : 4).

The chromatogram (TLC) were assayed either for fungitoxic compounds with a heavy suspension of spores of Cladosporium cucumerinum or relevant zones were removed and extracted for spectrophotometry as described earlier.

Cotyledons sprayed with suspensions of spores of non-pathogenic Colletotrichum spp. became dark brown within 24 h whereas cotyledons sprayed with race β became light brown in colour.

Cladosporium bioassay of the plates showed 6 inhibition zones. The compounds were identified from their R_f values and from their colour under UV (short and long waves). The purity of the compounds was assessed from their absorbance peak at a certain wave length. Compound Kievitone had a peak absorbance at 293 nm and phaseollin at 279 nm (Bailey, 1973) and (Bailey and Deverall, 1971) respectively.

Phaseollin appeared at the end of the TLC plates uncontaminated by other compounds under chloroform : methanol solvent system (25 : 1) but kievitone was not clearly separated in the above solvent. It was eluated and re-run under Benzene : ethyleacetate : methanol solvent (25 : 8 : 4). Kievitone and phaseollin were measured from the extinction coefficients of pure compounds.

Results as means of 4 replicates/treatment are given in Table 73.

Table 73. Production of antifungal compounds by bean cotyledons sprayed with pathogenic and non-pathogenic Colletotrichum spp.

Treatment	Incubation	Antifungal compounds ** (µg/g of fresh necrotic tissue)	
		Kievitone	Phaseollin
C. lagenarium	24	131.4	17.3
	48	73.9	31.0
C. coffeanum	24	102.2	17.3
	48	73.0	56.6
C. lindemuthianum	24	64.6	10.7
(race β)	48	48.2	24.1
Control		0	0

* Cotyledons sprayed with suspension of spores of Colletotrichum spp.; necrotic brown material collected and extracted

** Antifungal compounds were determined from extinction coefficient of pure compounds (Kievitone ; 55.6 µg/ml and Phaseollin = 32.9 µg/ml)

Loading of chromatogram extract from 1 g fresh necrotic tissue per cm base line

In cotyledons inoculated with C. lagenarium and C. coffeanum, the production of kievitone after 24 h was increased by 103% and 58% respectively and after 48 h, the production of kievitone was increased by 53% and 51% respectively compared to the cotyledons inoculated with race β. Amounts of kievitone decreased with time. After 48 h, amounts of kievitone in cotyledons inoculated with C. lagenarium, C. coffeanum and race β was decreased by 44, 39 and 25% compared with amounts present after 24 h.

In cotyledons inoculated with non-pathogenic Colletotrichum spp. amounts of phaseollin after 24 h were greater by 62% compared with amounts for race β. In cotyledons inoculated with C. lagenarium, C. coffeanum and race β, amounts of phaseollin after 48 h were greater by 29, 135 and 25% compared with amounts after 24 h.

Summary

i) Cotyledons inoculated with non-pathogenic Colletotrichum spp. produced within 24 h considerably more kievitone and phaseollin than did cotyledons inoculated with pathogenic, race β.

ii) Amounts of kievitone decreased with time, with greater decrease in cotyledons inoculated with non-pathogenic Colletotrichum spp.

iii) Amounts of phaseollin increased with time with greater increases in cotyledons inoculated with non-pathogenic Colletotrichum spp.

1.2 Hypocotyls

R and S cultivars of bean hypocotyls irradiated with UV for 1 h and inoculated with pathogenic and non-pathogenic Colletotrichum spp., produced lesions but hypocotyls remained resistant to Colletotrichum spp. when inoculated 24 or 72 h after irradiation with UV (Section 6.1). This change in resistance was investigated as follows.

Hypocotyls of R and S cultivars were prepared and irradiated with UV for 1 h as described earlier. Some of the hypocotyls were inoculated immedia with suspension of spores of Colletotrichum spp. and the rest were tely incubated in light but were not inoculated. Samples for extraction were taken from inoculated and uninoculated hypocotyls over a period of time. The extracts were prepared for TLC and antifungal compounds were assayed as described earlier.

Results as means of 4 replicates/treatment are given in Table 74.

Table 74. Effect of UV irradiation on production of antifungal compounds in bean hypocotyls inoculated with Colletotrichum spp.

Treatment	Incubation (h)*	S[a]		R[b]	
		Antifungal compounds (µg/g)			
		K[c]	P[d]	K	P
C. lagenarium	48	40.8	18.8	64.2	63.3
	72	63.0	31.5	47.3	123.4
C. coffeanum	48	34.6	32.8	73.4	67
	72	48.3	74.4	29.3	140.1
C. lindemuthianum (race β)	48	23.2	10.5	36.5	45.2
	72	38.9	31.3	81.0	227.1
None	48	6.7	31.1	10	40.5
	72	8.3	53.8	12	53.3

a - Susceptible cultivar of bean, Canadian Wonder

b - Resistant cultivar of bean, Kievit

c - Antifungal compound , Kievitone

d - Antifungal compound, phaseollin

* - Hypocotyls irradiated with UV for 1 h, incubated and later extracted for TLC

In S hypocotyls irradiated with UV for 1 h and inoculated with C. lagenarium and C. coffeanum at 0 h and extracted 48 h later, amounts of kievitone were increased by 76% and 49% respectively whereas in hypocotyls extracted after 72 h amounts of kievitone were increased by 62% and 24% respectively compared to hypocotyls inoculated with race β. Amounts of kievitone increased with time. In S hypocotyls inoculated with C. lagenarium, C. coffeanum and race β, 72 h later amounts of kievitone after 72 h as compared with 48 h were increased by 54, 40 and 68%.

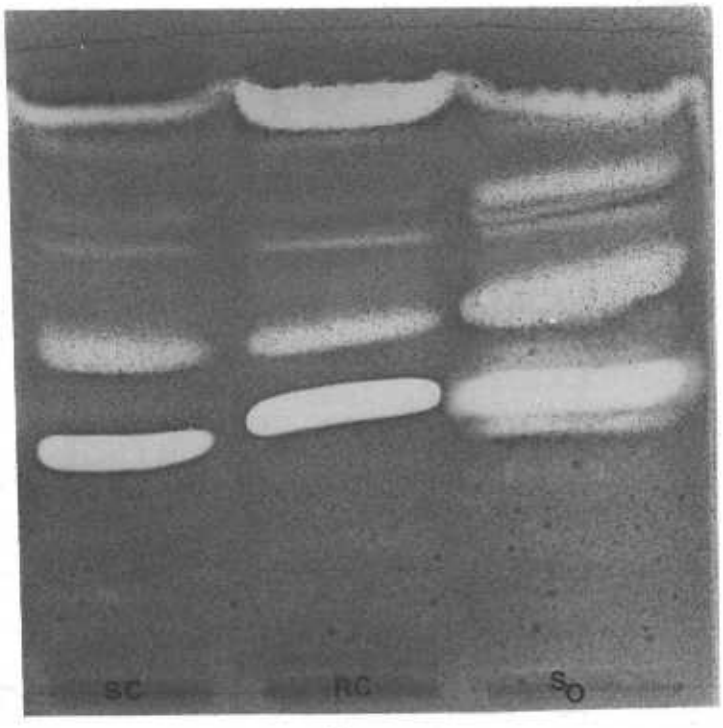
In hypocotyls irradiated with UV for 1 h and inoculated with C. lagenarium and C. coffeanum at 0 h and extracted 48 h later, amounts of kievitone were increased by 75% and 101% respectively whereas in hypocotyls extracted 72 h later, amounts of kievitone were decreased by 42% and 64% respectively compared to hypocotyls inoculated with race β. In R hypocotyls inoculated with race β, after 72 h the amount of kievitone was increased by 121% and in hypocotyls inoculated with C. lagenarium and C. coffeanum, amounts of kievitone were decreased by 26% and 60% respectively.

In S hypocotyls irradiated with UV for 1 h and inoculated with C. lagenarium and C. coffeanum at 0 h and extracted 48 h later, amounts of phaseollin were increased by 79% and 212% respectively whereas in hypocotyls inoculated with C. coffeanum and extracted 72 h later, the amount of phaseollin was increased by 138% compared to the hypocotyls inoculated with race β. In S hypocotyls inoculated with C. lagenarium, C. coffeanum and race β, after 72 h amounts of phaseollin were increased by 68, 127 and 198% compared to the hypocotyls extracted after 48 h.

In R hypocotyls irradiated with UV for 1 h and inoculated with C. lagenarium and C. coffeanum at 0 h and extracted 48 h later, amounts of phaseollin were increased by 40% and 48% whereas in hypocotyls extracted after 72 h, amounts of phaseollin were decreased by 46% and 38% respectively compared to the hypocotyls inoculated with race β. In R hypocotyls inoculated with C. lagenarium, C. coffeanum and race β, after 72 h, amounts of phaseollin were increased by 95, 109 and 402% compared to the hypocotyls

extracted after 48 h.

In hypocotyls irradiated with UV for 1 h and not inoculated with Colletotrichum spp., amounts of antifungal compounds increased with time and this was confirmed in chromatogram plates bioassayed with Cladosporium cucumerinum. The numbers of inhibition zones increased with time and areas of inhibition zones also increased with time. This increase in numbers of inhibition zone was next investigated in relation to activity of phenylalanine ammonia lyase (PAL), which is involved in the synthesis of secondary phenolic compounds.



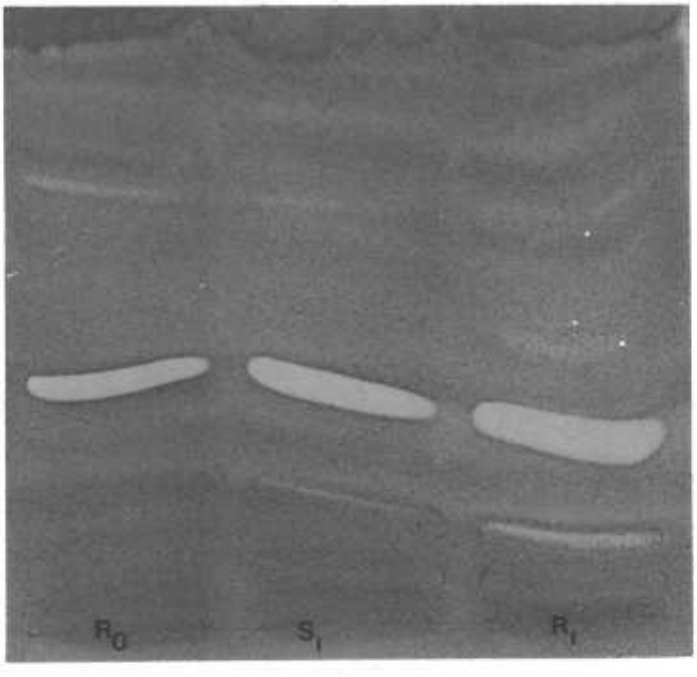
Loading of chromatograms 1 g fresh wt. of hypocotyls per cm; developed in ch : me (25 : 1)

Plate 40. Inhibition zones from extracts of etiolated bean hypocotyls

SC = Canadian Wonder hypocotyls not treated with UV

RC = Kievit hypocotyls not treated with UV

SO = Canadian Wonder hypocotyls irradiated with UV for 1 h and extracted at 0 h

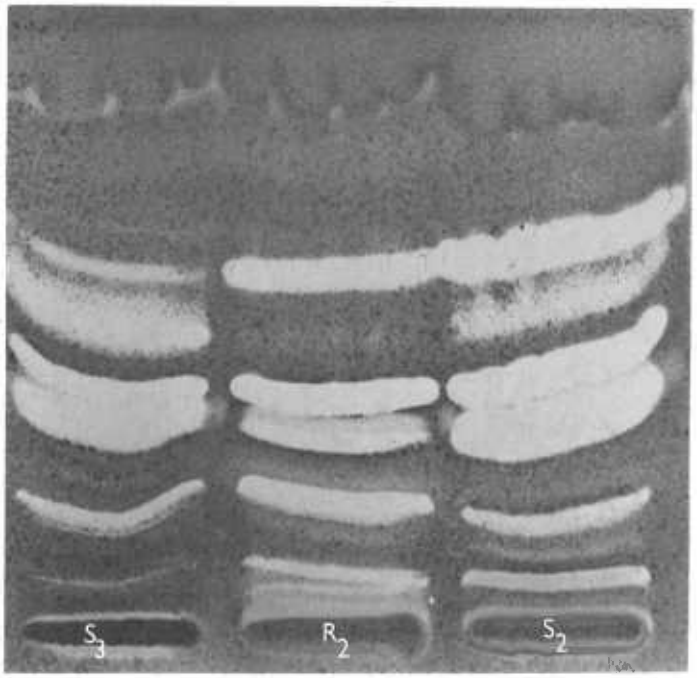


Loading of chromatograms 1 g fresh wt. of hypocotyl per cm; developed in ch : me (25 : 1)

Plate 41. Inhibition zones from extracts of etiolated Canadian Wonder (S) and Kievit (R) hypocotyls irradiated with UV for 1 h

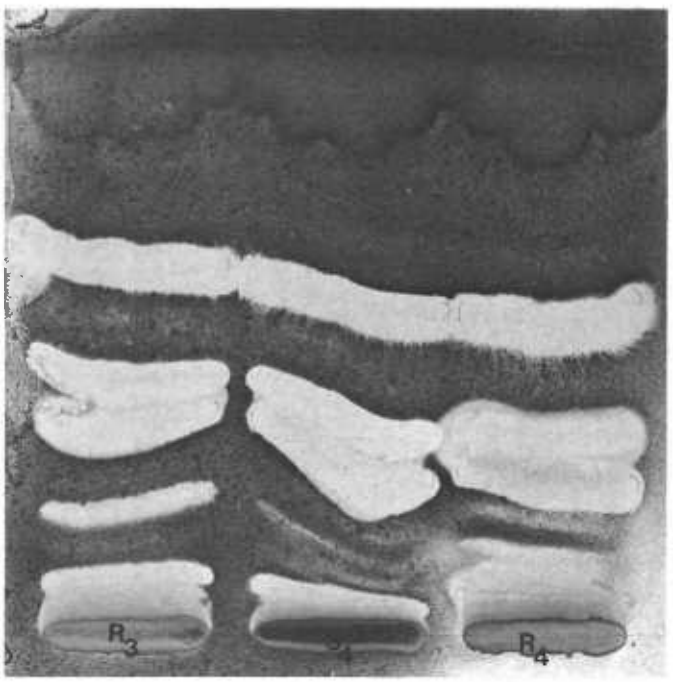
0 = hypocotyls extracted 0 h after irradiation

1 = hypocotyls extracted 24 h after irradiation



Loading of chromatograms
1 g fresh wt. of
hypocotyls per cm;
developed in ch : me
(25 : 1)

Plate 42. Inhibition zones from extracts of etiolated Canadian Wonder (S) and Kievit (R) hypocotyls irradiated with UV for 1 h
2 and 3 = hypocotyls extracted 2 and 3 d after irradiation



Loading of chromatograms
1 g fresh wt. of
hypocotyls per cm;
developed in ch : me
(25 : 1)

Plate 43. Inhibition zones from extracts of etiolated Canadian Wonder (S) and Kievit (R) hypocotyls irradiated with UV for 1 h
3 and 4 = hypocotyls extracted 3 and 4 d after irradiation with UV for 1 h respectively

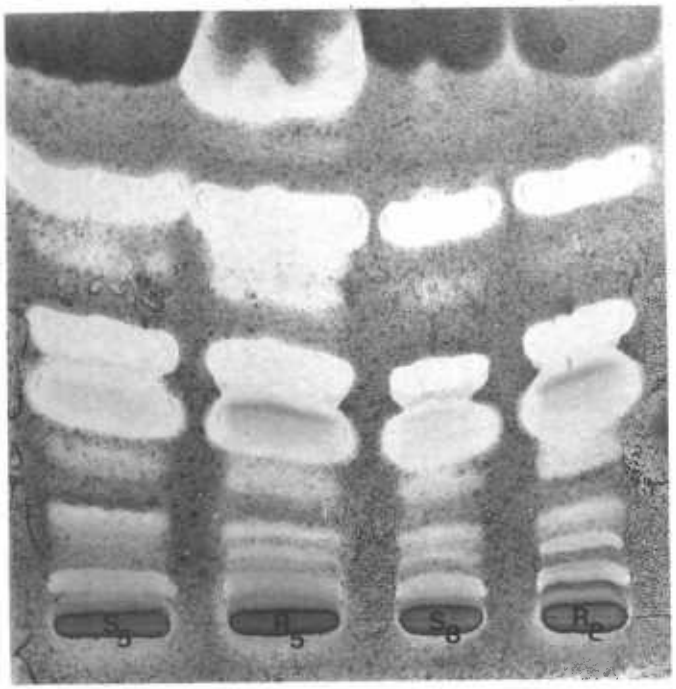


Plate 44. Inhibition zones from extracts of etiolated Canadian Wonder (S) and Kievit (R) hypocotyls irradiated with UV for 1 h

5 and 8 = hypocotyls extracted 5 and 8 d after irradiation with UV for 1 h respectively

Loading of chromatograms 1 g fresh wt. of hypocotyls per cm; developed in ch : me (25 : 1)

1.3 Phenylalanine ammonia lyase (PAL)

Changes in phenol content may depend upon alterations in the activity of this enzyme which is involved in their synthesis.

R and S bean hypocotyls were irradiated with UV for 1 h and were incubated in the dark at 24° - 25°C. They were not inoculated. One g of the hypocotyls were collected over a period of time, extracted and the enzyme activity was measured as described in Materials and Methods.

Results as means of 4 - 5 determinations/treatment are given in Fig. 7.

Results in Fig. 7 show that S and R hypocotyls irradiated with UV for 1 h and extracted at 0 h, PAL activity was increased by 166% and 273% respectively compared to hypocotyls not treated with UV. Activity of PAL in S and R treated hypocotyls was highest after 48 h and then decreased. But even after 96 h PAL activity in S and R hypocotyls was 50% and 25% respectively more than in hypocotyls extracted at 0 h.

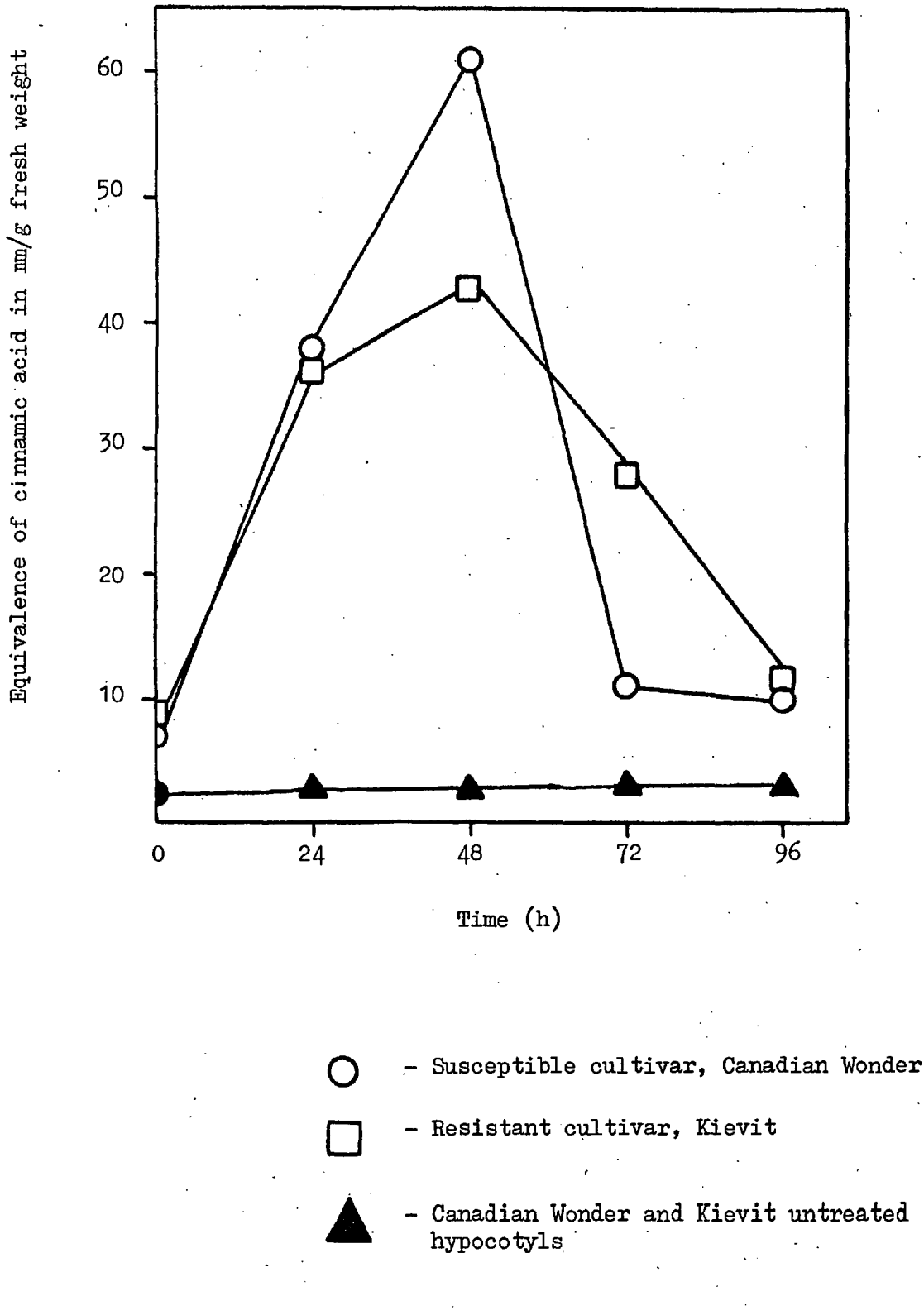
Summary

Notable points from sub-sections 1.2 and 1.3 are as follows.

i) In S hypocotyls treated with UV for 1 h and inoculated with non-pathogenic Colletotrichum spp. there was considerably more kievitone and phaseollin than in hypocotyls inoculated with race β.

ii) In R hypocotyls treated with UV and inoculated with non-pathogenic Colletotrichum spp. after 48 h there was more kievitone and phaseollin whereas after 72 h there was less kievitone and phaseollin than in hypocotyls inoculated with race β.

Figure 7. Effect of UV irradiation upon the amount of phenylalanine ammonia lyase in bean hypocotyls



iii) In S hypocotyls, treated with UV, amounts of kievitone and phaseollin increased with time whereas in R hypocotyls, inoculated with non-pathogenic Colletotrichum spp., amounts of kievitone decreased and amounts of phaseollin increased with time.

iv) There was a relationship between the increase in number of antifungal compounds and increase in PAL activity.

2. Cucumber

2.1 Cotyledons

R and S cucumber cotyledons were prepared and inoculated with suspension of spores of C. lagenarium (1×10^5 ml).

Disks were collected from the inoculated areas and comminuted in ethanol in a Sorval omnimixer. The final extract was made ready for TLC as described earlier.

The chromatograms (TLC) were run in chloroform : methanol solvent (25 : 1) and plates were bioassayed with Cladosporium cucumerinum.

From the bioassay, there was no indication of presence of antifungal compounds.

2.2 Hypocotyls

Hypocotyls from R and S cucumber plants were treated with UV for 1 h and then extracted for TLC as described earlier.

Cladosporium assay of the plates showed two inhibition zones from R and one zone from S UV treated hypocotyls. But from the areas of the inhibition zones, there was no significant difference between R and S hypocotyls.

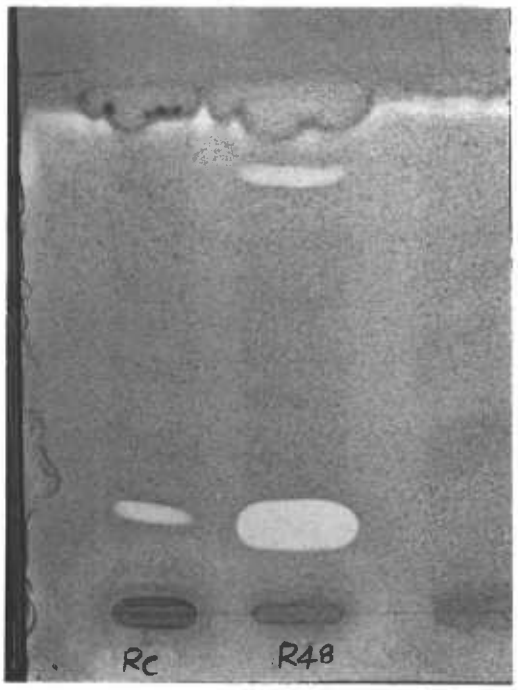


Plate 45. Inhibition zones from Sumter hypocotyls (R)

c = Sumter hypocotyls not treated with UV

48 = Sumter hypocotyls treated with UV for 1 h and 48 h later extracted

Loading of chromatograms 1 g fresh wt. of hypocotyls per cm;

developed in ch : me (25 : 1)

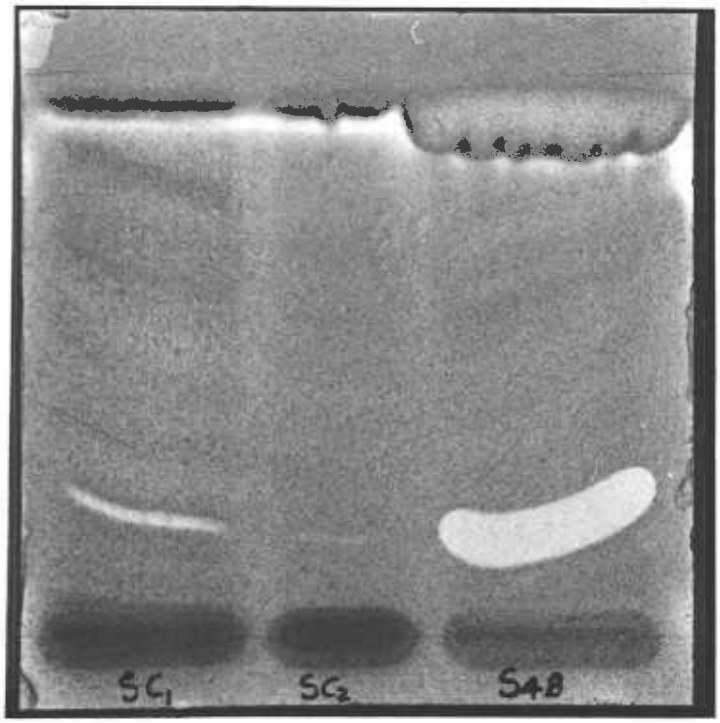


Plate 46. Inhibition zones from Baton Vert hypocotyls (S)

C_1 = Baton Vert hypocotyls not treated with UV

C_2 = Baton Vert hypocotyls treated with UV and extracted at 0 h

48 = Baton Vert hypocotyls treated with UV and extracted 48 h later

Loading of chromatograms 1 g fresh wt. of hypocotyls per cm; developed in ch : me (25 : 1)

2.3 Total Phenols

R and S cotyledons were prepared and inoculated with a suspension of spores of C. lagenarium (1×10^5/ml). Inoculated areas were removed with a cork borer, extracted and partitioned as described in Materials and Methods. Total phenols in the extract were determined by the Folin Ciacalteu reagent method using a Colorimeter (red filter).

Total phenols in R and S cotyledons 24 h after inoculation increased by 50% and 163% respectively. But 72 h after inoculation, total phenols in S cotyledons were lower than in controls and had levelled off 5 d after inoculation, whereas in R cotyledons, total phenols remained higher than in controls by 72% 4 d after inoculation compared to the controls.

This experiment was repeated with similar results.

2.4 Gel electrophoresis of proteins

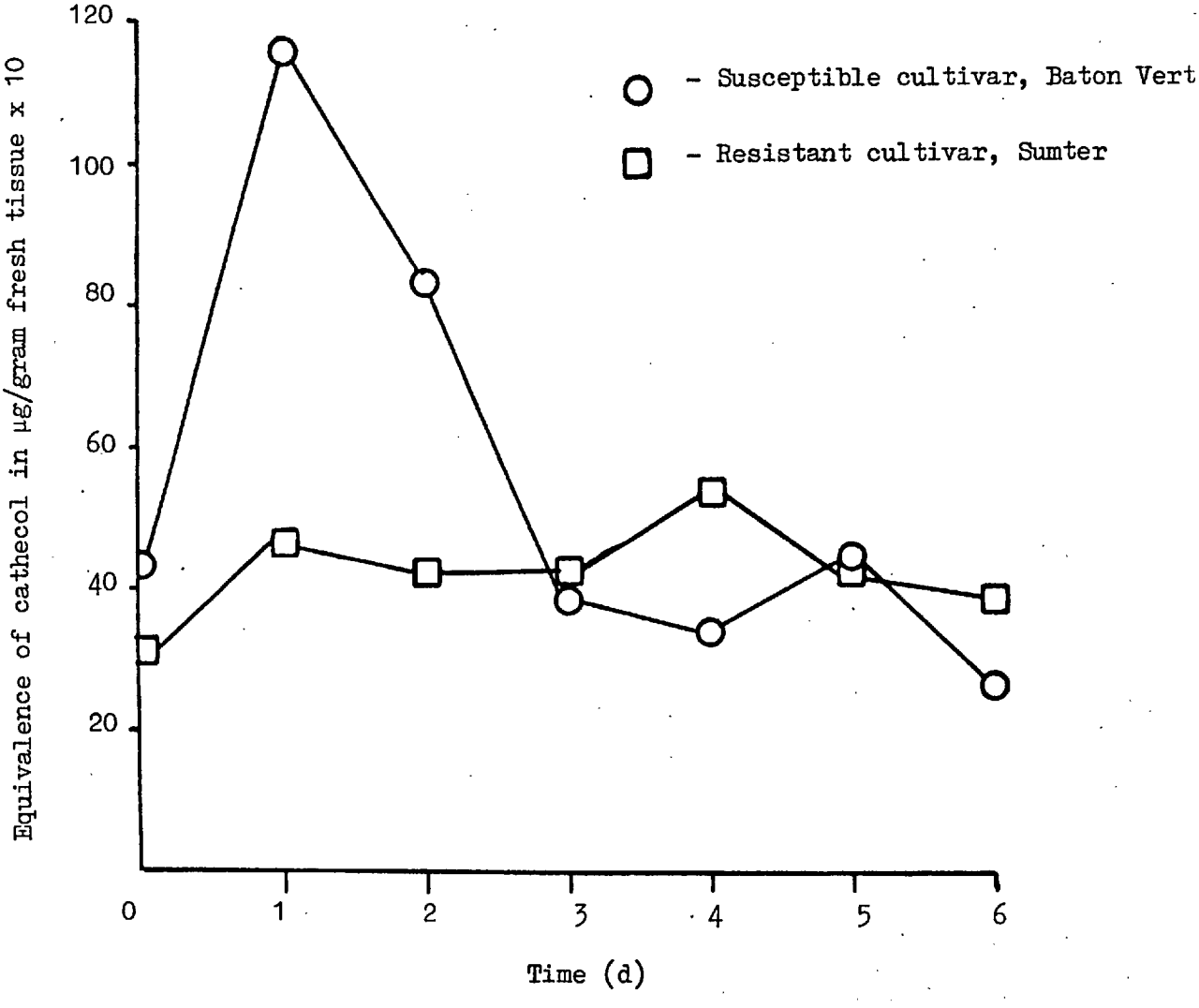
After first true leaves of S cucumber plants had been inoculated with a suspension of spores of C. lagenarium (1×10^8/ml), the second leaves were resistant to C. lagenarium (1×10^5 spores/ml) inoculated a few days later. Attempts were made to detect production of new proteins in the second leaves by gel electrophoresis.

Disks from lesions, from areas between lesions, from challenged (second) leaves, and from controls were extracted in buffer and applied to slab and cylindrical gels prepared as described in Materials and Methods.

The cylindrical gels were then stained with Coumasir blue for 24 h and destained with 10% acetic acid. The gels were scanned in a spectrophotometer at 280 nm.

The newly formed protein from the lesion extract was found at 70 units and had an R_f value of 0.74 - 0.75. Extracts from challenged leaves, areas between the lesions and controls did not show this new protein. But in the challenged leaves, there was a change in the pattern of protein metabolism

Figure 8. Total phenols in cucumber cotyledons inoculated with C. lagenarium

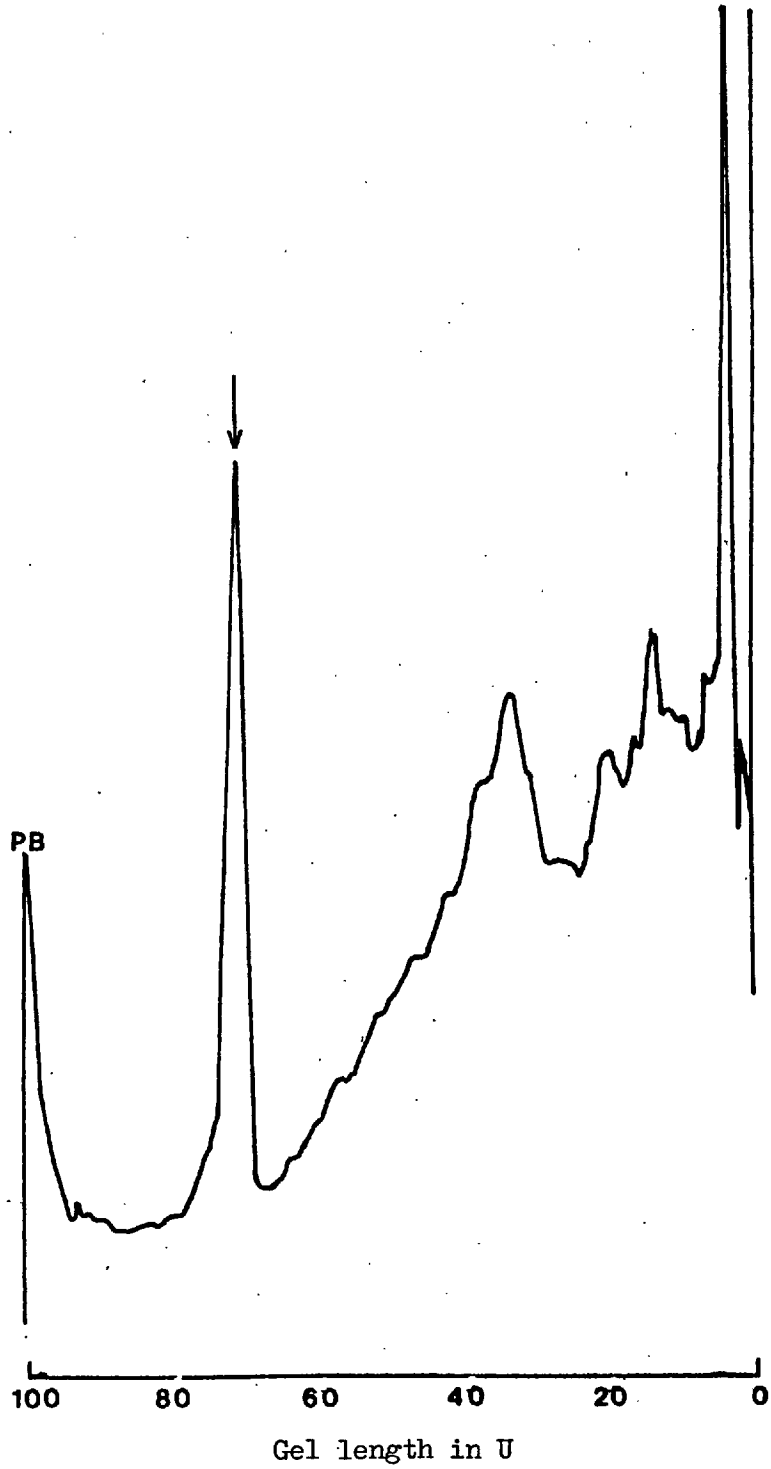


Note - the level of total phenols in the controls remained the same

compared to the controls (Figs. 10 and 11).

Plate 47. Gel electrophoresis from extracts of Baton Vert lesions (LE), uninoculated (CO) and challenged (second) leaves (CH). New protein (arrowed) from lesion extracts and none in CO and CH.

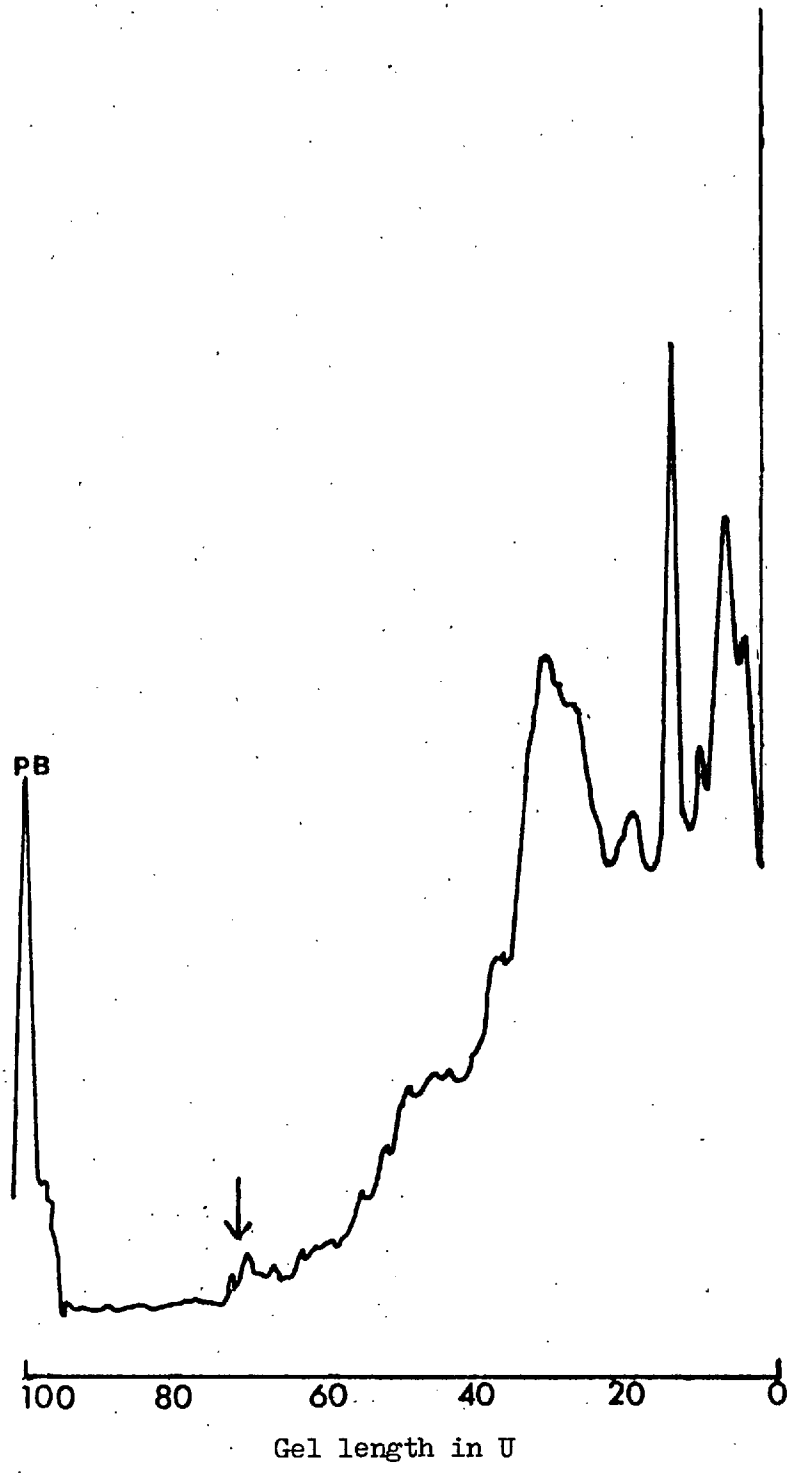
Figure 9. Gel electrophoresis of extracts of tissue from lesions of cucumber leaves inoculated with C. lagenarium



New protein (arrowed) formed in lesions of Baton Vert cucumber leaves

PB = Bromophenol blue used as a marker

Figure 10. Gel electrophoresis of extracts of tissue from cucumber leaves* not inoculated with C. lagenarium

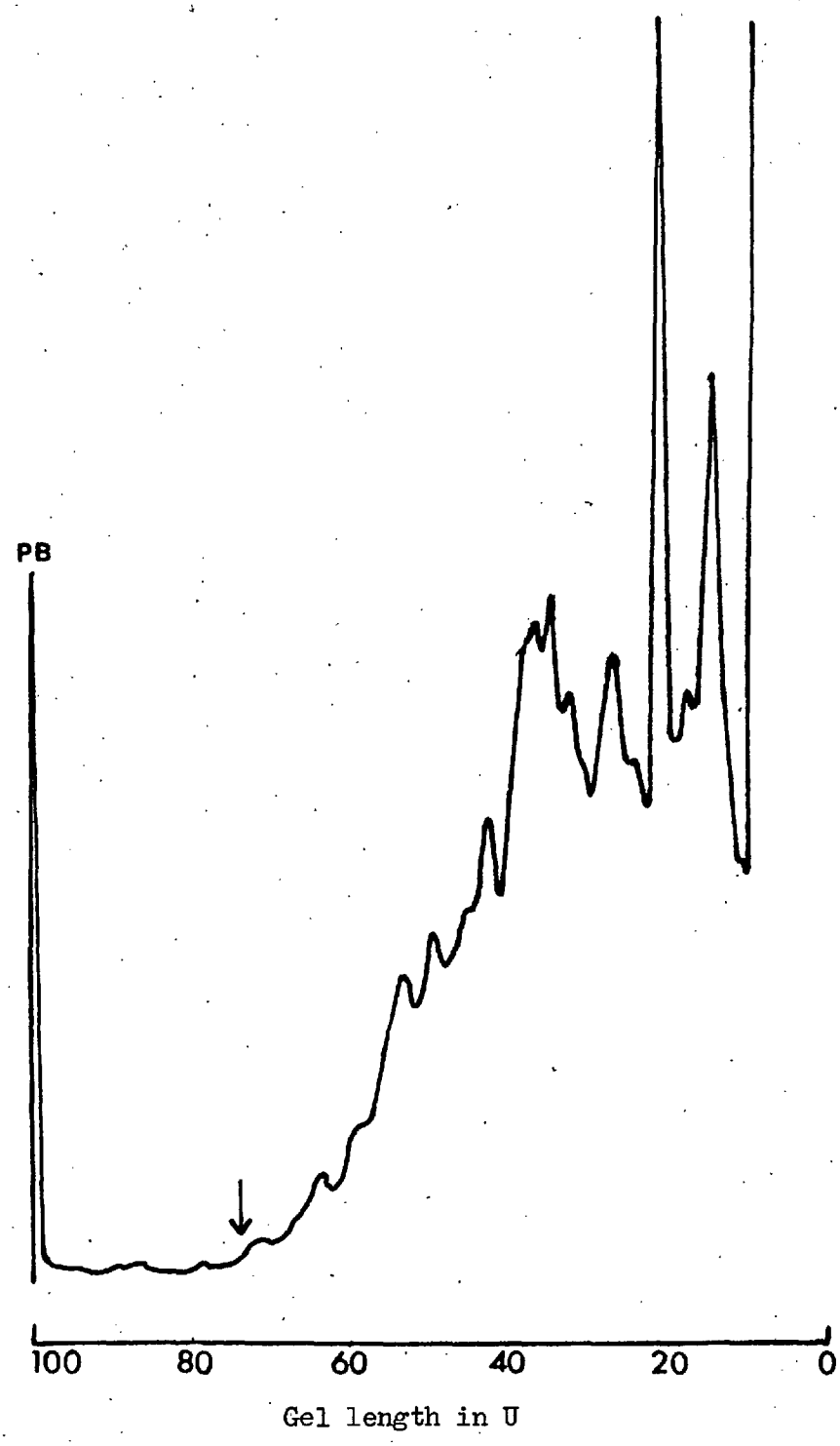


PB = Bromophenol blue used as a marker

→ = Location of the new protein in tissue from lesions

* = Susceptible cultivar, Baton Vert

Figure 11. Gel electrophoresis of extracts of tissue from second leaves of cucumber leaves* where first leaves were inoculated with C. lagenarium



PB = Bromophenol blue used as a marker

⟶ = Location of the new protein in tissue from lesions

* = Susceptible cultivar, Baton Vert

G. Pectic enzymes of Colletotrichum spp.

Known mechanisms of pathogenicity include the production by micro-organism of extracellular hydrolytic enzymes and toxins which affect host structure or metabolism, or both. Attempts were made to characterise and compare pectic enzymes produced by Colletotrichum spp. in vitro.

1. Polygalacturonase (PG) and pectin trans-eliminase (PTE) production

C. lindemuthianum (race δ), C. lagenarium and C. coffeanum were grown in a liquid medium containing 1% carbon source. The medium was prepared at two pH values, flasks were seeded with a suspension of spores of Colletotrichum spp. and incubated at 24° - 25°C.

In a second series of experiments, 0.5% glucose was added to the medium which already had either pectin (0.5%) or NaPP (0.5%) as a carbon source.

Protein solutions were prepared from culture filtrates, dialysed and tested for PG and PTE activity as described in Materials and Methods.

Results as means of 5 to 6 determinations are given in Tables 75 and 76.

The synthesis of PG in the above Colletotrichum spp. was very low and was induced by pectic substrates. The conducive substrate for PG production was pectin for C. lindemuthianum and C. coffeanum and NaPP for C. lagenarium. PTE production was only observed in C. lagenarium and not in the others. The addition of glucose to the medium considerably improved PG production by C. lindemuthianum and PTE by C. lagenarium.

pH of the medium played a great role in determining the efficiency of enzyme production.

As with time, enzyme production of the Colletotrichum spp. increased.

Table 75. Production of PG and PTE by Colletotrichum spp. grown for 10 d in a liquid medium

Carbon source (1%)	Colletotrichum spp.	Enzyme activity (RVU/ml)			
		PG		PTE	
		pH of medium			
		3.5	6	3.5	6
Glucose	C. lagenarium	0	0	0	0
	C. coffeanum	0	0	0	0
	C. lindemuthianum (race β)	0	0	0	0
Pectin	C. lagenarium	0	0	1	1.5
	C. coffeanum	8.3	5	0	0
	C. lindemuthianum (race β)	8.9	16.7	0	0
NaPP	C. lagenarium	3.6	0	3	2
	C. coffeanum	1.7	0	0	0
	C. lindemuthianum (race β)	0	0	0	0
Pectin + glucose	C. lagenarium	1.8	0	0	0
	C. coffeanum	3.5	5	0	0
	C. lindemuthianum (race β)	37	40	0	0
NaPP + glucose	C. lagenarium	10.4	4	22	31.2
	C. coffeanum	2.2	3	0	0
	C. lindemuthianum (race β)	6.6	9	0	4.3

Table 76. Production of PG and PTE by Colletotrichum spp. grown in a liquid medium

Colletotrichum* spp.	Time after inoculation (d)**	Enzyme activity (RVU/ml)	
		PG	PTE
C. lagenarium	4	4.3	0
	8	13.9	0.53
	12	27.8	1.75
C. coffeanum	4	4.5	0
	8	6.6	0
	12	10.0	0
C. lindemuthianum (race δ)	4	13.2	0
	8	29.9	0
	12	76.0	0
C. lindemuthianum (race β)	4	17.9	0
	8	34.7	0
	12	43.1	0

* C. lagenarium was grown in liquid medium with NaPP (1%) as a carbon source; pH = 7 - 7.2

C. coffeanum and races δ and β of C. lindemuthianum were grown in liquid medium with pectin (1%) as a carbon source; pH = 5.0

** A suspension of spores of Colletotrichum spp. was used as inocula; culture filtrates assayed after times shown

1.1 Improving production of PG and PTE

Colletotrichum spp. grown in Czapex Broth liquid medium, production of PG and PTE was low and to improve the production, Colletotrichum spp. were grown in shaken cultures prepared as described in Materials and Methods. Pectin (1%) and NaPP (1%) were used as carbon sources and pH was adjusted to 5 and 7 respectively. After 10 d culture filtrates were assayed for their enzyme activity as described earlier. Results are given in Table 77.

Table 77. Production of PG and PTE by Colletotrichum spp. grown for 10 d in shaken liquid medium

Colletotrichum spp.	Carbon source			
	Pectin		NaPP	
	Enzyme activity (RVU/ml)			
	PG	PTE	PG	PTE
C. lagenarium	7.1	31.3	5.2	0
C. coffeanum	17.9	0	6.3	0
C. lindemuthianum (race β)	100	0	222	0
C. lindemuthianum (race δ)	200	1.9	83.3	0

As shown in Table 77, production of PG by C. lindemuthianum was greatly increased when grown in shaken cultures. In stationary and shaken cultures enzyme production by C. lagenarium and C. coffeanum remained very low.

Summary

Notable points from Tables 75 - 77 are as follows.

i) Enzyme production by Colletotrichum spp. was higher in a liquid media containing pectic substrates rather than glucose as a carbon source.

ii) Enzyme production by Colletotrichum spp. was affected by the pH of the medium.

iii) Enzyme activity was higher in shaken compared with stationary cultures.

2. Optimum pH and Thiobarbituric acid test (TBA)

The optimum pH of enzyme activity from culture filtrates of Colletotrichum spp., prepared from shaken cultures was determined by viscometry and the TBA test.

Results in Fig. 12 and 13 show that the optimum pH of PG activity was between 5 and 5.5 and the spectrum at pH 5 exhibited a maximum activity at 510 nm which was good evidence for PG activity. The peak obtained at 550 nm at pH 8 indicated lyase activity which was also present in the culture filtrates of C. lindemuthianum (race β).

Similar results were obtained from culture filtrates of C. lagenarium and C. coffeanum.

Figure 12. pH optimum of PG of C. lindemuthianum (race β) determined by viscometry

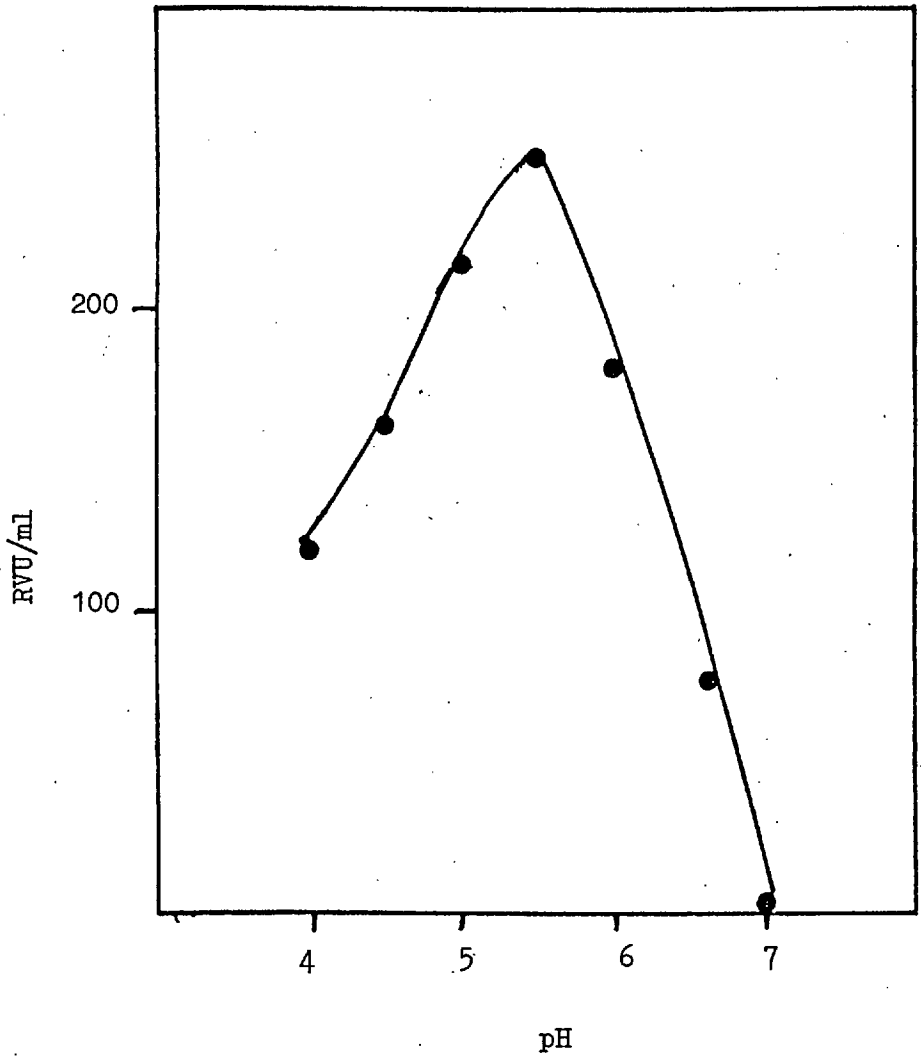
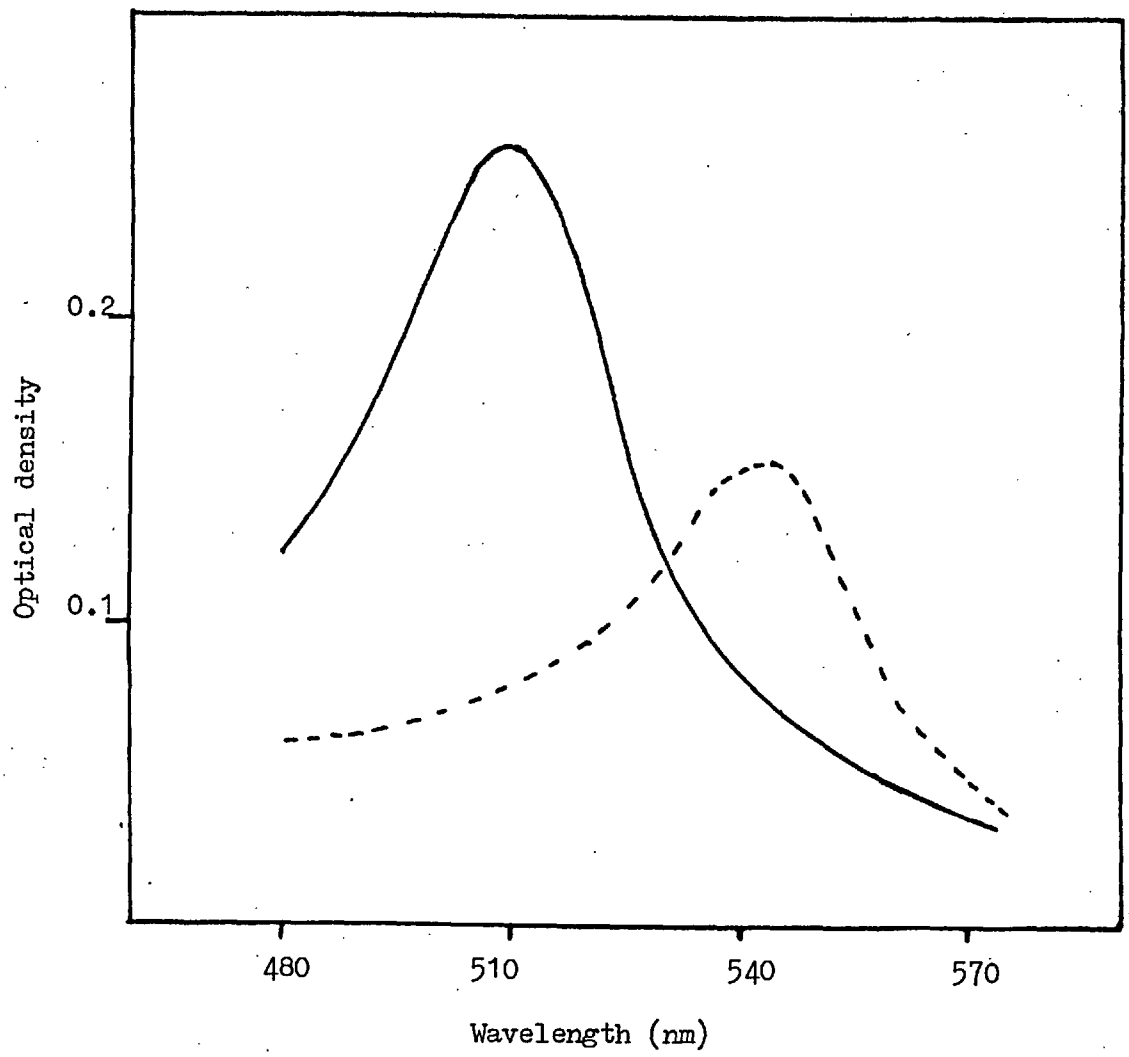


Figure 13. TBA Test of PG and PTE activity of C. lindemuthianum (race β) (——) pH 5 (----) pH 8



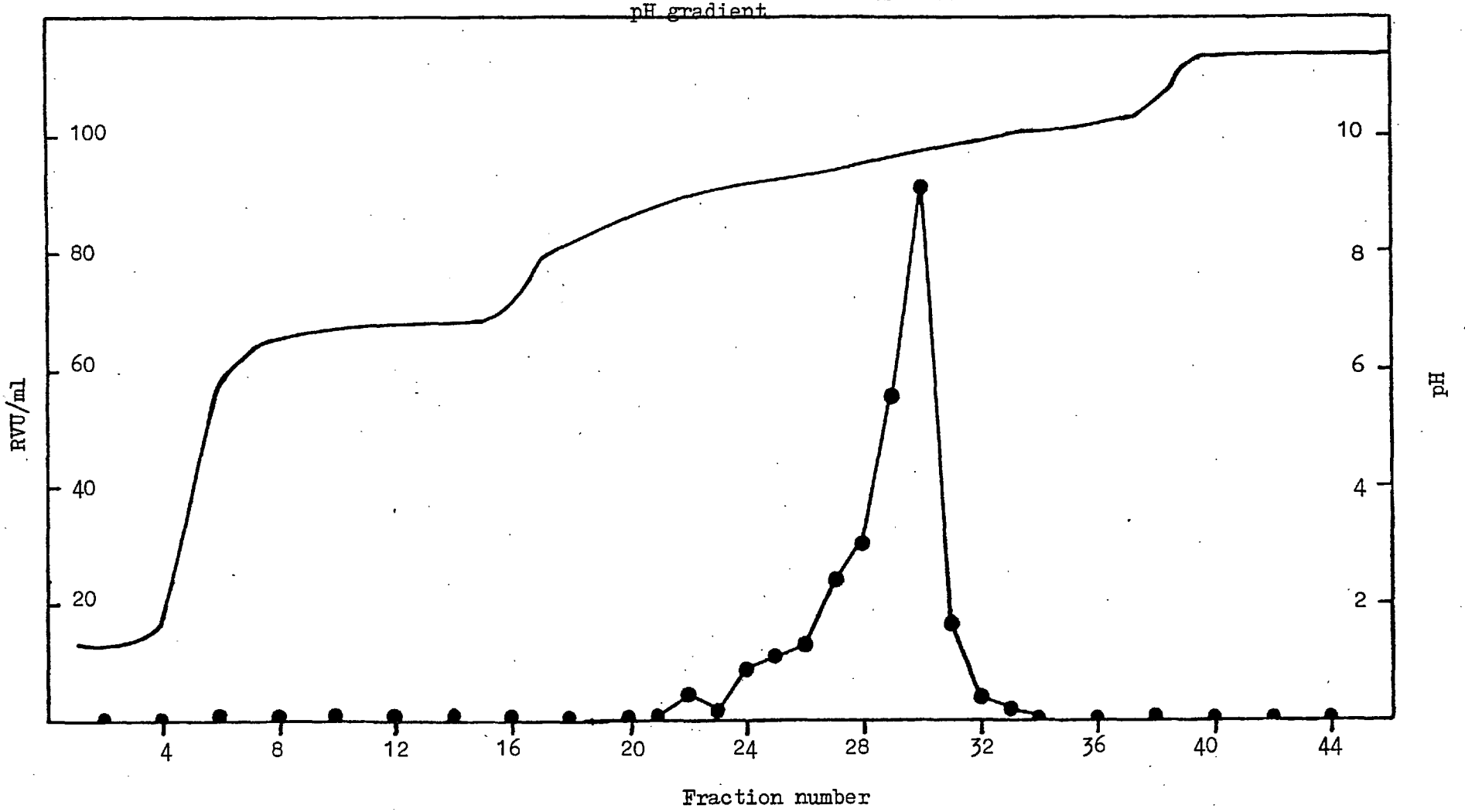
3. Purification and characterisation of PG from *C. lindemuthianum*

In order to eliminate the interfering lyase activity, culture filtrates of *C. lindemuthianum* (races δ and β) were subjected to isoelectrofocussing which was done as described in Materials and Methods.

PG was focussed as a single peak at pH 9.7 (Fig. 14). When filtrates of cultures of race β, race δ or of a combination of both were subjected to isoelectrofocussing, purified PG had an optimum pH of 5.5 (Fig. 13) and was free from lyase activity.

Where slices of germinated seeds or hypocotyls of susceptible and resistant cultivars of bean were incubated with PG from races β and δ of *C. lindemuthianum*, enzyme activity of the supernatant liquid decreased rapidly and at similar rates.

Figure 14. Isoelectric focussing of culture filtrates of C. lindemuthianum (race δ)(●-●) activity in u/ml (-) pH gradient



V. DISCUSSION

Host responses and germination of conidia of *Colletotrichum* spp.

Susceptible French bean hypocotyls inoculated with suspensions of spores of *C. lindemuthianum* were penetrated within 72 - 96 h after inoculation by primary hyphae and subsequent invasion was intracellular by secondary hyphae. Inoculation with incompatible races of *C. lindemuthianum* or with non-pathogenic *Colletotrichum* spp. led to hypersensitive reactions. Patterns of cellular responses to infection were as described by Skipp (1971). Patterns of symptom development in susceptible and resistant cucumber hypocotyls were the same as that in bean hypocotyls except that cucumber tissues reacted much more quickly.

Spores of *C. lindemuthianum* (races β and δ), *C. lagenarium* and *C. coffeanum* were compared for their ability to produce appressoria or germ tube in solutions of carbohydrates, most of which were components of plant cell wall polymers. Nevins *et al.* (1968) found that significant changes take place in the content of sugars of bean hypocotyl cell walls during their extensive growth. Glucose, galactose and arabinose declined to a basal level whereas xylose increased as with age. Arabinose and galactose decreased the production of appressoria by germinating conidia of races β and δ whereas glucose had little effect. The fact that the amount of xylose increases with age may be related to its effect in reducing production of appressoria by *C. lindemuthianum* and may explain, at least in part, why older bean hypocotyls become resistant to infection. The above carbohydrates decreased production of appressoria by conidia of *C. coffeanum* at low concentrations.

The formation and action of the appressorium are integral parts of infection by many parasitic fungi (Emmett and Parbery, 1975). In some species, appressoria may be obligatory for infection whereas in others it may only promote infection or be unnecessary. For *Colletotrichum* spp.

which cause anthracnose diseases, appressorium production is necessary.

Conidia of Colletotrichum spp. tested, produced appressoria equally and abundantly on host and non-host plants. Appressoria of C. lagenarium form equally well in susceptible and resistant cucumber cotyledons. Anderson and Walker (1952); White and Baker (1954) also found no difference in production of appressoria by C. lagenarium and Erysiphe graminis var. hordei on host and non-host and between resistant and susceptible cultivars. Therefore, the failure of C. lagenarium, C. lindemuthianum and C. coffeanum to attack non-host plants does not depend on their inability to produce appressoria.

Of the many factors involved in resistance, the extent to which the epidermis provides a physical barrier to invasion has long been debated. Epidermis from the lower surface of cucumber cotyledon was removed and then inoculated with suspensions of spores of pathogenic and non-pathogenic Colletotrichum spp. It was found that the non-pathogenic Colletotrichum spp. still failed to parasitise whereas removal of the epidermis enhanced parasitism by C. lagenarium (pathogenic). Purkayastha and Deverall (1965) reported that damage to or removal of cuticle from leaves of broad bean before inoculation did not enable Botrytis cinerea to cause spreading lesions.

One explanation for the specificity of pathogens for their hosts is the presence of compounds that stimulate their growth in one way or another (Strange and Smith, 1971; Strange et al., 1974). Water extracts of healthy parts of resistant and susceptible tissues of host and non-host were used for germination of conidia. Results show no consistent relation between germination and resistance and susceptibility of coffee and cucumber plants to their respective pathogens. Nutman and Roberts (1960) obtained similar results with extracts of coffee leaves. Extracts from a resistant cultivar were stimulatory whereas susceptible extracts were inhibitory. Similarly, Kirkham (1959) reported that fungitoxicity of

healthy leaf extracts of apple and pear was not related with scab resistance. However, Khan and Strange (1975) claimed a relationship between fungal stimulants from water extracts of healthy tissues of jute and resistance and susceptibility to C. corchori.

Colletotrichum spp. as inducers of resistance

Hypersensitive resistance to fungi in plants is characterised by the production of metabolites at the sites of infection which are inhibitory to a broad range of microorganisms; it is then suggested that induction of their synthesis to the inducing microorganism (Kuć, 1963; Cruickshank, 1965). In the current work bean hypocotyls were protected against C. lindemuthianum (race β) by prior inoculation with non-pathogenic C. coffeanum and C. lagenarium. In etiolated hypocotyls, for a wide range of concentrations of the suspension of spores of non-pathogenic Colletotrichum spp. and different intervals between the application of non-pathogen, disease development was decreased by 75 - 100%. Skipp (1971) also found that avirulent races of C. lindemuthianum caused localised protection to a virulent race applied later or up to one day earlier. Protection affected the growth of the intracellular hyphae but not spore germination or penetration. Spurr (1977) also protected tobacco leaves against Alternaria alternata by non-pathogenic isolates of Alternaria and protection was maximum when the protective conidia were applied to the leaves 2 - 3 d before inoculation. When bean hypocotyls were inoculated with mixed inocula of C. lindemuthianum (race β) and non-pathogenic Colletotrichum spp., disease development was greatly reduced. The greatest protection was obtained when C. lagenarium and C. coffeanum were at 5×10^5 spores/ml and race β at 1×10^6 spores/ml. Skipp (1971) found the reverse with virulent and avirulent races of C. lindemuthianum.

From histopathology work, it was found that cells of bean hypocotyls reacted hypersensitively within 24 - 48 h to non-pathogenic Colletotrichum

spp. As a result it seemed that the adjacent healthy cells are induced to produce antifungal compounds which inhibited the growth of the intracellular hyphae of C. lindemuthianum (race β). Helminthosporium carbonum and Alternaria spp., non-pathogenic to bean hypocotyls induced resistance to C. lindemuthianum when inoculated 24 h earlier (Rahe et al., 1969). There was a relation between the synthesis of phenolic substances and histological changes occurring in bean hypocotyls following inoculation with H. carbonum.

In cucumber cotyledons inoculated with non-pathogenic C. coffeanum and C. lindemuthianum (race β) and inoculated 24 h later with C. lagenarium, the lesion size was decreased by 30 - 45%, but when C. lagenarium was applied 72 h later, the decrease in lesion size was less. Although Deverall (1977) failed to characterize post-infectional antifungal substances in cucumber leaves, it seems that these substances are produced in cells reacting hypersensitively to the non-pathogenic Colletotrichum spp., in view of the effects on growth of C. lagenarium (pathogenic). The low level of protection after 72 h might be the result of degradation of antifungal substances at the site of inoculation.

C. lindemuthianum (race β) was more effective than C. coffeanum in protecting cucumber cotyledons against C. lagenarium.

Higgins and Millar (1969) reported that when alfalfa leaves were inoculated with a mixed inocula of Stemphylium botryosum and Helminthosporium carbonum, the infection drop contained low amounts of phytoalexin and that the lesions produced spread at a greater rate than did lesions produced by S. botryosum alone. This suggests that S. botryosum degraded the phytoalexin so that the normally non-pathogenic H. carbonum was also able to grow well. In the current work, when C. lindemuthianum (race β) was applied after 72 h to sites of cucumber cotyledons already inoculated with C. lagenarium and incubated at 17° - 19°C, lesion size was greater than when produced by C. lagenarium alone. But when inoculated cotyledons were incubated at 24° - 25°C, lesion size was not affected.

An effect of the incubating temperature may have been on production of appressoria by the germinating conidia of race β.

When a suspension of spores of C. lagenarium (1×10^6/ml) was mixed in equal volume with C. coffeanum (5×10^5/ml) or race β (2×10^5/ml) and used to inoculate cucumber cotyledons, lesion size was increased by 25% and 27% respectively compared with C. lagenarium alone. This increase in lesion size may be explained as proposed by Higgins and Millar (1969) (see above).

Remote cross-protection has been demonstrated in anthracnose diseases of bean and cucumbers and with bacterial diseases of pome fruit. Avirulent races of C. lindemuthianum caused sites 5 mm away on etiolated bean hypocotyls to become resistant to virulent races (Elliston et al., 1971). Skipp and Deverall (1973) failed to get similar results on green hypocotyls. Hecht and Bateman (1964) induced local and systemic resistance to TMV in tobacco by Thielaviopsis basicola; resistance to T. basicola was induced in opposite half leaves but not in the leaf above by localized TMV infection. In this work systemic protection was induced against C. lagenarium in the second leaves of cucumber by first inoculation of the first leaf with C. lagenarium. This finding was in accord with the report of Kuć et al. (1975). Hammerschmidt et al. (1976) protected cucumber against disease C. lagenarium by first inoculating with Cladosporium cucumerinum. Cucumber plants first inoculated with C. lindemuthianum were protected against C. cucumerinum. Systemic protection against C. lagenarium developed also in one cotyledon after the other cotyledon had been sprayed with C. lagenarium, C. coffeanum and C. lindemuthianum (race β). The greatest protection was obtained 96 h after spraying the other half of the cotyledon and non-pathogenic Colletotrichum spp. were more effective than C. lagenarium. But this system failed to induce systemic resistance against C. lagenarium in first leaves by prior inoculation of the second leaves with large inocula of C. lagenarium.

The induced change in the host plant which increase resistance could depend on diffusion of antimicrobial substances or others which affect cell metabolism, from sites of inoculation with the protectant organism. The induced protection in cucumber is unlikely to depend on phytoalexins because of the failure of Deverall (1977) to detect such compounds. But it is possible that other substances with appropriate solubility might diffuse through necrotic cells, where barriers to permeability and movement of molecules have been destroyed to other cells where they cause a change in sensitivity to attack by C. lagenarium. Skipp and Deverall (1973) came to similar conclusions for cross-protection in anthracnose of bean. In the current work, a new protein band was found in gel electrophoresis of lesion extracts at R_f = 0.75 in 10% acrylamide gel; it was not detected in extracts of the controls (healthy) or second leaves (first leaf inoculated with C. lagenarium). Extracts from conidia of C. lagenarium did not contain this protein. Coutts (personal communication) obtained a new protein from TNV inoculated cucumbers at the same R_f as the above; he could not detect this protein in TNV extracts. This protein could be synthesized as a result of host-pathogen interaction; it could be water soluble and translocated to the leaf above causing physiological changes in the second leaf upon which induced resistance depends.

Although this is the first report of a new protein following inoculation of cucumber leaves with fungi, several investigators have found new proteins in cucumbers after inoculating with virus. Tas and Peters (1977) detected a protein component in the soluble protein fraction of cucumber cotyledons infected with tomato spotted wilt virus; there was a close correspondence between the amount of this new protein and the severity of symptoms. Kassanis et al. (1974) also analysed the soluble protein fraction in Nicotinia tabacum cv. Xanthi-nc infected with TMV, CMV and potato virus X. The new protein bands which were found were also obtained after injection of polyacrylic acid into plants. Induced

resistance developed simultaneously with the appearance of the new proteins which appeared in inoculated and in non-inoculated leaves (Van Loon and Vari Kammen, 1970; Gianinazzi et al., 1970). Kassanis and White (1974) also showed that the appearance of the new proteins and induction of resistance in tobacco were inhibited by actinomycin D. They suggested that the proteins induce resistance as a plant "interferon".

Fungal material in induction of resistance

Various workers have reported that fungal metabolites produced in culture stimulate synthesis of phytoalexins and that fungi influence the metabolism of cells neighbouring those penetrated (Cruickshank and Perrin, 1963; Müller, 1956). In etiolated bean hypocotyls treated with diffusates, from conidia of Colletotrichum spp. incubated on cotyledons of resistant and susceptible cucumber cotyledons, and later inoculated with C. lindemuthianum (race β); disease symptoms were reduced greatly when application of diffusate preceded the challenge inoculation by 72 h. Berard et al. (1972) reported the same type of protection with incompatible interactions of bean hypocotyls with races of C. lindemuthianum.

But in susceptible cucumber cotyledons treated with diffusates from conidia of Colletotrichum spp. incubated on susceptible cotyledons, and later inoculated with C. lagenarium, there was little effect on lesion size; diffusates from resistant cotyledon reduced the lesion size by 15 - 28% depending on the combinations of sources of diffusates and time of treatments.

Similarly, diffusates from cultures of Colletotrichum spp. reduced disease in etiolated bean hypocotyls by 37 - 50% and in resistant cucumber cotyledons by 45 - 55%. The culture diffusates did not protect susceptible cucumber cotyledons against C. lagenarium.

When diffusates protected bean hypocotyls, phytoalexins accumulated following hypersensitive responses in cells below the drops. In cucumbers

it is difficult to explain how diffusates decrease lesion size in light of past failures to detect phytoalexins.

Klement and Goodman (1967) in their review of hypersensitive reactions (HR) to bacterial plant pathogens concluded that hypersensitivity is a general defence reaction and that HR operates only in incompatible host-parasite combinations and that development of HR in plants is associated with a loss of cell turgor which reflects a loss in membrane permeability. Susceptible and resistant cucumber cotyledons infiltrated with diffusates of cultures of non-pathogenic Colletotrichum spp., released more electrolytes than did cotyledons infiltrated with diffusates of cultures of the pathogenic, C. lagenarium. Cucumber cotyledons resistant to C. lagenarium, generally released more electrolytes than did susceptible cotyledons when infiltrated with diffusates of cultures of C. lagenarium and C. coffeanum.

Keen et al. (1972) reported that pathogens produce molecules which stimulate phytoalexin production in plant tissue and Skipp and Deverall (1973) showed that application of culture filtrates to bean tissues caused them to become resistant to otherwise virulent races of C. lindemuthianum. Ultrastructure studies of culture filtrate treated tissues by Mercer et al. (1974) showed changes similar to those in hypersensitive responses of an incompatible interaction with a race of C. lindemuthianum. In the current work in bean hypocotyls treated with elicitors prepared from mycelial cell walls of Colletotrichum spp. (Materials and Methods) disease was reduced by 100% with higher concentrations. Because phytoalexins accumulate in hypersensitive responses, this finding must be considered in light of the report by Anderson and Albersheim (1975) that elicitors can initiate the symptoms of incompatible interaction. Ayers et al. (1976) also reported accumulation of the phytoalexin, glyceollin, in soybean treated with elicitors from Phytophthora megasperma var. sojae. In the current work there was no significant difference in the ability of the elicitors of C. lagenarium, C. coffeanum and C. lindemuthianum (race β)

to induce resistance in etiolated bean hypocotyls, and in the content of total carbohydrate and protein in crude preparations. Theodorou (personal communication) also did not obtain significant differences in total protein and carbohydrate of elicitors of four races of C. lindemuthianum; results in Table 67 compare well with his.

Interesting results were obtained when seeds of cucumber were soaked with elicitors from Colletotrichum spp. and the cotyledons inoculated later with C. lagenarium. Lesion sizes were reduced by 50 - 58% at lower concentrations of the elicitors. Kumamato (1948) reported that in rice seedlings raised from seeds treated with the extract of cultures of Pyricularia oxyzae, susceptibility to blast disease was reduced. Protection of rice seedlings and mature plants against Helminthosporium oryzae was also obtained when seeds were first soaked in mycelial extracts or filtrate of cultures of the pathogen (Ganguly and Padmanabhan, 1962; Trivedi and Sinha, 1976). In addition to being stimulated to produce fungitoxic substance, embryonic tissue may be sensitized so that new tissues developing from the embryo may be more sensitive to the pathogen and produce fungitoxic substance in larger amounts than normal.

Plant growth regulators as inducers of resistance

In bean and cucumber plants treated with plant growth regulators (PGR), disease was either increased or decreased. In gibberellic acid (GA) treated bean plants on internode length was increased by 114%. Disease in hypocotyls was increased by amounts depending on concentration of GA and time. But when GA was applied in drops to etiolated hypocotyls, disease was greatly decreased.

When susceptible and resistant cucumber plants were treated with GA in different ways, lesion size was increased or decreased depending on the method of application and time of treatments. The most interesting effect was in susceptible cucumber cotyledon disks floating with their upper

surface on a solution of GA when lesion size was increased by 48 to 233%. Tobacco plants treated with GA showed more brown spots caused by Alternaria alternata than are the controls (Lucas and Ramm, 1963). Sinha and Wood (1964) also reported that GA increases susceptibility in Verticillium wilt in tomato.

The increase in susceptibility in GA treated plants may depend on its effect in increasing cell wall plasticity through wall softening effects of enzymes released from the cotyplasm into the cell wall (Phillips, 1971). Supporting this idea, Paleg (1960) reported that GA increases synthesis of α-amylase in barley. GA is so much involved with other growth regulators and with metabolic processes that it is difficult to attempt any explanation of its role in resistance.

In hypocotyls from bean plants treated with IAA, disease was either reduced or as in controls. In cucumber, its effects depended on mode of application.

Increase in susceptibility could depend on the changes in permeability reported by Power and Cocking (1970). Gayler and Glasziov (1969) suggested that regulation of invertase synthesis by IAA and GA would affect reducing sugars and thus susceptibility. Growth regulators may increase resistance by altering pectic polysaccharides in cell walls through increased bonding through calcium (Corden and Diamond, 1959); it was shown that the chemotherapeutic effect of naphthalene acetic acid (NAA) was related to calcium content (Corden and Edgington, 1960). Glasziou (1957) also reported that high concentration of IAA can reduce absorption of pectin methylesterase by the cell wall so that pectic polysaccharides become less esterified thus permitting carboxyl groups to be cross-linked through calcium cations. Induction of resistance in wheat to stem rust by IAA was reported by Samborski and Shaw (1957). They suggested that IAA can control direction and rates of transport of critical defence materials.

When kinetin was applied to bean plants, disease expression was decreased or increased depending on mode of application. Cucumber plants

treated with kinetin remained resistant and interestingly there was no chlorotic zone around the area of inoculation which may be attributed to the prevention of loss of chlorophyll (Jacoby and Dagan, 1970). Bushnell and Allen (1962) described retention of chlorophyll around fungal colonies in detached or senescent leaves of barley infected with Erysiphe graminis. The effects of kinetin have been described by Lovrekovich and Farkas (1963) for tobacco and Pseudomonas tabaci. Kinetin reversed protein degradation and chlorosis; in treated tobacco leaves, the levels of protein were doubled. Dekker (1963) also concluded that kinetin prevents protein breakdown and thus resistance. He found that powdery mildew would not grow on cucumber leaf disks floated in kinetin solution, although germination of conidia and penetration were normal. Other effects are suggested by Kenede (1971). Müller and Brautigam (1972) state that it promotes the movement of many metabolites to treated sites and Elsadi and Kuipper (1972) showed that kinetin applied to the roots of bean plants stimulated the transport of ^{22}Na from the tip of a bean leaf to stems and roots.

The growth inhibitors, maleic hydrazide and Cycocel increased or decreased disease development in bean and cucumber plants depending on concentration, time of treatment and mode of applications. Maleic hydrazide is said to be anti-auxin and Cycocel anti-gibberellins. Their antagonistic effects have been demonstrated in host-parasite relationship. In wheat plants treated with IAA, resistance to stem rust was increased whereas maleic hydrazide increased infection (Samborski and Shaw, 1957); they suggested that maleic hydrazide counteracts the effects of IAA. Sinha and Wood (1964) increased susceptibility in Verticillium wilt by treating tomato plants with GA; Cycocel increased resistance. In the current work GA increased susceptibility in bean hypocotyls whereas Cycocel induced resistance. Similarly, IAA induced resistance in bean hypocotyls whereas maleic hydrazide increased susceptibility.

Ethylene applied to plants works in one or more of three ways :

i) As an inducer of resistance;

ii) In breaking natural resistance;

iii) As an inducer of symptoms

(Pegg, 1976a)

When ethylene, from Ethrel was applied to the roots of bean plants and hypocotyls were inoculated later with C. lindemuthianum, susceptibility was increased except at 5 and 1 mg/l for 24 h which induced resistance. But in etiolated bean hypocotyls treated with Ethrel in drops, disease development was reduced. It has been reported that ethylene increases activity of phenylalanine ammonia lyase, peroxidases and polyphenoloxidase (Abeles, 1971) all of which have been implicated in disease resistance. The induced resistance caused by locally applied ethylene may depend on the induction of synthesis of phytoalexins, (Chalutz et al., 1969; Chalutz and Stahman, 1969; Emmanouil, 1977). Polysaccharide hydrolases increase in healthy beans after treatment of petioles with 1 ppm ethylene (Abeles and Forrence, 1970) and ethylene may alter the quantity or quality of cellulolytic or pectolytic enzymes produced by the pathogen, which subsequently could affect its ability to colonize host tissue. A similar suggestion was made by Hislop et al. (1973) in studies of brown rot of apples caused by Sclerotinia fructigena.

In resistant and susceptible cucumber plants treated with Ethrel, disease was increased. The greatest effect of Ethrel occurred in a resistant cultivar in which resistance was greatly decreased. Daly et al. (1970) reported that ethylene treatment changed a resistant reaction at 20°C. to a susceptible response rust infection in wheat despite a 60% increase in peroxidase activity usually associated with resistance. Abeles et al. (1971) also found that ethylene can increase membrane permeability in plants; this is a common disease symptom.

Little of the work on the role of ethylene in pathogenesis has been conclusive probably because it takes part in many basic plant processes so that it is difficult to decide which change in function has affected resistance or susceptibility.

GENERAL COMMENTS

Sugar content has been considered important in the reactions of a host to a particular pathogen. Horsfall and Dimond (1957) classified diseases as high and low sugar so that a plant would be susceptible or resistant according to the demands of a pathogen for these compounds; they give a number of examples in which alterations in resistance induced with growth-regulating substances is paralleled by expected changes in the sugar content of the tissue. This approach was beyond the scope of the current work but any future work on the effect of growth-regulators in host-pathogen relationships should consider this factor. The present work has revealed some points which may be significant in this connexion. The capacity of the PGR to induce resistance in one host-parasite combination need not imply that it acts similarly in others. The future of these compounds as practical chemotherapeutants will be influenced by several factors. The most obvious, of course, is whether deleterious effects of the compound are more important than losses caused by the disease. Relative deleterious and beneficial effects will be controlled by the nature of the compounds, the disease, environment, dosage and method of application. Much more work, therefore, is necessary before any firm statements can be made on the practicality of PGR as chemotherapeutants.

Ultra-violet light (UV) and disease resistance

Pathologists have used UV for inducing resistance in plants through the synthesis of phytoalexin. Pisatin in pea, hydroxyphaseollin in soybean and resveratol in grape vines are induced by treating host-plants with UV

(Hadwiger and Schwochau, 1971; Bridge and Klarman, 1973; Langcake and Pryce, 1977). But in the current work in UV treated bean hypocotyls, the non-pathogenic Colletotrichum spp. were able to penetrate and caused expanding lesions. This seems to be the first report of the use of UV to break specificity of a host-parasite relationship. C. coffeanum and C. lagenarium under natural condition do not parasitize bean hypocotyls but when etiolated bean hypocotyls are irradiated with UV for 1 h and immediately inoculated, lesions were produced within 48 - 72 h. Moreover, inoculating with an incompatible race of C. lindemuthianum, also led to lesions comparable to those of susceptible reactions.

Infection by pathogens is accompanied by major changes in the metabolism of the host which have often been related to the appearance of new isozymes or enzymes with altered properties (Scott, 1972; Shaw, 1967). Host-parasite interactions that lead to susceptibility or resistance of a host plant to a pathogen are likely to take place at a very early stage during transcription and translation (Chakravortry and Shaw, 1977). Nucleic acids are the genetic basis for specificity in biological systems and in most diseases their involvement is indirect, regulating the production of cell components, toxins or phytoalexins and it is through these agents that many hosts and pathogens interact. The presence of receptors in plasma membranes of plants as factors in specificity has been claimed by Strobel and Steiner (1971; 1973; 1974), who found that susceptible cultivars of sugar cane have plasma membranes proteins which bind helminthosporoside (host-specific toxin of Helminthosporium sacchari). Cell walls of dicotyledons have cell wall proteins which inhibit endopolygalacturonases secreted by plant pathogens (Albersheim and Anderson, 1971; Fisher et al., 1973; Jones et al., 1972). It has been suggested that a pathogen will not attack a plant unless the environment in vivo permits the secretion of sufficient endopolygalacturonase to nullify the inhibitors in the cell walls. UV radiation causes conformational changes in cellular DNA which may affect the genetic code so that an existing protein is altered, or a

new protein is synthesized, or particular proteins are not synthesized. As a result, the protein pool in a cell will be affected. If there are conformational or other changes in the receptor site caused by UV radiation, the cells may fail to recognize a pathogen, there is no hypersensitive response, and hyphae will successfully develop inside the cells, and cause lesions. The work of Rohringer et al. (1974) with wheat leaves and rust suggested that specificity depends on RNA because RNase treatment of certain fractions that caused a HR-like response destroyed specificity. Albersheim and Anderson (1975) in their work with elicitors have proposed that recognition is mediated by carbohydrate-containing molecules and proteins, and that small changes in the terminal structure of elicitor molecules such as might be caused by gene mutation, would destroy the elicitor-receptor relationship. The host cell would then fail to recognize the pathogen, defensive responses would not be initiated and the mutation would be avirulent races behaving as virulent races. Complementary this may happen when non-pathogenic Colletotrichum spp. colonize UV irradiated etiolated bean hypocotyls; mutation altering recognition sites in the host.

When bean hypocotyls were inoculated 24 - 48 h after irradiation with pathogenic or non-pathogenic Colletotrichum spp., disease resistance was induced. This was associated with the accumulation of phytoalexins. Protection was also observed in untreated tissue close to irradiated tissue. This may have again depended on diffusion of phytoalexins from treated cells as reported by Elliston et al. (1971). However, according to Deverall (1977) phytoalexins are not known to diffuse from sites of production.

Antifungal compounds and incompatibility in host-parasite relationship

Antifungal compounds, phytoalexins, have been implicated as important components of resistant responses of bean tissue to fungal parasites. Their synthesis is associated with host necrosis which is an

early indication of a high degree of incompatibility between fungus and host (Deverall, 1977). This was confirmed in the current work in which bean cotyledons inoculated with non-pathogenic Colletotrichum spp. produced more phytoalexin than did those treated with pathogens. Stholasuta et al. (1971) also reported that incompatible races of Psueodmonas glycinea, P. mors-prunorum or incompatible races of P. phaseolicola introduced into bean leaves, induced 10 times more phaseollin than did a compatible race of P. phaseolicola. Similarly, amounts of kievitone and phaseollin produced by bean hypocotyls, UV-irradiated and then inoculated with non-pathogenic Colletotrichum spp., were much higher than in those inoculated with a pathogen. Moreover, a resistant bean cultivar (Kievit) irradiated with UV and inoculated with an incompatible race of C. lindemuthianum (β), produced more kievitone and phaseollin than did a susceptible cultivar.

In cucumbers, phytoalexins could not be detected by the methods used for extracting antifungal compounds from bean tissues. Because the accumulation of aromatic compounds in host tissues invaded by parasites is very common; it was investigated that the level of total phenols remained higher in a resistant reaction than in the controls whereas in a susceptible reaction the level of total phenols decreased with the development of the disease. However, although a sharp, post-infectional increase in the concentration of phenolics is a common response to fungi (Farkas and Kiraly, 1962), the results in the current work were not conclusive in this respect. Further work on this line is needed.

Cell wall degrading enzymes

Most phytopathogenic micro-organisms are able to produce enzymes which degrade a number of polymeric carbohydrates found in higher plant cell walls (Bateman and Miller, 1966; Albersheim et al., 1969; Wood, 1967). C. coffeanum and C. lindemuthianum (races α, β and δ) produced endo-polygalacturonase (PG) in vitro when grown in a solution containing 1%

pectin whereas C. lagenarium produced pectate-trans-eliminase (PTE) in a solution containing 1% NaPP. The enzymes from races of C. lindemuthianum were studied for their role in cultivar specificity. As described by Anderson and Albersheim (1972), the PG secreted by several races of C. lindemuthianum were indistinguishable by isoelectric points which was pH 9.75 for each. Also indistinguishable on this basis were the inhibitors of PG of C. lindemuthianum from several cultivars of bean as shown when slices of germinated seeds or hypocotyls of susceptible and resistant cultivars were incubated with PG from races α and δ. Enzyme activity of the supernatant liquid was lost at the same rate so these factors could not explain the specific reactions of the different cultivars.

In light of the main results of the research, summarized in this thesis, the following are suggested as promising lines for further study.

1. In the systemic protection of cucumber leaves what is the nature and mode of action of the factors which pass from the inoculated leaf ?

2. What changes are induced in the protected leaves ?

3. Does the new protein present in lesion extracts have a role in inducing systemic resistance ?

4. What is the molecular nature of the action of UV in breaking the resistance of bean hypocotyls with non-pathogenic Colletotrichum spp. ?

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