POTATO TISSUE RESISTANCE TO THE GROWTH OF *Phytophthora infestans* (Mont.) De Bary

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by

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SUMMARY

This thesis describes an investigation of aspects of race non-specific resistance which determines the rate at which tuber tissue is colonized by Phytophthora infestans. It was mainly concerned to determine whether active resistance mechanisms are involved or whether resistance could be explained by passive factors only. Five clones with different levels of race non-specific resistance were used.

Hyphal growth rates in all clones and in all tissues tested were similar and thus growth rate can play no part in the differences in race non-specific resistance between these clones. The growth rates in all experiments at all temperatures used were linear indicating that resistance does not involve active factors accumulating in the tissue ahead of the lesion to concentrations high enough to affect hyphal growth. Thus resistance to hyphal growth would appear to depend upon passive factors only. The growth rate on different media was always faster than that in tuber tissue indicating that hyphal growth in vivo may be affected by inhibitory factors, but if so, these factors are equally active in all clones.

The total time required for the penetration and initiation of hyphal growth at the inoculated surface and for the establishment of visible sporulating mycelium on the opposite surface (Growth phases I and III) differed between clones and thus is a factor in the differences in race non-specific resistance between the clones.

The rate of initiation and spread of lesions in the tubers differed between clones. These rates, in all clones, were slower than those calculated for the initiation of hyphal growth and for hyphal growth itself, indicating that the hyphae are growing ahead of the fluorescent

tissue surrounding the nectoric tissue.

The tuber tissues of the different clones were colonized to an equal extent and thus there was no evidence to indicate that active factors might accumulate to different levels in the tissues behind the hyphal front in the different clones and thus affect the extent to which lateral branches developed to further colonize the tissues.

Hyphae were distributed non-randomly in the medullary tissue in the tubers and stems of Craigs Alliance and in the tubers of Pentland Dell and Pentland Squire, indicating that this tissue is not uniformly susceptible to colonization, but that there are pockets of tissue more resistant than others.

Four types of haustoria were found in tuber and stem tissue but only two types were found in leaf tissue and there was no obvious relation between haustorial type and resistance. Haustoria were distributed non-randomly between cells indicating that cells may differ in their resistance to penetration. There were also differences between clones in total number of haustoria produced which appeared to be correlated with resistance to growth phases I and III and to resistance to lesion growth. Thus factors affecting haustorial formation may be important in the differences in race non-specific resistance between the clones.

GENERAL INTRODUCTION

GENERAL INTRODUCTION

Phytophthora infestans (Mont.) de Bary is the most important of about forty known fungal parasites of the potato in Britain (Moore, 1959). It causes serious economic losses in every country where the potato is grown and where the climatic conditions are suitable for its growth and dispersal. The most effective means of control would be to grow resistant clones and a knowledge of the physiological basis of resistance might aid plant breeders in producing these clones.

Resistance to P. infestans can be broadly grouped into two categories:-

1. Race specific resistance
2. Race non-specific resistance.

1. Race specific resistance

Potato clones which contain specific resistance genes (R genes) derived from wild species of Solanum are susceptible to certain races of P. infestans only. Other races are unable to establish a parasitic relationship but instead induce a hypersensitive resistance reaction which inhibits fungal growth immediately after penetration. This type of resistance is called race specific resistance and a lot of work has been done on the host reaction involved (Müller & Black, 1952; Müller, 1953; Tomiyama, 1966, 1967, 1971; Tomiyama & Stahmann, 1964; Tomiyama et al., 1958, 1968; Kitazawa & Tomiyama, 1969; Friend, 1973; Friend et al., 1971).

2. Race non-specific resistance

On the other hand, clones which are susceptible to a certain set of races are generally not equally susceptible to them all, some clones being more resistant than others. This resistance appears to depend upon a number of factors as reported by Van der Zaag (1959).

1. Resistance to penetration, which includes all factors which affect spore germination, growth on the surface and penetration through the outer surfaces to the living cells underneath.

2. Tissue resistance, which includes all factors affecting the rate of growth of the fungus and the extent of colonization of the internal tissues.

3. Resistance to the formation of reproductive structures, which includes all factors which affect the formation of reproductive structures and thus the potential of the parasite to spread and cause further infections.

Detailed studies of these phenomena have been made by Umaerus (1959, 1960, 1963); Lapwood (1961a, 1963); Lapwood & McKee (1966); Guzman (1964); Guzman et al. (1960); Knutson (1962); Jeffery et al. (1962); Thurston et al. (1962); Hodgson (1962); and Thurston (1971).

However, in any particular clone, resistance based upon these factors is expressed equally against all races of P. infestans capable of infecting that clone. For this reason it is known as race non-specific resistance.

Physiological Basis of Resistance

The resistance factors operating in both race specific and race non-specific resistance may fall broadly into two groups:-

1. Passive mechanisms depending on properties of the plant which are present prior to infection.
2. Active mechanisms which depend on properties of the host plant, that develop only after the parasite contacts the living tissues.

Either one or both mechanisms may be involved in resistance to any stage of infection from penetration to the formation of reproductive structures.

(1) Resistance to penetration

Plant resistance mechanisms may start to operate as soon as the plant comes into contact with the pathogen. Thus the chemical or physical features of its surface may prevent or reduce the probability of spores or other propagules from germinating and penetrating. Some plants contain substances which prevent spore germination of the parasite as in the case of Onion Smudge caused by the fungus Colletotrichum circinans, where resistant varieties generally having red outer scales containing Catechol and Protocatechuic acid (Angell et al., 1930; Walker & Link, 1933). These phenols are water soluble and diffuse from the dead cell layers into the infection drop where they inhibit spore germination (Walker & Link, 1933).

It is well known that the leaves of some clones of potato are less readily penetrated by P. infestans than are the leaves of other clones (Umaereus, 1960) and that the upper surface of the leaf is less readily penetrated than the lower surface (Lapwood, 1968). The cuticle has been regarded as principle obstacle to entry by parasites but Martin (1964) considers that its contribution to prevention of penetration by fungal parasites is not great, and does not constitute

a serious barrier to any fungus except perhaps if it is very hard and thick. However, the chemical and physical features of the potato leaf and stem surfaces which are responsible for their resistance to penetration by P. infestans are still unknown.

The outer peridermal tissues of the tuber also appear to be effective barriers against infection by pathogenic microorganisms. The unbroken skin of the potato tuber is almost impenetrable by fungal or bacterial parasites. The entry of P. infestans is restricted to the buds or to regions around the buds (eyes) where the periderm is thin, or to the lenticels, or wounds, prior to the formation of a wound periderm. Fusarium solani var. coeruleum can only invade through fresh wounds (Lapwood & Hide, 1971).

The resistance of the potato tuber to infection by P. infestans has been attributed to the thickness of the periderm (Pethybridge, 1913; Simonet, 1925; Bond et al., 1940; and Toxopeus, 1958), but Lohnis (1925) found no correlation between resistance and the number of cork layers composing the skin. On the other hand, Nagdy & Boyd (1965) reported a significant correlation between the periderm thickness and resistance to Oospora pustulans.

Another character which is associated with tuber resistance to P. infestans is the degree of suberization of the lenticels (Lohnis, 1923, 1925; Bond et al., 1940; and Zan, 1962). Lohnis found much higher degrees of infection in the tubers of the clone Eigenheimer grown on clay soil than those grown on sandy soil. On staining with Sudan III, he found that the lenticels in the tubers grown on clay were filled with unsuberized parenchyma cells, while those in the tubers from the sandy soil were closed with suberized cells.

The periderm layers of tuber tissue which develop in response to wounding become as resistant to penetration as the unbroken skin. However, the time taken for this resistance to develop varies with

the parasite. Thus Müller (1957) reported that P. infestans is still able to penetrate wound periderm of the tuber six days after wounding but the probability that individual spores will be able to penetrate is reduced by at least 92%. On the other hand resistance of most clones to F. solani var. coeruleum, in the early stage of storage, is more or less fully expressed 24 hours after cutting (McKee, 1954).

The resistance of the periderm may be due to its mechanical toughness, its chemical composition, or the ability of associated living cells to produce inhibitors in response to attempted penetration. There is evidence to indicate that the periderm contains significant amounts of inhibitory compounds such as phenols (Farkas & Kiraly, 1962) and steroid glycoalkaloids (Allen & Kuc, 1968; Ozeretskovskaya et al., 1971; McKee, 1955). The steroid glycoalkaloids have been found to be present in concentrations at least eight times that necessary to inhibit the growth of Helminthosporium carbonum by 50%, a fungus which does not attack potato (Allen & Kuc, 1968).

(2a) Tissue resistance in relation to race specific resistance

Parasites which manage to penetrate the plant surface by one route or another may or may not be able to establish a parasitic relationship and colonize the tissue further. This will depend upon other resistance mechanisms which only come into operation after penetration and which may differ between clones and in response to different parasites.

Clones having race specific resistance to P. infestans react hypersensitively to infection by incompatible races. The term "hypersensitivity" was first used by Stakman (1915) to describe the responses of the cells of certain varieties of wheat to penetration by incompatible isolates of wheat rust Puccinia graminis f. sp. tritici. This involved a number of morphological and physiological changes in the infected cell and in the cells surrounding them, which resulted

in their rapid collapse and death. Hypersensitivity is discussed by Müller (1959) and Klement & Goodman (1967) who described this process in diseases caused by many parasites including fungi, bacteria and viruses.

The cytological and chemical events which occur in the hypersensitive response of potato tissues to incompatible races of P. infestans have been studied in detail by Tomiyama and his colleagues (Tomiyama, 1957, 1966, 1967, 1971; Kitazawa & Tomiyama, 1969; 1973; Kitazawa et al., 1973; Tomiyama & Stahmann, 1964; Yamamoto, 1974). They observed that in tissues infected by incompatible races rapid degenerative changes begin within minutes of the hyphae entering the cell. Brown pigments accumulate both in the cytoplasm and the cell wall. Some cells die within 10 minutes of infection while the majority die within 40 to 60 minutes (Tomiyama, 1967). On the other hand compatible races have little or no effect on susceptible cells for two or more days after inoculation. Kitazawa & Tomiyama (1969) reported that cell death in the hypersensitive reaction in itself does not inhibit fungal growth. Hyphal elongation was not markedly retarded until about nine hours after inoculation, that is about seven to eight hours after cell death, but growth had almost stopped two hours later, that is eleven hours after inoculation. Shimony & Friend (1975) in their study on a comparison of the ultrastructure of the reaction of a susceptible and resistant potato to race 4 of P. infestans also found that cells of the clone Orion died at about seven to nine hours, but death of the fungus did not occur until 12 hours after inoculation. Hyphal growth was often confined to the initially penetrated cell, but if growth into the surrounding intercellular spaces occurred then these surrounding cells reacted hypersensitively so that the infecting hyphae were rapidly enclosed by necrotic tissue.

The hypersensitive response is usually associated with the accumulation of certain compounds most of which are aromatic in nature. Some of these compounds may be associated with the cessation of hyphal growth, others may simply be the result of the disordered metabolism of the penetrated surrounding cells and play no role whatsoever in resistance. The problem is in differentiating between those compounds which are involved and those which are not.

Compounds directly involved in resistance have been termed phytoalexins by Müller & Borger (1940) who defined them in a restricted sense to denote chemical compounds produced only when the living cells of the host are invaded by a parasite and undergo necrobiosis. However, the term is now defined to cover a much wider range of chemical compounds. It now includes those which are formed in response to infectious agents or which are the products of such agents, or which are formed in response to wounding, provided that they contribute to disease resistance (Kuc, 1972).

Many studies of the chemical basis of hypersensitive resistance in the potato have been concerned with changes in the concentrations of phenolic compounds after penetration (Farkas & Kiraly, 1962; Tomiyama, 1963; Kosuge, 1969). It has been found that phenols accumulate much more rapidly and to higher levels in potato tissue infected with incompatible races than with compatible races (Tomiyama, 1963; Rubin & Artsikhovskaya, 1963). Chlorogenic and Caffeic acids and related compounds have been extensively studied (Kuc, 1966, 1967, 1971, 1972; Wood, 1967; Sokolova *et al.*, 1958; Kosuge, 1969; Cole, 1970; Stoessel, 1970; Cruickshank *et al.*, 1971), but doubt still exists regarding their role in disease resistance (Kuc, 1973). Friend (1973) found lower chlorogenic acid levels in the clone Orion in the early stages after inoculation with the compatible race,

race 4, than in uninoculated tissue. He considered it unlikely, therefore, that chlorogenic acid is being produced as an antifungal compound, and thus it cannot be considered to be involved directly in hypersensitive resistance. Another three phenolic acids, P-hydroxybenzoic acid, Vanillic acid and Salicylic acid, were reported to accumulate in callus cultures of the clone Orion after infection by P. infestans (Robertson et al., 1968, 1969). It is difficult to assess the significance of this result since none of these compounds have been reported in hypersensitive lesions from whole plants.

At least four sesquiterpenes have been isolated from potato tubers after inoculation by incompatible races of P. infestans. They are Rishitin (Katsui et al., 1968; Tomiyama et al., 1968; Varns, 1970; and Chalova et al., 1971), Rishitinol (Katsui et al., 1971), Phytuberin (Varns et al., 1971b), and Lubimin (Metlitskii & Ozertskaya, 1970). Rishitin was also found to accumulate in response to inoculation with numerous non-pathogens of potato (Tomiyama et al., 1968 ; Varns, 1970; Varns et al., 1971a). Phytuberin has been found to accumulate in potato tubers of susceptible clones invaded by Erwinia carotovora var. atroseptica (Lyon, 1972; Coxon et al., 1974). Sato and his colleagues (1971) consider cell death may be a trigger for the synthesis of rishitin.

Some phenolic compounds, particularly in their oxidised state, are inhibitory to many micro-organisms and their activity is probably largely due to their ability to denature proteins, and thus inhibit the extra-cellular enzyme activity of the parasite. Thus inhibition of extra-cellular enzymes involved in the degradation and penetration of cell walls by P. infestans may contribute to confining the fungus to the lesion and the deposition of lignin-like compounds in the

cell walls in the hypersensitive lesion may almost certainly act in this way (Friend et al., 1971; Friend, 1973). Nothing is known about the mechanism of inhibition by the sesquiterpenoids but they are highly toxic in vitro at fairly low concentrations where it has been found that the median effective dose (ED_{50}) of rishitin required to inhibit the growth of spore germ tubes of P. infestans (race 0) was about 2.1×10^{-4}M. Germination was completely inhibited by rishitin at 10^{-3}M. Rishitin also inhibited spore germ tube growth in Alternaria kikuchiana by 50% at 1.3×10^{-4}M and F. solani f. sp. phaseoli at 10^{-4}M (Tomiyama et al., 1968). Sato et al. (1971) found that a concentration of 100µg rishitin/g fresh weight was sufficient to inhibit hyphal growth in vitro and they reported that rishitin seems, therefore, to play an important role in the inhibition of hyphal growth and lesion development. However, Friend et al. (1973) stated that the amount of rishitin (342µg/Kg fresh weight in the resistant clone Orion two days after inoculating with P. infestans) is lower than the 2×10^{-4}M which inhibits fungus spore germination in vitro, and so rishitin is not very important in the inhibition of P. infestans.

It is not clear so far whether hypersensitivity is a cause or a consequence of plant resistance. Ersek et al. (1973) and Kiraly et al. (1972) found that the growth of P. infestans was inhibited in potato tissue after treatment with chloramphenicol (50 - 800 PPM) or Streptomycin (3 - 50 PPM). The inhibition was accompanied by hypersensitive-like reactions. Increases in the activities of polyphenoloxidases, peroxidases and in the amount of the phytoalexin rishitin indicated a similarity of this reaction to the natural hypersensitive reaction. They concluded that hypersensitivity associated with phytoalexin production is a consequence rather than the cause of host resistance to infection.

(2b) Tissue resistance in relation to race non-specific resistance

The problem now arises as to how compatible races of P. infestans and other microbial parasites of potato tissue, after they have penetrated the external barriers, can then continue growth without triggering the hypersensitive response. Tomiyama (1966) found that when a potato plant cell had been infected by a compatible race of P. infestans and was reinoculated 15 - 20 hours later with an incompatible race it did not react hypersensitively to this second inoculation. He concluded, therefore, that the metabolic activity of the host may be retarded by contact with the compatible race and thus lose its ability to react rapidly to infection by the incompatible race. Varns & Kuc (1971) reported that prior inoculation with a compatible race not only inhibited the hypersensitive necrotic response to a subsequent inoculation by an incompatible race but also the ability of the tissue to synthesise rishitin and phytuberin. A mechanism more complex than direct inhibition may be involved in which the compatible race may be able to channel synthesis in the host into the formation of less toxic compounds or to toxic compounds which accumulate in regions not in direct contact with the invading organism. Thus Clarke (1972, 1973) suggests that the coumarin glycosides such as scopolin which accumulate in potato tissue in response to invasion by compatible isolates of P. infestans and other virulent pathogens does so at the expense of the more fungitoxic lignins. These coumarins accumulate in the cell vacuole and are thus not in contact with the invading pathogen. Therefore, the fungus is able to grow further and colonize the tissue.

However, as mentioned earlier all clones are not equally susceptible to all compatible isolates or indeed to all pathogens. Thus in the field, lesions have been found to develop more slowly

in the leaves of resistant clones than in susceptible clones and lesion size has been reported to be correlated with resistance (Jones et al., 1912; Vowinckel, 1926; Kammermann, 1951; Guzman et al., 1960; Umaerus & Lihnell, 1976). However, this is not always the case since Van der Zaage (1959) in greenhouse tests found the same rate of spread of lesions in the leaves of the susceptible clone Eigenheimer as in the more resistant clone Voran. Knutson (1962) found that although the relative resistance in the field of the potato clones Pontiac, Sebago, and Ostbote, was associated in most greenhouse tests with size of lesion, this was not true of the clone Cobbler. Main & Gallegly (1964), however, did not find any correlation between degree of resistance measured in the field and the rate of invasion.

Most work on fungal growth in tubers was done either by recording the density of mycelium on the inoculated surface of tissue slices or by estimating fungal invasion from the depth of necrotic tissue of the tubers. Besides differences in the rate of development of necrotic symptoms between clones, differences have also been reported in the rate of development of necrosis in different regions of the tuber. Boyd and Henderson (1953) reported that the cortex of tubers in general is more susceptible to P. infestans than the medulla. Lapwood & McKee (1961) also found that the cortex in some R gene clones was more susceptible than the medulla but in other clones there was no difference. Lapwood (1965c) reported that the cortex of clone (Record) was more resistant than the medulla, while Gausuzzaman (1966) found that differences between the rate of spread of lesions through the cortex and medulla of the tubers of Up-to-Date, Home Guard and Arran Victory were small. Medullary tissues have also been reported to be more susceptible to Phoma exigua var. foveata than cortical tissue (Jellis, 1975; Pietkiewicz & Jellis, 1975). According to De Bruyn

(1943) necrotic lesions of P. infestans spread more in the heel end than in the rose end. Boyd (1952) reported that the heel end was more susceptible to dry rot, caused by Fusarium solani var. coreuleum, than the rose end.

The differences in the amount of necrotic tissue certainly does not reflect differences in the rate at which the tubers of different clones or in which different regions within the tubers of each clone are colonised by fungal hyphae since the internal spread of the fungus is not always directly related to the amount of necrotic tissue in the lesion. The hyphae are in fact established in the living tissue ahead of the lesion and variation in the amount of necrotic tissue produced in a given time may be due to variation in the rate at which the living cells die after invasion (Lapwood, 1965c).

Little work has been done on the physiological basis of race non specific resistance but both passive and active factors may be involved. Among the passive factors are the anatomical characters and the chemical composition of the tissue. The cell wall is one of the anatomical characters which may play an important role in the resistance of the tissue. Szymanek (1927b) indeed reported that there is a close correlation between resistance to P. infestans and the thickness of the walls of the parenchyma cells of the tuber tissues. Hawkins & Harvey (1919) stated that Pythium de baryanum penetrates tuber cell walls mechanically, and that its rate of growth in different clones depends on differences in the resistance of the walls to penetration. A resistant clone had a higher fibre content and more secondary thickening of the walls than did a susceptible clone. However, Wood & Gupta (1958) reported that this parasite readily secretes cell-wall degrading enzymes which macerate cells and tissues of potato tuber so that Hawkins & Harvey's conclusions are now less

convincing.

Mechanical penetration of the cell wall is probably unimportant but the chemical composition of the cell wall may affect the rate of enzymic degradation and thus the spread of P. de baryanum through the tuber. Neither mechanical nor enzymic wall penetration is very important for parasites such as P. infestans where the hyphae grow intercellularly except in so far as they may affect wall penetration by haustoria or in the case at which the hyphae make their way through the middle lamella as reported by Gaumann, (1950) and Gausuzamann (1966).

Other chemical factors besides those associated with the cell wall in relation to penetration may play a role in resistance to colonization. Thus the invading hyphae may fail to grow readily due to the presence of inhibitory compounds or to the presence in inadequate amounts of substances which are essential for their nutrition. Lewis (1953) proposed a balance hypothesis of parasitism, in which the host-parasite relationship is governed by a combination of the inhibitory factors of the host and the nutritional requirements of the parasite. Garber (1956) examined this hypothesis and suggested that from the four combinations of the two factors, nutritional environment and inhibitory environment, only one combination will favour successful invasion, that is when the environment is nutritionally adequate and non-inhibitory.

Kock (1931) found that the hyphae of P. infestans grown in susceptible tuber tissue were straight with long branches, but those in more resistant tuber tissue were twisted with a strikingly gnarled appearance and with numerous short branches. He considered that the latter may result from "inhibition" or "starvation" but he did not investigate this matter further. So far few investigators have been able to link resistance to the ability of the resistant clones to

supply the factors necessary for the growth of the parasite. If the host does not contain the substances required for the growth of the parasite in an available form, or they are present but in the wrong proportions (Wood, 1967), then either parasitism is restricted or it does not occur. The extent of nutrient availability will determine the varying degrees of resistance of the host to the parasite.

The most convincing evidence to support these notions comes from studies by Kline et al. (1957) and Keitt et al. (1959) using mutants of Venturia inaequalis, the causal organism of Apple Scab. They found that mutants deficient in certain growth factors would not infect apple unless these factors were supplied artificially and Keitt et al. (1959) proposed that the loss of virulence was based on these nutritional deficiencies. They found that of seven nutritionally deficient avirulent mutants, six grew well on sterile sap expressed from apple leaves, and the only one which did not grow on this medium was the histidine mutant. Thus for six of the mutants the substances required for growth were present, but apparently in an unavailable form.

Grainger (1956, 1962) has associated resistance to several diseases including potato blight and Helminthosporium leaf spot of Oats with a low ratio of total carbohydrate to the residual dry weight of the plant. His interpretation of these observations is that with increasing ratios of total carbohydrate to residual dry weight there is a greater amount of carbohydrate available to support the growth of the parasite. Thus the tissue would become increasingly more susceptible as the available carbohydrate increases. This relationship does not hold for all host-parasite systems as in some cases, for example target spot disease of tomato caused by Alternaria solani and Dutch elm disease caused by Ceratocystis Ulmi, reducing

the sugar levels in the plant increases disease severity (Horsfall & Dimond, 1957). Horsfall & Dimond have classified diseases into high sugar and low sugar diseases, and clearly the relationship between carbohydrate levels and susceptibility or resistance is not direct and is probably fairly complex.

Lepik (1939); Griesinger (1941); Swiniarski et al. (1959); Yamamoto & Honda (1960) and Sakai (1961) suggested that the nitrogen containing components of the tuber, especially the proteins and amino acids, are likely to be the main sources of energy for P. infestans since this organism appears to be unable to degrade starch, the major reserve carbohydrate of the tuber. However, later work by Knee & Friend (1970) revealed that P. infestans is able to produce galactanases and, to a lesser extent, polygalacturonases, and thus carbohydrates derived from the host cell wall may be used as a source of energy.

A plant may not be parasitized, or be parasitized to a lesser extent, if it contains substances in concentrations high enough to prevent or seriously reduce the growth of the parasite. Pre-infectional levels of phenolic substances have been implicated in the resistance of potato tissue to P. infestans. A comparative study by Rubin et al. (1947) showed that leaves of resistant clones contained more tannins than the leaves of susceptible clones.

Valle (1957) found appreciable concentrations of caffeic and chlorogenic acids in potato leaves. Virtanen et al. (1957) also reported that there was enough chlorogenic acid in the leaves of some potato varieties to reduce significantly the growth of P. infestans. However, Sokolova et al. (1960) stated that chlorogenic acid at concentrations of up to 2×10^{-3}M is not toxic to P. infestans and even tends to stimulate its growth, and they suggested that chlorogenic acid can only be significant in resistance if it is converted to

other compounds after infection. Friend's work (1973) on post-infectional changes, mentioned earlier, supports this view. There is evidence for a role of chlorogenic acid in the resistance of potato roots to Verticillium albo-atrum (Patil et al. 1964, 1966).

Alkaloids in potato tuber tissue such as α-Solanine and α-Chaconine may play an important role in the resistance of the tuber to invasion by fungi. McKee (1954, 1955) found that potato tubers are susceptible to Fusarium solani var. coeruleum whereas growth of Fusarium avenaceum is impeded. The latter fungus grows intracellularly and its limited growth may be due to inhibitory factors within the cell. McKee found that sap extracted from tissues contained fungitoxic substances, with most of the toxicity being due to the alkaloid Solanine.

Solanine also has been suggested to be a factor in the resistance of green tomato fruit to Colletotrichum phomoides (Allison, 1952). Another glycoalkaloid, α-tomatine, has also been suggested to be a factor in the resistance of tomato to fungi (Arneson & Durbine, 1968).

In addition to any pre-formed factors which affect the extent of tissue colonization, post-infectional changes may also occur leading to increased tissue resistance. Thus compounds which are absent, or present in low concentration, may accumulate later in response to invasion and play a role in reducing the growth of the parasite. However, there is no work which so far clearly implicates post-infectional changes in tissue resistance to P. infestans.

(3) Resistance to the formation of reproductive structures

After the pathogen has colonized the host tissues to varying extents depending upon the clone, it finally produces sporangia. The period between inoculation and sporulation has been termed "reproduction period" by Gaumann (1950) or "latent period" by Van der Plank (1963). This period and the amount of sporulation of

P. infestans in potato tissue has been studied as a feature of field resistance and differences have been found between some resistant and susceptible clones in the field. The fungus has been reported to produce more spores and to produce them faster on susceptible clones than on resistant clones (Schaper, 1951; Kammerman, 1951; Deshmuth & Howard, 1956; Van der Zaag, 1959: Lapwood, 1961b; Hodgson, 1962; Thurston et al., 1962; Weihing & O'Keefe, 1962; Guzman et al., 1960 and Guzman, 1964).

However, Knutson (1962) found that although the relative resistance to late blight in the field of potato clones Pontiac, Sebago and Ostabote was associated in most greenhouse tests with abundance of sporulation, this was not the case for the susceptible clone Cobbler. Main & Gallegely (1964) also found no correlation between degree of resistance measured in the field and amount of and time required for sporulation. Nothing is known about the physiological basis of the difference between clones which determine the differences in the levels of sporulation.

In conclusion, while there is clear evidence to implicate active resistance factors in the race specific resistance of potato to P. infestans, and in fact to indicate some of the metabolic events involved, virtually nothing is known about the physiological basis of race non-specific resistance to this fungus. There is no evidence to indicate the relative roles of nutritional factors or inhibitory factors in race non-specific resistance or indeed whether it is dependent entirely upon passive factors or whether active mechanism are involved.

This thesis reports an investigation which attempted to investigate one aspect of race non-specific resistance, that aspect which affects the rate at which tuber tissue is colonized by P. infestans. It attempted to determine whether active resistance mechanisms could

be involved or whether resistance could be explained by passive factors only.

PART I

THE DIFFERENTIATION BETWEEN ACTIVE AND PASSIVE FACTORS IN TUBER TISSUE RESISTANCE

THE DIFFERENTIATION BETWEEN ACTIVE AND PASSIVE FACTORS IN TUBER TISSUE RESISTANCE

INTRODUCTION

The tissue resistance component of race non-specific resistance may depend upon factors which are normal components of the tissue, i.e. passive factors, or on factors such as phytoalexins which are induced to form by infection, or by a combination of both passive and active factors.

If resistance is based entirely on passive factors then one would expect that the growth rate of the fungus within the tissue would be linear with time although a linear growth rate would not rule out active resistance. Thus a linear rate of growth would occur with active resistance, if the induction of inhibitory factors occurred on initial contact and continued to occur at a constant rate in the cells at the edge of the lesion when the fungus came into contact with them. On the other hand, if the resistance is based on systems which develop in the uninfected tissue ahead of the fungus, gradually building up with time, as appears to be the case for some leaf spot diseases (Kurosaki, 1957), then this might be reflected in a gradual reduction in the rate of hyphal growth.

This work was carried out to investigate whether or not post-infectional changes do occur in tuber tissue, leading to change in resistance, and also to determine if resistance to hyphal growth is an important factor in race non-specific resistance.

GENERAL MATERIALS AND METHODS

1. Potato Clones

The clones listed in Table 1 were selected, after discussion with Dr. J. F. Malcolmson, Scottish Plant Breeding Station, Pentlandfields, to cover a range of race non-specific resistance from highly susceptible to highly resistant. The number after each clone is the resistance category to which it belongs according to the classification devised by the Scottish Plant Breeding Station. This classification ranges from 1, highly resistant, through to 4^+, highly susceptible. This assessment is based on observations made over a number of years on the extent to which each clone is affected by P. infestans, and thus is an assessment of the effects of all resistance factors operating at all stages of growth. It is not, therefore, necessarily related to the resistance of the tuber tissue to colonization, which is in fact the subject of this thesis, but it was considered the only basis on which the selection of suitable material could easily be made. The first clone in the Table was obtained from the Scottish Plant Breeding Station while the remaining four clones were obtained as foundation stock from various seed potato merchants. The clones were grown in the experimental gardens of the Botany Department at Garscube and stored in a cool place until use.

Table 1 The resistance categories of the clones used according to the Pentlandfield classification of race non-specific resistance

	Resistance
6003ab(33)	1-2
Pentland Raven	2
Pentland Squire	3
Pentland Dell	4
Craigs Alliance	4^+

2. The fungal culture

The culture of *P. infestans* used throughout this work was also originally obtained from Dr. J. F. Malcolmson. It was a complex race and was shown to be pathogenic on all the clones used.

Cultures were maintained on French bean agar slopes, incubated for three to four days at 20°C before transferring to an incubator at 11°C. Cultures were subcultured at fortnightly intervals.

The French bean agar was prepared as follows:-

> 150g Birds Eye frozen green beans were mixed in a Waring blender with a small volume of water and then filtered through muslin. The volume was made up to 1 litre with distilled water and 15g Oxoid agar (No. 3) then added. The medium was autoclaved at 10lb for 10 minutes to dissolve the agar before dispensing into large boiling tubes. It was finally autoclaved at 15lb for 20 minutes and the tubes then sloped.

3. Inoculation procedures for hyphal growth studies

Tubers were surface sterilized in 10% chloros solution for 10 minutes before washing in tap water. They were then dried with blotting paper. Slices of the required thickness were cut using a knife sterilized by flaming, in the apparatus shown in Figure 1. The apparatus gave slices 7, 12, 17, 22, 27 and 33 mm thick. The slices were then placed on the plastic mesh base of the wooden trays (Figure 2).

The trays were covered, top and bottom, with sheets of glass lined with damp blotting paper. Where tuber slices were too thick to lie in one tray, two trays joined together were used (Figure 3). Prior to use, the trays and glass sheets were cleaned and sterilized

by immersing them in a saturated solution of sodium hypochlorite for 10 minutes.

After cutting, the slices were left for 24 hours before inoculation to allow a suberised layer to form over the cut surfaces. This procedure reduced the level of bacterial contamination which sometimes occurred, and at the same time prevented the spore suspension from spreading across the slice from the point of inoculation.

The inoculum was prepared by flooding 14 day old cultures with about 10 ml of sterile distilled water. The suspension was kept at 12°C for 3 hours to promote zoospore formation and emergence. Zoospore counts were made using a haemocytometer slide and when necessary the suspension was diluted with sterile water to give a zoospore concentration of approximately 30,000 spores/ml.

Slices were inoculated by putting 0.5 ml of the zoospore suspension onto the cut surface. The trays containing the slices were then covered with glass sheets as described above and the whole assembly was wrapped in polythene to maintain a high humidity throughout the period of incubation. The slices were incubated in the dark in a constant temperature incubator maintained at the required temperature. They were examined three times daily at 9 a.m., 3 p.m. and 9 p.m. for the appearance of sporulating mycelium in the lower surface of the slices using a stereoscopic microscope.

Figure 1 The apparatus used for cutting slices of different thicknesses. The apparatus gives slices 7, 12, 17, 22, 27 and 33 mm thick.

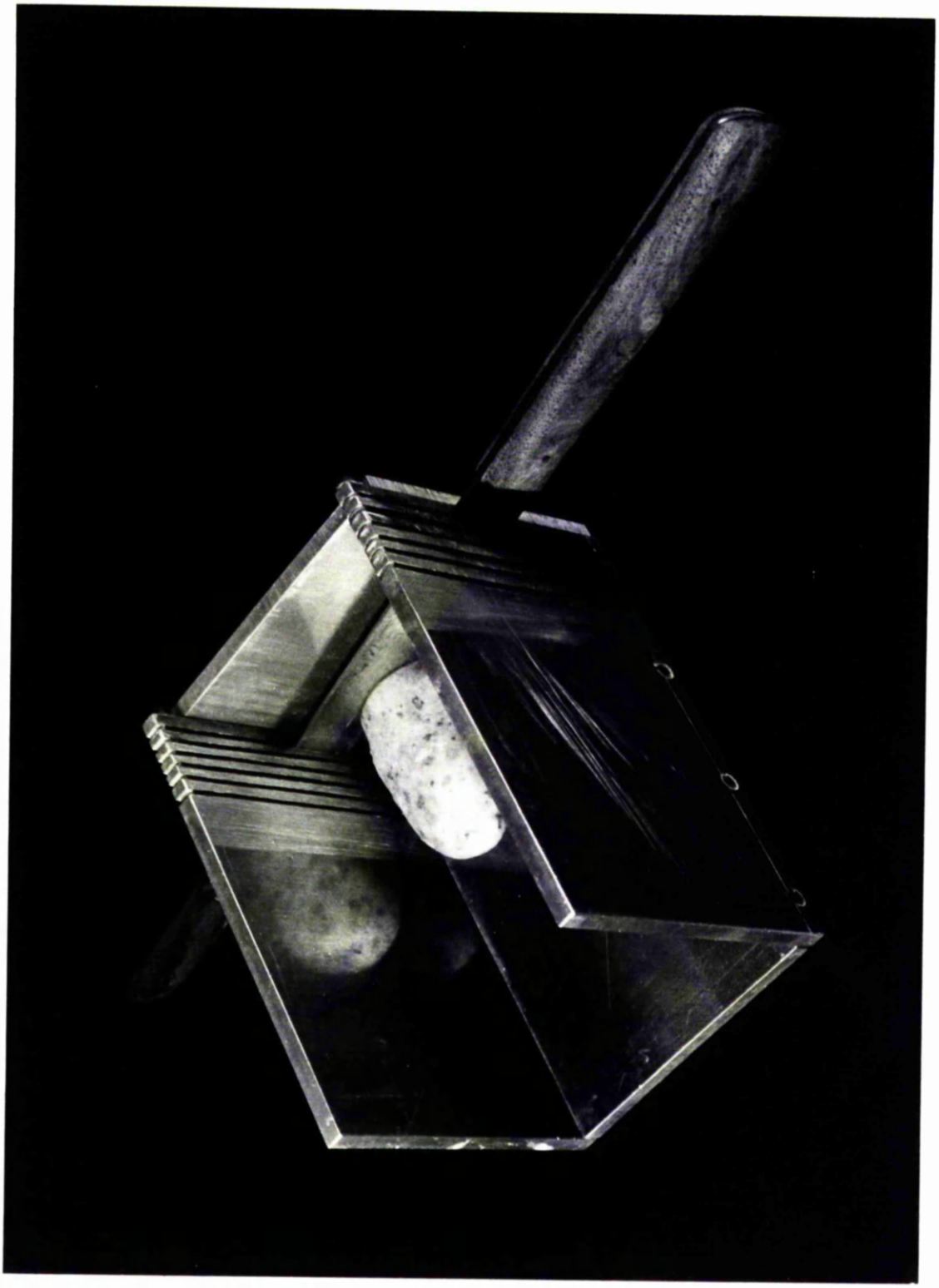
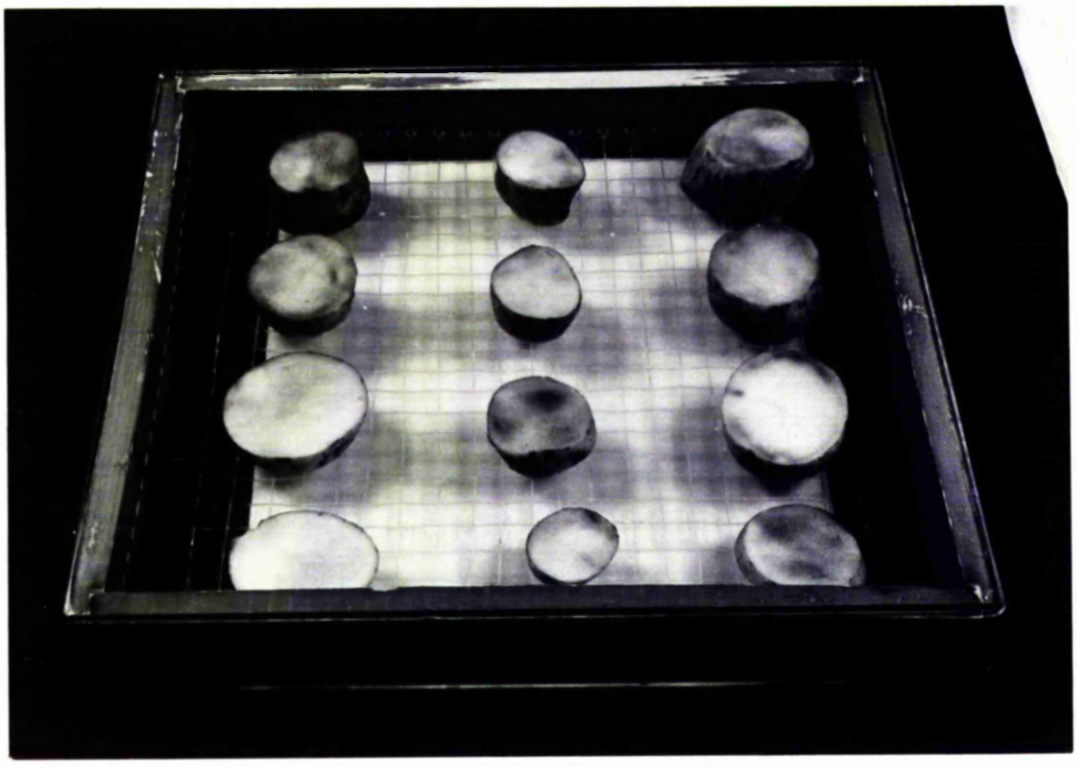
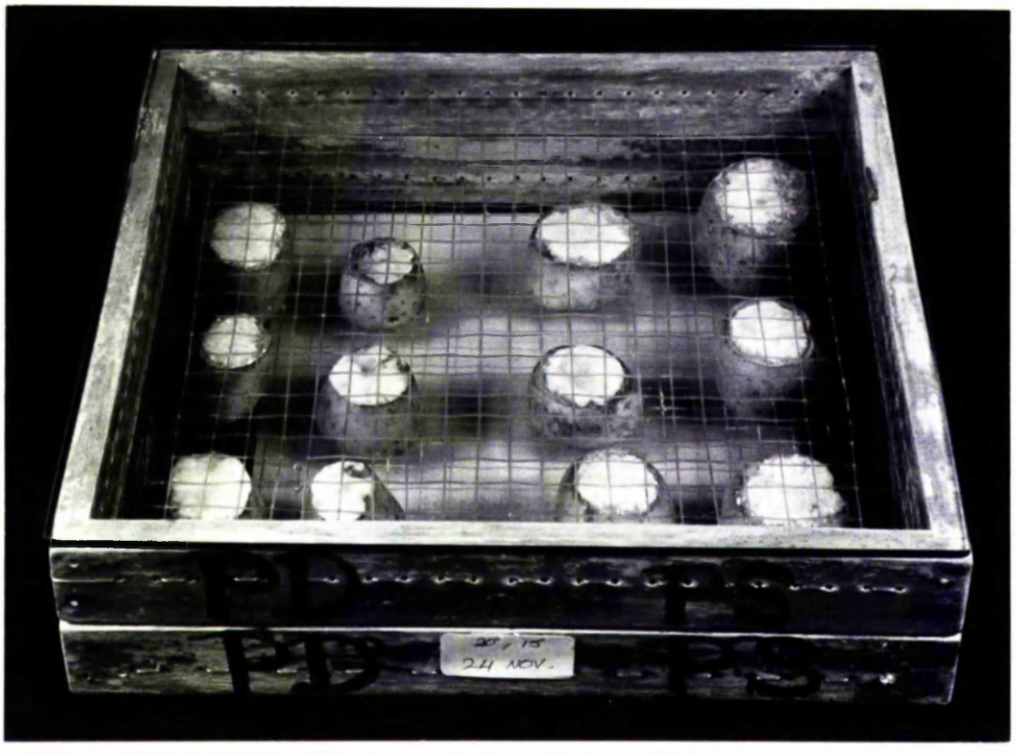


Figure 2 The wooden tray used for incubating the slices. The trays were covered top and bottom with sheets of glass lined with damp blotting paper to maintain a high relative humidity.

Figure 3 Two wooden trays joined together. Used when incubating slices too thick to fit in a single tray.




22/15
24 NOV.

1 - Hyphal Growth in Tuber Medullary Tissue

Materials and Methods

Slices 7 mm, 12 mm, 17 mm, 22 mm, and 33 mm thick cut from tubers of the five clones were used. The slices, after inoculation, were incubated at 20°C and the time for sporulating mycelium to appear on the lower surface of the slices of different thickness was recorded. This experiment was carried out twice using all five clones and repeated once more for all clones except Pentland Raven.

Results

The time required for hyphae to grow through the slices and form sporangia on the lower surfaces in all experiments are given in Tables (1, 2, 3) in the appendix. An analysis of the correlation between time for sporangia to develop on the lower surface, and slice thickness shows that the two are significantly correlated in all cases ($P < 0.001$).

In general the results of the three experiments are in agreement and so the results of one experiment only is presented in detail here. The regression lines of the mean time required for hyphae to grow through and form sporangia on the lower surface of the slices of the five clones against slice thickness are plotted graphically in Figure 5.

The regression equations are:

Craigs Alliance	$Y = 6.16x + 93.00$
Pentland Dell	$Y = 5.74x + 114.42$
Pentland Squire	$Y = 5.99x + 103.80$
Pentland Raven	$Y = 5.06x + 119.30$
6003ab(33)	$Y = 6.37x + 123.30$

Y = mean time from inoculation until the appearance of sporulating mycelium on the opposite surface of slice

x = Slice thickness

An analysis of joint regression, based on the method given by Mather (1949) shows that the slopes of the regression lines are not significantly different (See Table 2 of the text and Tables 2a, 3a of the Appendix) showing that the growth rate in the medullary tissue of tubers of all clones was more or less identical.

When the regression lines are extrapolated back to the time axis, however, they do not intercept the axis at the same point. The distance along the axis represents the time required for two of three phases of fungal growth (See diagram 4). These two phases are:-

I. The phase of germination and initiation of hyphal growth

III. The phase of establishment of visible mycelium on the lower surface of the slice.

By subtracting the time required for phases I and III from the total time taken from inoculation until sporangia develop on the lower surface it is possible to calculate the time required for phase II, that is for the fungus to grow through the slice. From this the growth rate in mm/day can be calculated.

$$\text{Growth rate mm/day} = \frac{\text{Slice thickness} \times 24}{\text{Total time} - \text{Time at point of intercept}}$$

An analysis based on the method by Mather (1949) was carried out to determine if the differences between the points of intercept were statistically significant. The results (Table 3) show that there are significant differences between Craigs Alliance and 6003ab(33) and Pentland Squire and 6003ab(33), but not between the other clones. However, the same differences are not found in the other two experiments, for in one experiment none of the differences are significant (See Table 2b of the appendix), while in the other experiment the differences between the points of intercept for Pentland Squire and 6003ab(33), Pentland Dell and 6003ab(33) and

and Craigs Alliance and Pentland Squire are significant (See Table 3b of the Appendix), the difference between Craigs Alliance and 6003ab(33) is not.

The times required for the growth phases I and III from all experiments involving the four clones which have been carried out over the three years 1973-1975 are listed in order of rank in Table 4. A Friedman two-way analysis of variance (Siegel, 1956) was carried out to determine whether the association with rank was due to chance variation or due to varietal differences. The value of $Xr^2 = 8.7$ was computed using the formula:-

$$Xr^2 = \frac{12}{NK(K + 1)} \qquad (R_j)^2 \quad - \quad 3N(K + 1)$$

The result indicates that association with rank was most likely due to varietal differences ($P = 0.014$). Thus this test reveals significant differences between clones. Pentland Raven was not included in the analysis because it was not included in two of the four experiments.

Discussion

The growth rate in the five clones tested was not significantly different in any experiment and so resistance factors affecting hyphal growth rate would appear to play little or no role in the differences in race non-specific resistance between these clones.

The differences between the points of intercept on the graphs (Figure 5) indicates that the time required for the fungus to penetrate and establish itself in the tissues and to develop visible mycelium on the opposite surface once it has grown through (phases I and III) may be significantly shorter in some clones than in others. In general these phases of growth took longest in 6003ab(33) while they were shortest in Pentland Squire. The small differences between the points of intercept of some clones may be due to the big inoculum used in

the experiments and so by using a smaller inoculum bigger differences might be obtained.

6003ab(33), which appears to have the greatest race non-specific resistance according to the Pentlandfield scale also appears most resistant to growth phases I and III in these tests. However, Pentland Squire which has an intermediate level of resistance according to the Pentlandfield scale appears most susceptible in these tests, while the most susceptible clone, Craigs Alliance, according to the Pentlandfield scale, has a moderate level of resistance.

Although these experiments do not differentiate between the importance of the time required for each of the two phases, germination and initiation of hyphal growth (phase I), and establishment of visible aerial mycelium (phase III) in tissue resistance, other work indicates that it is the first phase which is the most important (Personal communication from Dr. Clarke). Further work on this aspect is required.

In one of the experiments the hyphal growth rate appeared to be slightly slower in the thicker slices than in the thinner slices in all the five clones. Although the differences in growth rates were not statistically significant it was considered that they could have been due to the developing wound periderm on the lower surfaces of the slices, which, by the time the fungus had grown through, had developed to a stage where it was just beginning to impede growth.

The next experiment was carried out to determine if the wound periderm could be the site of resistance to hyphal growth, or whether a more general resistance involving more of the tissues was developing in the thicker slices.

Figure 4 The three phases involved in the colonization of tuber tissue slices from inoculation until the appearance of sporangia on the opposite surface

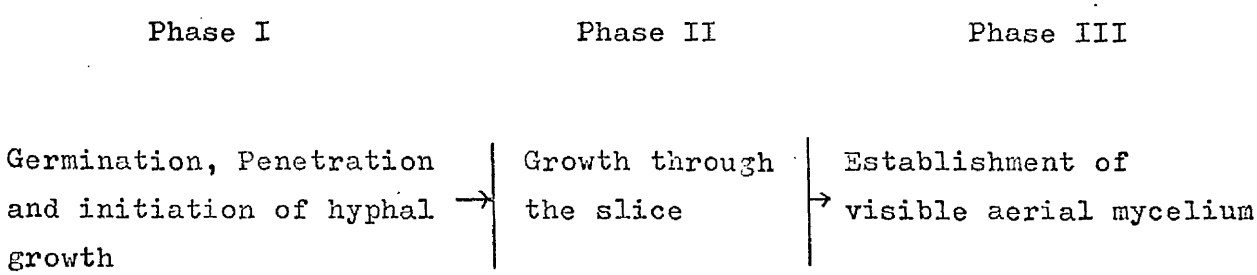


Table 2 Joint regression analysis of variance of hyphal growth rate in tuber slices of the five clones

Source of Variance	N	M.S.	V.R.	P
Joint regression	1	85189.45	2031.706	
Heterogeneity of regression	4	24.94	--	NS
Differences between means	4	1121.90	26.756	
Error	20	41.93		

Table 3 Analyses of differences between points of intercept of the regression lines of pairs of clones

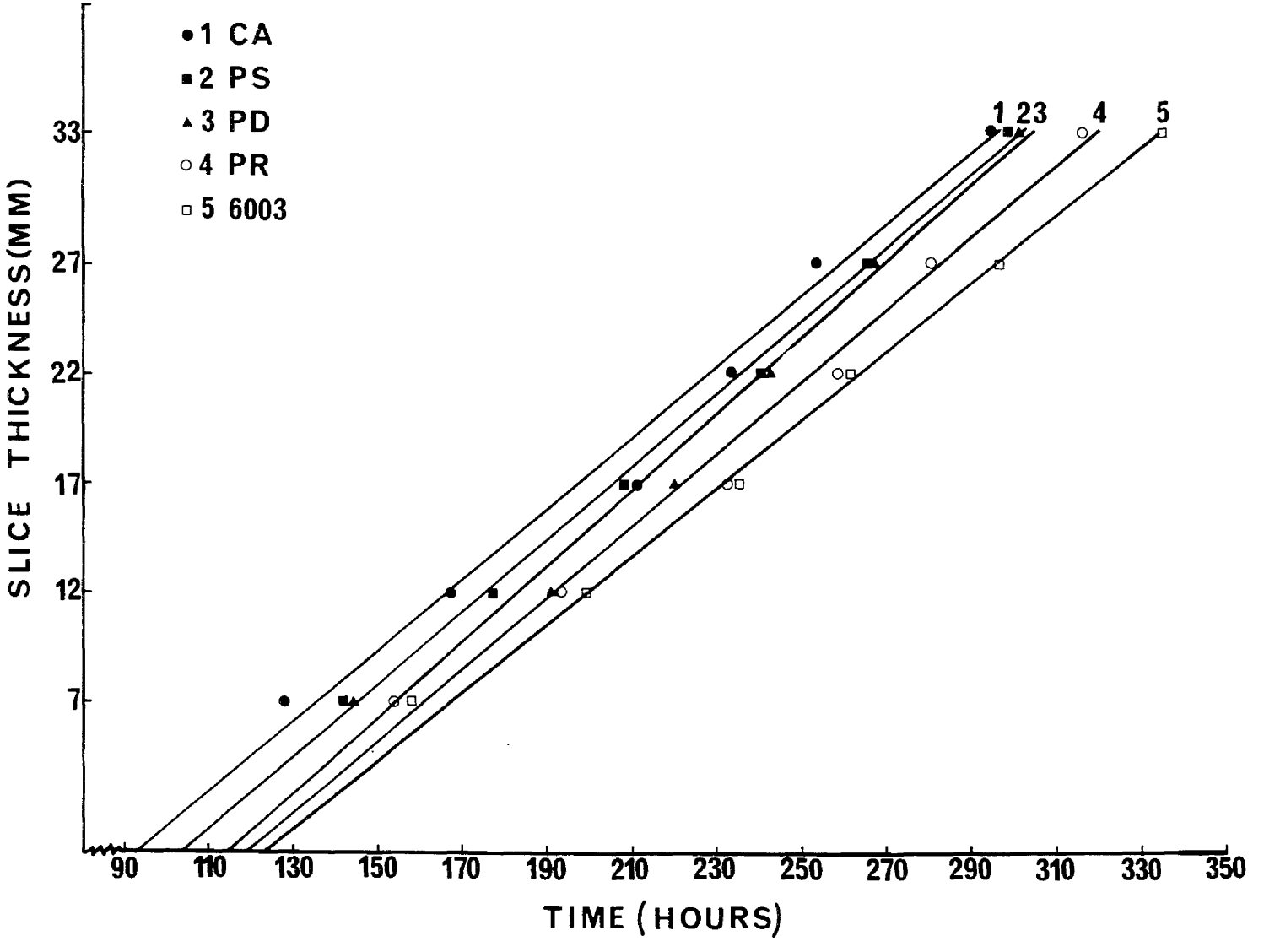
Clones being compared	Degrees of freedom	Value of t	P
Craigs Alliance vs. 6003ab(33)	8	3.30	$P < 0.02$
Craigs Alliance vs. Pentland Raven	8	1.79	NS
Craigs Alliance vs. Pentland Dell	8	1.81	NS
Pentland Squire vs. 6003ab(33)	8	4.53	$P < 0.01$
Pentland Squire vs. Pentland Raven	8	1.26	NS
Pentland Squire vs. Pentland Dell	8	1.17	NS
Pentland Dell vs. 6003ab(33)	8	1.08	NS

Table 4 Friedman two-way analysis of variance by rank

Experiment	6003ab(33)	Pentland Dell	Pentland Squire	Craigs Alliance
1	1	2	3	4
2	1	2	4	3
3	1	3	4	2
4	2	1	4	3
R_j	5	8	15	12

$$Xr^2 = 8.7 \qquad P = 0.014$$

Figure 5 Regression lines of time required for hyphae to grow through and form sporangia on the lower surface of slices against slice thickness for Craigs Alliance (CA), Pentland Squire (PS), Pentland Raven (PR), Pentland Dell (PD) and 6003ab(33).


●1 CA
■2 PS
▲3 PD
○4 PR
□5 6003
1 2 3
4
5
SLICE THICKNESS(MM)
33
27
22
17
12
7
90
110
130
150
170
190
210
230
250
270
290
310
330
350
TIME (HOURS)

2 - Effect of wound periderm on the lower surface of the slices on hyphal growth

Materials and Methods

Tubers of each of the four clones, Craigs Alliance, Pentland Dell, Pentland Squire and 6003ab(33) were surface sterilized and slices 30 mm, 40 mm, 50 mm and 60 mm thick for each clone were cut and inoculated as described on page 21. Slices 23 mm, 33 mm, 43 mm and 53 mm thick were also cut and inoculated in exactly the same way as the other slices. All slices were incubated at 20°C. With the assumption that the hyphal growth rate was independent of slice thickness and occurred at approximately the same rate in thin and thick slices, the slices were incubated until it was judged that the hyphal front was about 10 mm from the lower surface. Then a slice 7 mm thick was removed from the lower surface of the slices 30 mm, 40 mm, 50 mm and 60 mm thick. This removed any wound periderm which had developed. The wound periderm of the 23 mm, 33 mm, 43 mm and 53 mm thick slices was not removed. The time taken for sporulating mycelium to appear on the lower surface of all slices was recorded.

Results and Discussion

The results are given in Tables (4i, 4ii) of the Appendix and the means are plotted graphically in Figure 6. The graph shows that growth through the slices of all thickness from which the wound periderm had been removed, was linear with time and indicated that the apparent reduced rate of growth through the slices with the periderm was due to the periderm or to adjacent tissues. In an attempt to determine whether there was any variation in the degree of resistance of this periderm between the clones, the growth rates in the last five millimeters of the slices, including the wound

periderm, was calculated. The calculation was based on the assumption that the growth rate in the slices up to the last five millimetres was uniform and equal to the growth rate in the slices from which the wound periderm had been removed. The growth rates are given in Table 5 and show a much lower growth rate in the periderm tissue zone which decreases with time after wounding, indicating that the resistance of the wound periderm increases with time, but this increase occurs at approximately the same rate in all the clones tested. The growth rate in the periderm zone of all the clones was similar indicating that there is no variation in the degree of resistance of the periderm in these clones. In conclusion the wound periderm appears to be responsible for any inhibition of hyphal growth in slices 23 mm and thicker. The effect of the wound periderm on the penetration of the pathogens into the tuber tissue is well documented (Müller, 1957; McKee, 1954), and its resistance has been attributed to the presence of inhibitory compounds such as phenols and steroid glycoalkaloids (see Page 5). However, in this investigation, periderm resistance has been investigated using hyphae which have colonized the tuber tissue internally before growing from the inside of the slice through the periderm to form sporangia on the outer surface. While this is not necessarily similar to the processes involved in the penetration from the outside surface into the tissue, the resistance factors involved may well be similar.

Thus this experiment shows that changes in resistance do occur during the formation of the periderm which affect hyphal growth. However, these changes are restricted to within or near the periderm and do not affect the rest of the tissue.

The linearity of hyphal growth found in this experiment and the earlier work indicates that the resistance of the uninvaded tuber

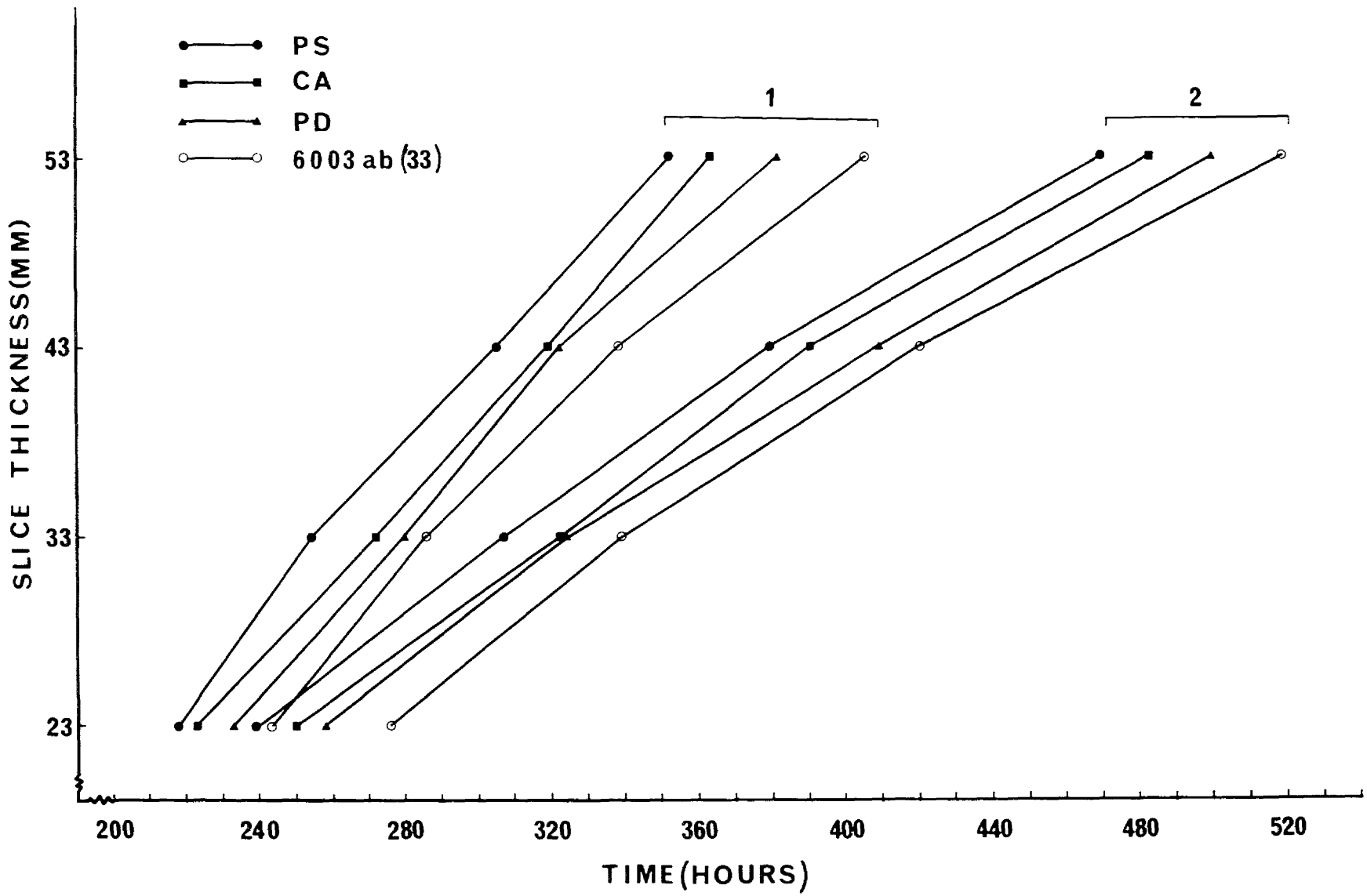
tissue in advance of hyphal growth does not increase or decrease on prolonged contact with adjacent invaded tissue. However, this work does not rule out the possibility of constant changes in tissue resistance occurring only after contact with the advancing fungal hyphae. If such changes do occur they are clearly not at a high enough rate to completely inhibit hyphal growth. Such changes, however, might be detected by using treatments which reduce hyphal growth rate but do not reduce, to the same extent, the rate of development of tissue resistance.

The next experiment was carried out to determine if temperature of incubation has a differential effect on hyphal growth rate and tissue resistance and could thus be used to investigate this point.

Table 5 Growth rate (MM/day) in the last five millimetres of the slice, including the wound periderm, in slices of three different thicknesses.

Clone	Growth rate (mm/day)		
	33 mm thick	43 mm thick	53 mm thick
Craigs Alliance	2.42	2.75	1.77
Pentland Dell	2.90	1.99	1.86
Pentland Squire	2.69	2.42	1.77
6003ab(33)	3.32	2.21	1.69

Figure 6 Mean time required for hyphae to grow through and form sporangia on the lower surface of slices of different thickness from which the wound periderm had (1) and had not (2) been removed.


PS
CA
PD
6003 ab (33)
1
2
SLICE THICKNESS(MM)
53
43
33
23
200
240
280
320
360
400
440
480
520
TIME (HOURS)

3 - Effect of temperature on the rate of hyphal growth through the medullary tissue of the tuber

Materials and Methods

Tuber slices of Pentland Squire 7 mm, 12 mm, 17 mm, 22 mm and 27 mm thick, were inoculated and incubated as before but at the different temperatures stated in the text. The slices were examined microscopically at intervals and the time required for the hyphae to grow through the slices and produce sporulating mycelium on the lower surfaces at the different temperatures was recorded.

Results and Discussion

A preliminary experiment was carried out using temperatures of incubation of 15°C, 20°C and 25°C. This experiment indicated that the optimum temperature for growth was around 20°C (see Table 6). The results of a more detailed experiment using temperatures of 15°C, 18°C, 20°C, 22°C and 25°C are given in Table 5 of the appendix. The regression mean times for hyphae to grow through and form sporangia on the lower surface of the slices against slice thickness was calculated and is plotted graphically in Figure 7.

The regression equations are:-

15°C	$Y = 8.26X + 76.18$
18°C	$Y = 7.48X + 59.24$
20°C	$Y = 6.08X + 65.64$
22°C	$Y = 7.42X + 54.86$
25°C.	$Y = 7.62X + 77.06$

when

Y = Mean time from inoculation until the appearance of sporulating mycelium on the opposite surface of slice

X = Slice thickness

An analysis of joint regression was carried out based on the

method given by Mather (1949) and the results show significant differences between the slopes of the regression lines at the different temperatures (Table 7) and so temperature clearly affects hyphal growth rate with a temperature of about 20°C appearing to be the optimum for hyphal growth (see Table 6).

However, the growth rate was linear at all temperatures and so if temperature has an effect on the rate of development of resistance factors then the rates of such changes and hyphal growth rate would appear to be equally affected by temperature or, even at the temperature giving the fastest accumulation, the levels accumulated are still not high enough to affect hyphal growth at the edge of the colony.

Thus this experiment does not provide any evidence for an active component in resistance to hyphal growth, but indicates that tissue resistance is most likely determined by passive factors with the tissue acting more or less as an inert substrate for fungal growth. However, the rate of growth is low compared to that which usually occurs in culture, and so the following experiment was carried out to determine the growth rate and the optimum temperature for growth <u>in vitro</u> of the isolate used in these studies to obtain some indication of the extent of the inhibitory effects of passive resistance factors in the host tissue.

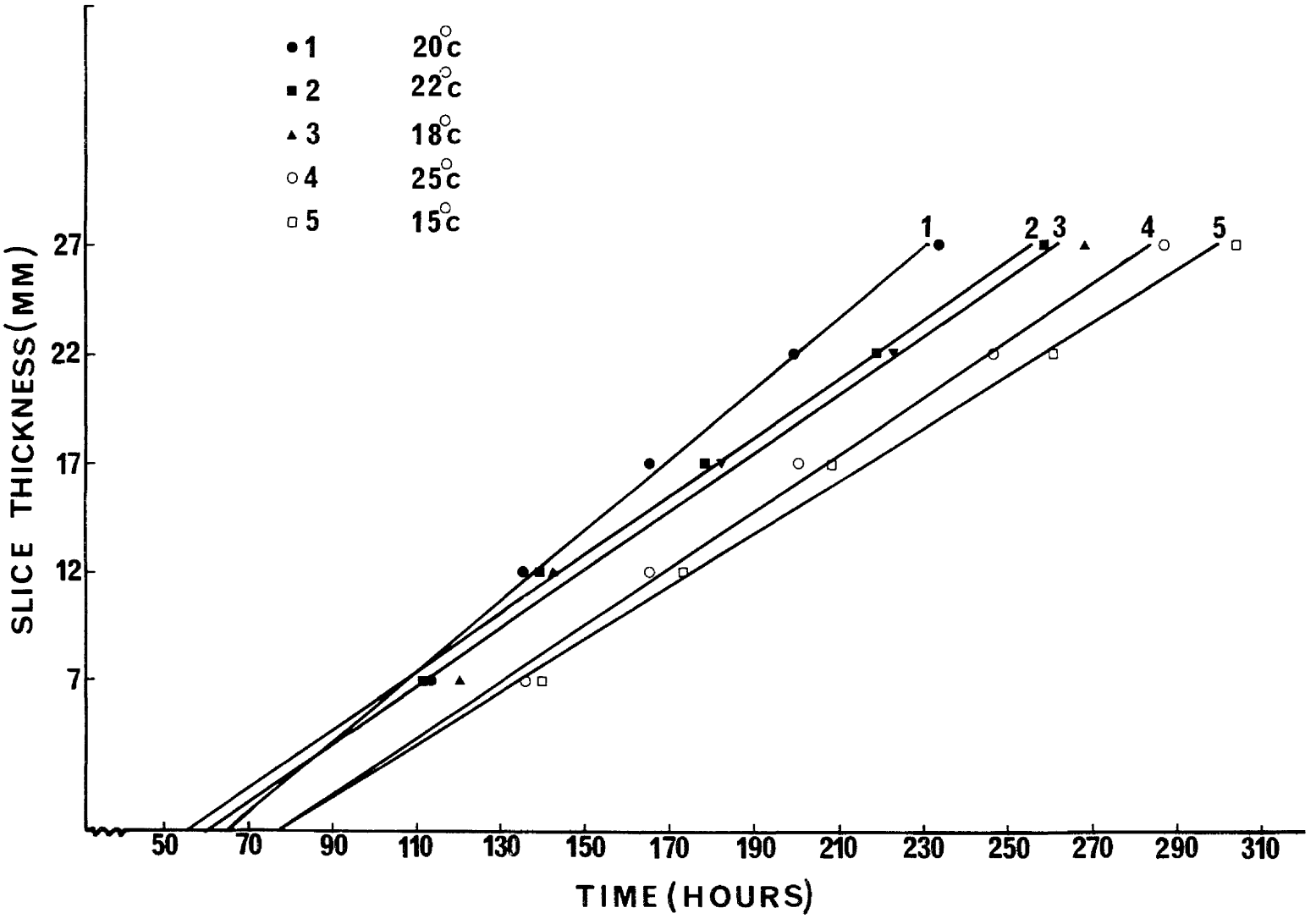
Table 6 The effect of temperature on hyphal growth rate

Temperatures	Growth rate mm/day	
	Expt. 1	Expt. 2
15°C	4.48	2.86
18°C		3.12
20°C	4.56	3.87
22°C		3.19
25°C	3.68	3.10

Table 7 Joint regression analysis of variance of hyphal growth rate in tuber slices of Pentland Squire incubated at five different temperatures

Source of Variance	N	M.S.	V.R.	P
Joint regression	1	67932.98	1796.218	
Heterogeneity of regression	4	158.33	4.186	<0.05
Differences between means	4	1874.56	49.565	
Error	15	37.82		

Figure 7 Regression lines of time required for hyphae to grow through and form sporangia on the lower surface of slices against slice thickness for Pentland Squire incubated at five temperatures.


● 1 20°c
■ 2 22°c
▲ 3 18°c
○ 4 25°c
□ 5 15°c
SLICE THICKNESS (MM)
27
22
17
12
7
1
2 3
4
5
50 70 90 110 130 150 170 190 210 230 250 270 290 310
TIME (HOURS)

4 - Effect of different nutrient sources on hyphal growth rate

Materials and Methods

The following media were used:-

1. French bean agar (Hall, 1959). This medium was prepared as described on page 21.

2. Pea sucrose agar (Keays, 1953). The medium consisted of:-

Frozen peas (Birds Eye)	250 g.
Sucrose	20 g.
Oxoid agar	15 g.
Distilled water to	1 l.

The peas were macerated in a Waring blender with water, and strained through muslin. The sucrose and Oxoid agar were added and the medium was then autoclaved for 15 minutes at 10lbs/in^2.

3. Minimal medium. This medium had the following composition.

KH_2PO_4	0.500 g.
$MgSO_4 7H_2O$	0.250 g.
Glucose	25.000 g.
Asparagine	1.000 g.
Thiamine	1 ml stock soln. (0.001/g)
Oxoid Agar	20.000 g.
Water	1 l.

The medium was adjusted to pH 5.5 using N.HCl or N.NaOH as required before autoclaving for 10 minutes at 10lbs/in^2.

4. Complete medium (Clarke, 1966). It had the following constituents in addition to those listed for the minimal medium above:-

Yeast extract	5.0 g.
Casamino acid	2.0 g.
Pectin	10.0 g.

This medium was also adjusted to pH 5.5 using N.HCl or N.NaOH

as required before autoclaving for 10 minutes at 10lbs/in^2.

After autoclaving, approximately 20 ml of each medium was poured into each of 15, 10 cm petri dishes. The plates were inoculated with 10 mm diameter agar discs cut from two week old cultures grown on French bean agar medium using a flamed No. 6 Cork borer. The plates were then incubated in the dark in constant temperature incubators maintained at the following temperatures:-

15°C, 18°C, 20°C, 21°C, 22°C and 25°C.

Two previously marked arbitrary diameters, at right angles to each other, were measured to the nearest millimetre, five, six and seven days after inoculation and the mean of these two diameters for each petri dish was recorded.

Results and Discussion

Colony diameters are given in Table 6 in the Appendix. The mean growth rates mm/day are plotted graphically in Figure 8. The fungus grew fastest and had the highest density of mycelium on Pea sucrose and French bean agar, while growth was slowest and the mycelium density was least on the minimal medium. However, the growth rates on all media were much greater at all temperatures than the growth rates in vivo (see Table 6).

The optimum temperature for hyphal growth was about 21°C on all media and thus is similar to the optimum temperature for growth in vivo (20°C). It thus appears not to be markedly affected by nutritional factors.

The optimum temperature for fungal growth in vivo found in these experiments is in agreement with that reported by Vowinckel (1926) who stated that a temperature of 19° - 21°C is most favourable for the spread of the mycelium in the tuber. A greater range of optimum

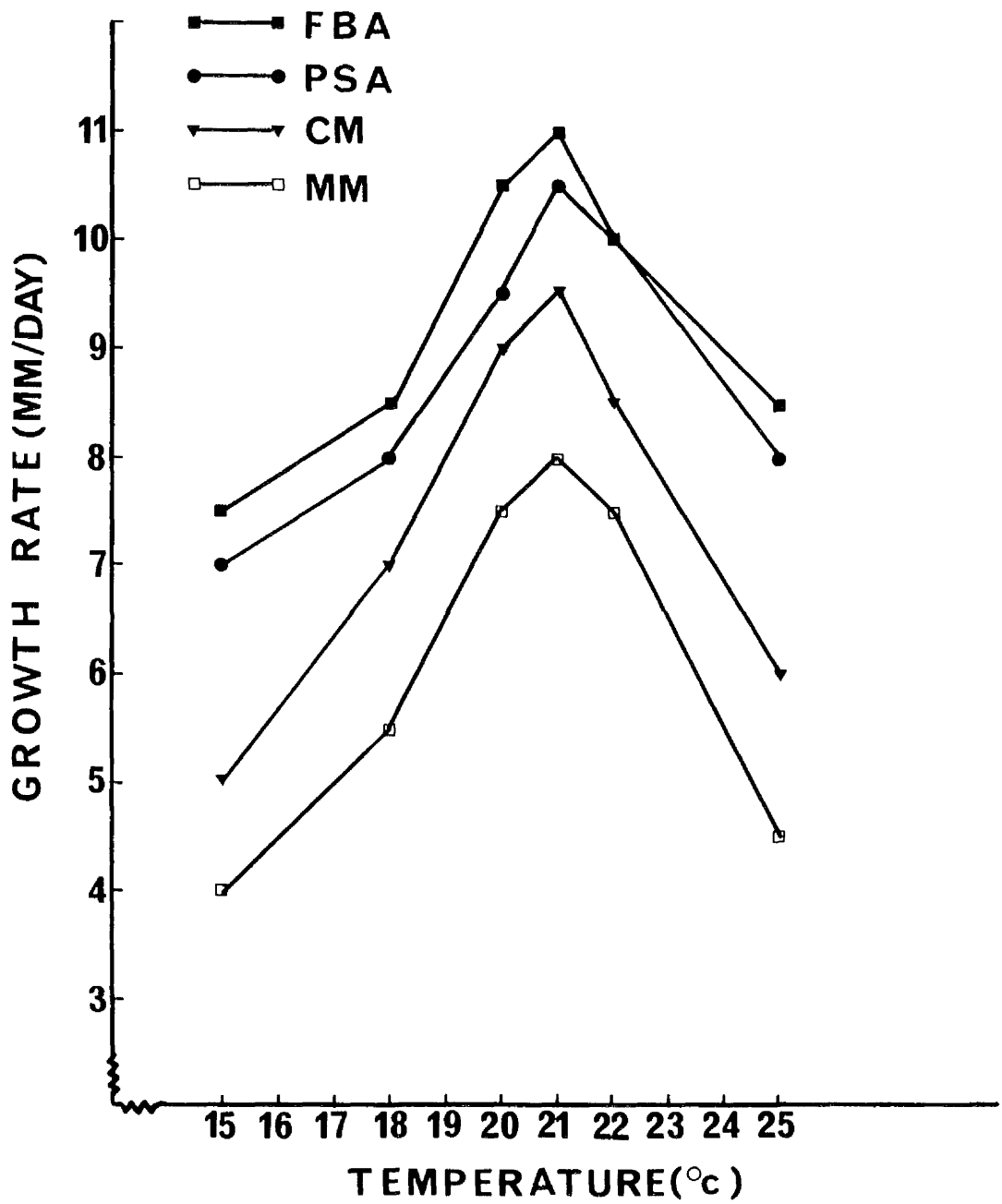
temperatures for hyphal growth *in vitro* has been reported in the literature. Jones *et al* (1912) reported that the best growth on Lima bean agar and other vegetable and synthetic media was between 16°C and 19°C. Crosier (1934) found that the optimum temperature for growth of the fungus on Oatmeal dextrose agar was 21°C. Kaung Zan (1962) found that the optimum temperature for hyphal growth on Chick-pea sucrose agar was 20°C and a similar optimum was found by Hodgson & Grainger (1964) for growth on a rye agar medium. Vodyanaya & Khromova (1973) reported that different strains of *P. infestans* had different optimum temperatures for mycelial mass formation in culture. Thus the differences between the optimal temperatures found by different investigators may be because different races were used.

The growth rate is lower *in vivo* than *in vitro* indicating that host tissue does have marked inhibitory effects on hyphal growth. This inhibition could be due to one or more of the following factors:-

1. Hyphae growing in the tissue may be restricted by the narrow intercellular spaces which physically inhibit to some degree, hyphal growth. However, it seems to be unlikely that the growth rate is physically restricted by the size of the intercellular spaces since observations show that the majority of the spaces are much wider than the hyphae. A more convincing reason is that hyphae growing between the parenchyma cells have to grow round the cells and thus can not follow the straightest path as they can on media. Thus they require more time to grow through the slice than would be expected if the shortest possible route were taken.

2. Hyphae may have difficulty in absorbing nutrients from the cells since they grow intercellularly. Thus the absorption of nutrients may not be so direct and easy as when the fungus is growing on media. Haustorial formation may be necessary in order to obtain nutrients.
3. The nutrients may be present but not in an available form, or present in inadequate amounts, or in the wrong proportions.
4. Inhibitory substances may be present affecting hyphal growth.

Figure 8 Optimal temperature for growth of P. infestans on French bean agar (FBA), Peas sucrose agar (PSA), Complete medium (CM) and Minimal medium (MM).


FBA
PSA
CM
MM
GROWTH RATE (MM/DAY)
11
10
9
8
7
6
5
4
3
15 16 17 18 19 20 21 22 23 24 25
TEMPERATURE(°c)

5 - Resistance of different regions of the tuber to hyphal growth

Introduction

In the previous experiments, considerable variation was found to occur in the times between inoculation and the development of sporulating mycelium on the lower surface of slices of the same thickness in each clone. Since slices of each thickness were taken from different regions of the tuber, some from the rose end, others from the heel end, and others more centrally, then this variation might be a reflection of differences in tissue resistance in different parts of the tuber. Some variation may also be due to differences in resistance of the tissues of tubers of different sizes. The following experiments were carried out to investigate these points.

5.1 Hyphal growth in the medullary tissue at rose and heel ends of the tubers

Materials and Methods

Ten tubers from each of the three clones Pentland Dell, Pentland Squire and 6003ab(33) were surface sterilized before cutting slices 7 mm and 12 mm thick from the rose and heel ends of the tuber. The slices were inoculated in the centre of the medullary tissue with a zoospore suspension before incubating at 20°C. The time for sporulating mycelium to first appear on the lower surface was recorded. The experiment was repeated using the clones Pentland Dell and Pentland Squire only.

Results

The time required for the fungus to grow through and form sporangia on the lower surface of the slices from the rose and heel ends are given in Table 7 in the Appendix. The analyses of variance (Table 8) shows that the differences in the total time (time required for the

three phases of the growth) between the two different regions in all three clones are not significant and thus the medullary tissue of both ends of the tuber appears to be equally susceptible either to fungal penetration and initiation of hyphal growth and the establishment of sporulating mycelium on the lower surface (phase I and III) or to hyphal growth (phase II).

Table 8 Analyses of variances between the hyphal growth in the heel end and rose end tissues

Clones	Source of Variation	N	M.S.	V.R.	P
a- Pentland Squire	Between thicknesses	1	2924		
	Between rose and heel end	1	1102	0.6	NS
	Residual	36	1832		
b- Pentland Dell	Between thicknesses	1	7290		
	Between rose and heel end	1	3	0.1	NS
	Residual	36	232		
c- 6003ab(33)	Between thicknesses	1	11425		
	Between rose and heel end	1	410	1.3	NS
	Residual	36	313		

5.2 Hyphal growth in the medullary tissue of small and large tubers

Materials and Methods

Five small tubers approximately 5.5-6.0 cm long x 3-4 cm diameter and five large tubers approximately 10.5-11 cm long x 6-6.5 cm diameter of each of the two clones Pentland Dell and Pentland Squire were surface sterilized before cutting into 7 mm, 12 mm and 17 mm thick slices. The slices were inoculated with a zoospore suspension and then incubated at 20°C.

Results

The time required for the fungus to grow through and form sporangia on the lower surface of the slices are given in Table 8 in the Appendix. An analysis of variance of the data was carried out (Table 9) and showed no significant differences which could be attributed to differences in resistance between the medullary tissue of large and small tubers. A repeat of this experiment gave essentially the same result. Thus in the two clones the resistance of the medullary tissue from large and small tubers would appear to be more or less identical.

Table 9 Analyses of variances between the hyphal growth in the small and large size tuber

Clone	Source of Variation	N	M.S.	V.R.	P
a-Pentland Dell	Between thicknesses	2	14289.5		
	Between small and large tubers	1	480	2.9	NS
	Residual	24	163		
b- Pentland Squire	Between thicknesses	2	27825		
	Between small and large tubers	1	43	0.3	NS
	Residual	24	141		

5.3 Hyphal growth in cortex and medullary tissue of the tuber

Materials and Methods

Five tubers from each of the two clones Pentland Dell and Pentland Squire were surface sterilized and slices 7 mm, 12 mm and 17 mm thick were cut. They were inoculated immediately after cutting with 10 mm discs cut (using a No. 6 flamed cork borer) from two week old petri dish cultures growing on French bean agar. Mycelium infested discs were used instead of a zoospore suspension because of the danger that a zoospore suspension would spread away from the inoculation point. For each slice one disc was put on the upper surface in the cortical region and another in the centre of the medullary tissue. The slices were incubated at 20°C. The experiment was repeated using the same clones.

Results

The time required for the fungus to grow through and form sporangia on the lower surfaces of both cortex and medullary regions are given in Table 9 in the Appendix. From the table it can be seen that in general the time required for hyphae to grow through the cortex appears to be shorter than that required for growth through the medullary tissue. Thus analysis based on the method by Mather (1949) was carried out to determine if the differences between the points of intercept (Time for growth phases I and III calculated by regression) were statistically significant, but the results showed that they were not (Table 10). An analysis of variance of the data also showed that the time required for all three phases of growth through the medullary tissue was not significantly different from that required for growth through the cortical tissue (Table 11). Thus it can be concluded that the cortex and medullary tissue of the tubers of the two clones are equally susceptible to all phases of growth.

Table 10 Analyses of differences between point of intercept of the regression lines for cortex and medullary tissues

Clone	Intercepts being compared	N	t	P
Pentland Dell				
Expt. 1	Cortex vs. medulla	6	0.001	NS
Expt. 2	Cortex vs. medulla	6	1.337	NS
Pentland Squire				
Expt. 1	Cortex vs. medulla	6	0.220	NS
Expt. 2	Cortex vs. medulla	6	0.887	NS

Table 11 Analyses of variance between the hyphal growth in cortex and medulla

Clone	Source of Variation	N	M.S.	V.R.	P
a-Pentland Dell	Between thicknesses	2	12950		
	Between cortex and medulla	1	464	3.4	NS
	Residual	24	138		
b-Pentland Squire	Between thicknesses	2	13839		
	Between cortex and medulla	1	30	0.2	NS
	Residual	24	159		

Discussion

The range of variation in the time taken from inoculation until the appearance of the sporulating mycelium on the opposite surface of the slices from the different parts of the tuber, and from tubers of different sizes, is similar to that variation obtained in the earlier experiments. Since the variation between different parts of the tuber and between tissues from small and large tubers were found not to be statistically significant, it cannot be due to differences in the resistance between the various parts of the tuber. Thus the variation found in the earlier experiments must have been due to error or chance variation alone.

Cheng & Hanning (1955) found that chlorogenic acid, caffeic acid and L-tyrosine are the most common phenols in potato tuber tissue. Hasegawa et al. (1966) found that nearly 70% of the total phenol content was chlorogenic acid and that the phenol concentration was low in the centre of tuber, and increased greatly towards the cortex. According to Umaerus & Olsson (1972) the heel end of the tuber usually has a higher chlorogenic acid content than the rose end, while the centre of the tuber has the least. Thus if the passive resistance of the potato tissue is based upon the presence of these phenols then the tissue of the cortex and of the heel end of the tuber would be expected to be more resistant to hyphal growth than the tissue of the central and rose end of the tuber. The tubers tested here did not show any variation in resistance of their different parts and this may indicate that these compounds are not involved in tissue resistance. This may be because the hyphae grow intercellularly and are not directly affected by these compounds which are located inside the cells. Such is the case of Fusarium solani var. coeruleum where McKee (1954, 1955) found that potato tubers were susceptible to this

fungus while they are resistant to Fusarium avenaceum. He suggested that because the latter fungus grew intracellularly its limited growth may be due to the inhibitory factors present within the cell which then come into contact with the hyphae. The intercellular growth of Fusarium solani var. coeruleum would enable it to avoid direct contact with intracellular inhibitors.

Storage period in relation to tuber tissue resistance

Introduction

It has been reported that the resistance of the flesh of tubers of some varieties increased with maturity until harvesting and then decreased again early during storage, while in others the increase was maintained over a longer period (Boyd & Henderson, 1953). McKee (1964) reported that infection of Ulster Ranger tuber discs by race 4, an incompatible race, occurred only in one experiment, in which the discs were obtained from old tubers, also indicating that resistance may be lost after prolonged storage. Gausuzzaman (1966) on the other hand, from measurements of the diameter, depth and weight of rot during the period from July to February found a peculiar fluctuation in the susceptibility of the three clones Up-to-Date, Home Guard and Arran Victory during this period. Thus, he concluded that there was no consistent relationship between resistance and maturity. However, his measurements were of symptom expression and not of resistance to fungal growth.

The work reported so far in this thesis has involved the measurement of hyphal growth rates in five clones between November and May in the years 1973-1975. Thus it is possible to examine the results obtained in relation to possible changes in resistance in storage both in terms of resistance to the establishment of growth and formation of sporangia (Growth phases I and III) and in terms of resistance to hyphal growth rate (Growth phase II).

Results and Discussion

Hyphal growth rates of experiments carried out between November and May in the years 1973-1975 in the five clones are given in Table 12. The differences in the growth rate for experiments in the same month of the same year are bigger than differences between the beginning

and end of the season. These results indicate that tissue resistance to hyphal growth in these clones does not change during storage and during the three different seasons.

The time required for germination and initiation of growth and formation of sporulating mycelium on the opposite surface of the slices (phases I and III) obtained by extrapolating the regression lines back to the point of intercept on the time axis, are given in Table 13. Although there are some exceptions, from the table it can be seen that in general the time required for phases I and III reduces during storage indicating that the tissues became more susceptible to these two phases of growth during prolonged storage. It can be seen also that, in general, the fungus required more time for the two phases of growth in most clones in the year 1975 compared to that in 1973 and 1974, and this may be due to differences in tissue resistance from season to season, possibly due to differences in the environmental conditions during plant growth in these seasons. There is also the possibility that the fungus may have become less active in the year 1975 than before due to its prolonged growth in culture. However, Jones et al. (1912) reported that some strains of P. infestans have been grown in pure culture continuously for over five years without evidence of change in pathogenicity.

Table 12 Growth rate (mm/day) in relation to length of time in store

Clone	Year	NOV.	DEC.	JAN.	FEB.	MAR.	APR.	MAY
Craigs Alliance	1973	3.94		4.39				
	1974		4.14					
	1975			5.17				
Pentland Dell	1973	4.24		4.15				
	1974		3.63					
	1975			4.86		4.65 (2)	3.17 (2)	3.2 (2), 3.24 (2)
Pentland Squire	1973	4.08		3.86				
	1974	3.97	3.74		4.56, 4.85, 5.35			
	1975			3.87, 5.28	3.85	4.60 (2)	3.35 (2)	4.50, 3.31 (2). 4.36 (2)
Pentland Raven	1973	4.05		3.86				
	1974							
	1975							
6003ab(33)	1973	3.76		3.81				
	1974		3.77			5.20, 5.14		
	1975			4.38		3.65 (2)		
Mean		4.01	3.82	4.36	4.65	4.65	3.26	3.72

(2) Mean of two measurements

Table 13 Time (hrs) for length of combined growth phases I and III in relation to length of time in store

Clone	Year	NOV.	DEC.	JAN.	FEB.	MAR.	APR.	MAY
Craigs Alliance	1973	93		71.81				
	1974		99.2					
	1975			116.8				
Pentland Dell	1973	114.42		77.24				
	1974		81.63					
	1975			119.2		108.07(2)	56.8(2)	57.15(2), 82.7(2)
Pentland Squire	1973	103.8		62.8				
	1974	108.5	67.31		47.96, 63.9, 65.1			
	1975			65.6, 111.2	82.2	103.15(2)	53.4(2)	74, 45.35(2), 92.5(2)
Pentland Raven	1973	119.3		67.62				
	1974							
	1975							
6003ab(33)	1973	123.3		82.26				
	1974		113.68			93.56, 103.03		
	1975			114.9		117.6		
Mean		110.39	90.45	88.94	64.79	105.08	55.1	70.34

(2) Mean of two measurements

The relationship between lesion production in whole tubers and hyphal growth

Introduction

In the previous experiments the time taken for hyphae to grow through slices of equal thickness varies from clone to clone, being generally slowest in 6003ab(33) and fastest in Pentland Squire, but hyphal growth rates in all clones was more or less similar. No difference were found in the resistance of different parts of the tubers of particular clones to hyphal establishment and growth. Other workers have shown that different clones and even different regions of the tuber in certain clones, either in the case of invasion by P. infestans or by other pathogens vary in the rate of development of the necrotic tissue (De Bruyn, 1943; Boyd, 1952; Lapwood, 1961, 1965c). This does not mean that the rate of hyphal growth in these clones or in the different regions of the tubers of these clones is different, since hyphal growth rate may not be directly related to the development of necrosis. In fact, P. infestans grows in the living tissues adjacent to the necrotic lesion. The necrotic lesion is always surrounded by a zone of fluorescent tissue which results mainly from the accumulation of scopolin in response to invasion (Clarke, 1972, 1973), and the fungus must be growing at least within this fluorescent tissue and even possibly in the tissues ahead. Clarke (1972) has shown for a number of clones infected with P. infestans, a direct relation between the width of the fluorescent zone surrounding the necrotic lesion and the size of this lesion produced in a given time.

This investigation was carried out in an attempt to determine the rate of development of necrotic tissue and of the surrounding fluorescent tissues in the four clones, Craigs Alliance, Pentland Dell, Pentland Squire and 6003ab(33), and in the different regions of the

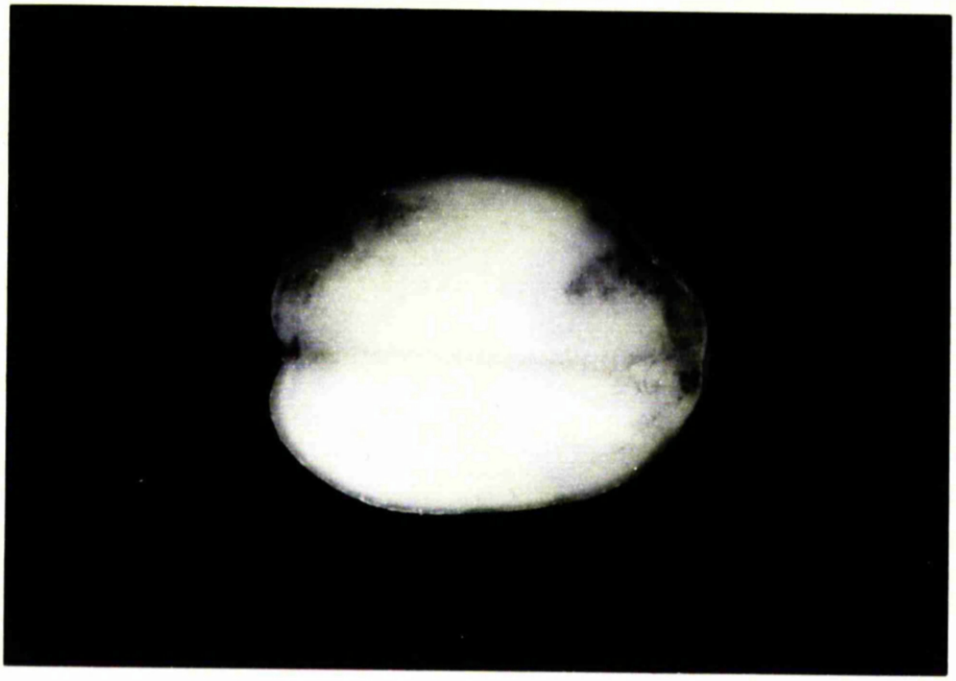
tubers of these clones in order to compare the rate of lesion development (necrosis plus fluorescence) with the rate of hyphal growth through tissue slices. Although we have no direct means of measuring hyphal growth rates in whole tubers, there is no evidence to indicate that the rate will be different from that in tissue slices since in all the experiments the rate has not been affected in any way by the thickness of the slices. Thus the comparison should be reasonably valid.

Materials and Methods

Ten tubers from each of the clones, Craigs Alliance, Pentland Dell, Pentland Squire and 6003ab(33) were surface sterilized in 10% chloros and then after drying in air, were washed with alcohol. Incisions were made at the rose and heel ends, about two to three millimetres below and parallel to the surface using a sterilized scalpel. The tubers were inoculated by inserting approximately similar amounts of masses of mycelium from French bean agar cultures, into the incisions. Pieces of filter paper soaked in sterile distilled water were sellotaped over the point of inoculation and the tubers were then placed in biscuit tins and covered with polythene bags to provide humid conditions conducive to infection. The tubers in the tins were then incubated at 20°C. Five days after inoculation, five tubers from each of the four clones were cut longitudinally through the middle of the incisions at both ends of the tubers (see Figure 9a). The other five tubers of each clone were cut in a similar way 15 days after inoculation. Radial measurements of the width of the necrotic tissue and of the surrounding fluorescent tissue were made in the cortex for each clone at both the rose and heel ends of the tuber (see Diagram 9b). The fluorescent zones were measured under a U.V. lamp.

Figure 9 (a) Necrotic lesions in a tuber of the clone 6003ab(33) cut longitudinally

(b) Diagram illustrating the position of the radial measurements of the width of the necrotic tissue and of the surrounding fluorescent tissue. The measurements recorded were the means of the measurements of the lesions at both sides of inoculation site.



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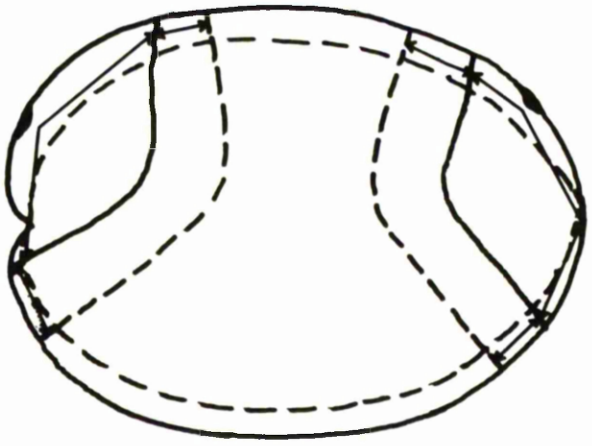


b

Results and Discussion

The results are given in Tables (10a, 10b) in the Appendix.

There was no significant difference in any clone between the size of the lesion (necrotic plus fluorescent tissue) produced at the rose and heel end of the tuber after five or 15 days of inoculation (Table 14a, 14b), and because of this the analyses for comparisons between pairs of clones were carried out on the combined measurements of lesion size at both ends. The analyses (Tables 15a, 15b) revealed significant differences in lesion size five days after inoculation only between clones Craigs Alliance and 6003ab(33), Craigs Alliance and Pentland Dell, and Pentland Squire and 6003ab(33). Differences between Craigs Alliance and Pentland Squire, Pentland Squire and Pentland Dell, and Pentland Dell and 6003ab(33) were not significant. However, there were significant differences in lesion size 15 days after inoculation between all clones, except between Craigs Alliance and Pentland Squire. Craigs Alliance had the widest lesion, while 6003ab(33) had the narrowest.

Subtracting the measurements of necrotic and fluorescent tissue obtained five days after inoculation from those obtained 15 days after inoculation gave the amount of necrotic and fluorescent tissue which had developed during the intervening ten day period. Thus it is possible to calculate the rate of lesion growth per day which occurs once active hyphal growth in the tissue is established.

$$\text{Lesion growth rate mm/day} = \frac{\text{Measurement of lesion obtained after 15 days} - \text{Measurement of lesion obtained after 5 days}}{\text{Intervening 10 days}}$$

Thus lesion development and growth can also be considered to occur in two phases:-

1 - Penetration and initiation of growth

2 - The rate of lesion growth

The minimum, maximum and mean rate of lesion growth and of hyphal growth calculated from the earlier experiments are given in Table 16. It can be seen from the Table that the rate of lesion growth appears to be lower than that of hyphal growth indicating that hyphae may be growing in tissue ahead of the lesion. Differences in the rate of lesion growth between clones are quite large with 6003ab(33) having the lowest rate.

It is also possible to calculate the time required for the penetration and initiation of growth (Phase I) from the following formula:

$$\text{Time (hrs)} = 360 - \frac{\text{Mean measurement of lesion at 15 days} \times 240}{\text{Mean measurement of lesion at 15 days} - \text{Mean measurement of lesion at five days}}$$

In the calculation of the two phases, the measurements of lesion size at both rose and heel end of the tuber were also combined since the differences in lesion size between both ends were not significant.

It can be seen from the table (17) that the time required for this phase (Phase I) was variable with Pentland Dell having the highest time. However, when the clones were classified according to speed of initiation of the lesion, the result was in approximate agreement with the classification according to the rate of lesion growth (Phase II).

The clones can be classified according to the rate of lesion growth in the following order of resistance. 6003ab(33) is the most resistant followed by Pentland Dell, with Pentland Squire and Craigs Alliance being most susceptible (Table 18). It must be borne in mind that resistance to lesion or symptom growth is being considered not resistant to the fungus itself. The classification obtained, however, is in agreement with the Pentlandfield Classification for the two

clones 6003ab(33) and Craigs Alliance and with Pentland Dell and Pentland Squire being intermediate between these two. However, Pentland Dell, which is more susceptible than Pentland Squire according to the Pentlandfield scale proved to be more resistant in this test.

The classification based on this test is in broad agreement with the result obtained earlier in this thesis which showed that 6003ab(33) had the highest level of resistance to the processes of fungal penetration and initiation of hyphal growth and formation of sporulating mycelium on the opposite surface of the slices (Phases I and III). However, Pentland Squire which showed an intermediate level of resistance based on rate of lesion spread, had the least level of resistance to fungal growth phases I and III (see Table 18).

Table 14 The mean width of the lesion (necrotic and fluorescent tissue) in the cortex at the rose end compared with that developed in the cortex at the heel end of the tuber

a- 5 days after inoculation

Clone	N	t	P
Craigs Alliance	8	0.20	NS
Pentland Dell	8	0.40	NS
Pentland Squire	8	1.00	NS
6003ab(33)	8	0.86	NS

b- 15 days after inoculation

Clone	N	t	P
Craigs Alliance	8	0.38	NS
Pentland Dell	8	0.07	NS
Pentland Squire	8	1.39	NS
6003ab(33)	8	0.77	NS

Table 15 Comparisons between pairs of clones of lesions size (necrosis and fluorescence) at the both ends of the tuber

a- 5 days after inoculation

Clones being compared	N	t	P
Craigs Alliance vs. 6003ab(33)	18	5.25	< 0.001
Craigs Alliance vs. Pentland Dell	18	3.71	< 0.01
Craigs Alliance vs. Pentland Squire	18	1.59	NS
Pentland Squire vs. Pentland Dell	18	1.48	NS
Pentland Squire vs. 6003ab(33)	18	3.31	< 0.01
Pentland Dell vs. 6003ab(33)	18	1.75	NS

b- 15 days after inoculation

Clones being compared	N	t	P
Craigs Alliance vs. 6003ab(33)	18	8.05	< 0.001
Craigs Alliance vs. Pentland Dell	18	4.62	< 0.001
Craigs Alliance vs. Pentland Squire	18	1.83	NS
Pentland Squire vs. Pentland Dell	18	2.50	< 0.05
Pentland Squire vs. 6003ab(33)	18	5.87	< 0.001
Pentland Dell vs. 6003ab(33)	18	3.80	< 0.01

Table 16 Comparisons of rates of lesion growth and hyphal growth in four clones

Clone	Minimum, Maximum and Mean rate of lesion growth (mm/day)			Minimum, Maximum and Mean hyphal growth rate (mm/day)		
	Minimum	Maximum	Mean	Minimum	Maximum	Mean
Craigs Alliance	2.74	4.14	3.38	3.94	5.17	4.41
Pentland Dell	2.26	3.56	2.90	3.20	4.86	3.89
Pentland Squire	2.66	4.06	3.31	3.31	5.35	4.23
6003ab(33)	1.76	2.96	2.37	3.26	5.20	4.24

Table 17 Time required for the initiation of lesion development in four clones

Clone	Time (Hours)
Craigs Alliance	10
Pentland Dell	26
Pentland Squire	20
6003ab(33)	25

Table 18 The order of resistance of the clones determined by the various tests compared with the Pentlandfield classification

Clone	Pentlandfield Classification	Classification according to resistance to fungal growth (phases I & III)	Classification according to speed of initiation of the lesion	Classification according to rate of lesion spread
6003ab(33)	1-2	1	2	1
Pentland Squire	3	4	3	3
Pentland Dell	4	2	1	2
Craigs Alliance	4^+	3	4	4

GENERAL CONCLUSIONS

Hyphal growth rate in all clones and in all tissues where tested was similar but much slower than in culture. Thus while there may be inhibitory factors in the tissue affecting hyphal growth they are equally active in all clones and can play no part in the differences in race non-specific resistance between the five clones. No obvious change occurred in the growth rate during prolonged storage of the tubers. The wound periderm formed on the surface of the slices was found to inhibit, to a certain degree, the growth of hyphae through to the outer surface and this effect increased with time.

The times required for the penetration and initiation of hyphal growth at the inoculated surface of the slice, and for the establishment of visible sporulating mycelium on the opposite surface, were different between clones usually taking longest in 6003ab(33) and being shortest in Pentland Squire and Craigs Alliance. Thus these phases of growth (phases I and III) constitute important factors in the differences in race non-specific resistance between the clones. In contrast to resistance to hyphal growth the time required for these latter phases of growth decreased towards the end of the storage season.

The rates of initiation and spread of lesions (necrotic and fluorescent tissue) in the clones were different. The rates were slower than those calculated for the initiation of hyphal growth and of hyphal growth itself, indicating that the hyphae grow ahead of the fluorescent tissue surrounding the necrotic tissue.

When clones were classified according to resistance to fungal growth (phases I and III), speed of initiation of the lesion and of rate of lesion spread, the results of all tests were in agreement with the Pentlandfield Classification which indicates that 6003ab(33) is the most resistant.

Pentland Dell, which is more susceptible than Pentland Squire according to the Pentlandfield classification, proved to be more resistant than Pentland Squire in all our tests. Pentland Squire was the most susceptible for the initiation of hyphal growth and the establishment of visible sporulating mycelium on the opposite surface (phases I and III), but Craigs Alliance proved to be the most susceptible to the speed of initiation of lesion and to the rate of lesion spread.

Temperatures of 15°C or 25°C reduced the rate of hyphal growth over that at 20°C, but the effect did not appear to result from alterations in the resistance of the tissues. This observation, together with the fact that the rate of hyphal growth in all clones in all experiments was linear with time, indicates that resistance does not involve active factors accumulating at the edge of the lesion to concentrations high enough to affect hyphal growth. Resistance would appear to be based on preformed passive factors entirely. However, active resistance may be involved with factors accumulating in the tissues behind the colony edge which have not yet been completely colonized by hyphae. If this does occur it might affect the extent to which lateral branches and haustoria develop to further colonise these tissues.

The work described in the next part was designed to investigate this aspect.

PART II

THE EXTENT OF TISSUE COLONIZATION AND HAUSTORIAL DEVELOPMENT IN RELATION TO TISSUE RESISTANCE

The extent of tissue colonization and haustorial development in relation to tissue resistance

Introduction

The results of the last part showed that there were no significant differences between colony extension rate in the tubers of the five clones. Thus it was considered that there might be differences in the extent to which their tissues were colonized and that might be a factor in resistance.

The general structure and appearance of hyphae, diverticula and haustoria of *P. infestans* in potato tissues have been described by a number of investigators. Much of the early work was reviewed by Blackwell (1953) and most of the following review is based largely on her account.

Hyphae

A number of workers have shown in some relatively general studies that hyphae of *P. infestans* can colonize almost all tissue systems including epidermis, cortex, vascular tissue and pith. The hyphae have been described as aseptate (Ward, 1887), intercellular (De Bary, 1863, 1876; Ward, 1887; Szymanek, 1927a, 1927b; Butler & Jones, 1949), with a diameter of 4 to 8μm (Butler & Jones, 1949; Blackwell, 1953) or 7.5 to 12.5μm (Gausuzzaman, 1966). Hyphae were found to branch freely in all parts of the potato, although in the tuber and stem they have been reported to form short branches (De Bary, 1863, 1876). Hyphae in all tissues have been described by Szymanek, (1927a, 1927b) as wide, palmately branched and rarely filamentous. In the leaf tissue hyphae are simply pressed against the walls of the cells (De Bary, 1863, 1876). They are usually dichotomously branched with occasional trichotomous branches, branching occurring whenever hyphae come into contact with cell walls (Blackwell, 1953).

Diverticula

Some workers have frequently observed that intercellular hyphae develop short lobed lateral branches which do not penetrate into the lumen of the host cell, but remain intercellular. These structures are clearly not haustoria and have been referred to as diverticula (Butler & Jones, 1949; Blackwell, 1949, 1953 and Gausuzzaman, 1966). Some workers have mistaken them for haustoria (Delacroix, 1903, 1904; Jones et al., 1912). The diverticulum is a simple or complex fan-shaped or palmate structure. The factors controlling their development are not understood. Gausuzzaman (1966) stated that diverticula were often found near the border line between necrotic tissue and healthy tissue, and so suggested that their formation may be due to unfavourable conditions for the growth of hyphae and is thus influenced by the host tissue.

Some limited work has been done on the relation between resistance and patterns of hyphal growth. Thus Kock (1931), as mentioned earlier, found that the hyphae of P. infestans grown on susceptible tuber tissue were straight with long branches, but those on resistant tuber tissue were twisted with a gnarled appearance and numerous short branches.

Haustoria

Haustoria in general are special hyphal branches which penetrate and grow to a limited extent in the lumen of the cell and they are presumed to have an absorptive function and to be the main channel for interchange of substances between host and parasite (De Bary, 1863, 1876; Rice, 1927). However, in Gaumann's opinion haustoria are not essential feeding organs because in artificial culture lateral hyphae also absorb nutrients and, in spite of this, do not become transformed into haustoria. Thus haustoria, as such, do not appear either in artificial culture or if the fungus continues to grow

saprophytically in the tissues of the host after death. He considers that they are extensions of intercellular hyphae which are deformed and inhibited in their growth by reactions in the host cells, and, further, that the life of haustoria is therefore shorter than that of intercellular hyphae (Gaumann, 1950).

De Bary (1863, 1876) was the first to describe the haustoria of P. infestans in stem and tuber tissues. He reported that they were rare and failed to find any in the leaves. Mangin (1895) described haustoria as slender simple suckers of variable lengths, but very frequent, and not rare as De Bary has reported them to be. According to Butler (1918) haustoria in tuber and stem tissues are common and easily seen. They are simple or branched, finger shaped, clavate or roundish. They are much harder to find in the leaves usually being filiform. Szymanek (1927a, b) also reported that haustoria were very variable in form, including club-shaped, hooked or spirally twisted types occurring singly or in pairs in the host cells. Schilberszky (1928) found short protuberances and rudimentary hyphal branches, no longer than the width of the hyphae. These were close together, sometimes in twos, threes or fours, and mostly on the same side of the hypha. Blackwell in her own work on infected tuber and stem tissues and in work done by Miss Godwin on infected leaves (1953) showed that haustoria were sporadically produced, and she described them in the tuber as simple and straight or curved, but rarely coiled or branched, and usually not longer than the width of the hyphae which bears them. Their diameter varies from 1 to 2µm but the width at the base is narrower than at the tip. In the leaf they are much thinner and longer than in the tuber. Gausuzzaman, (1966) found that haustoria, in the tubers of a number of clones, were frequent and varied in size and shape. He classified them on the basis of their size and

shape into four types:

1 - Knob - almost spherical with diameter, 2.5 to 5.0μm

2 - Peg - conical or finger-shaped structure, the length varying from 5.0 to 7.0μm

3 - Filiform - an elongated slender structure with a straight or twisted neck and length 12.0 to 25.0μm

4 - Hook-shaped - similar to the filiform type but bending gradually near the tip. Its length may be more than 25μm if the tip is included.

Haustoria in tuber and stem tissues are occasionally found to be surrounded by a cellulose sheath (De Bary, 1863; 1876; Butler, 1918; Szymanek, 1927a, b; Blackwell, 1949, 1953; Gausuzzaman, 1966). This sheath is believed to be formed by the host cell protoplasm (De Bary, 1863, 1876; Butler, 1918 and Blackwell, 1949, 1953). On the other hand, haustoria in the leaves are reported to be completely devoid of this thickening.(Blackwell, 1953).

All the studies mentioned above described the morphology of the mycelium and haustoria as seen by the light microscope but more recent studies using the electron microscope have revealed details of the fine structure of the host-parasite interface. The ultrastructure of hyphae and haustoria of P. infestans in the leaves have been described in detail by Ehrlich & Ehrlich (1966) who reported that haustoria consist of an expanded head surrounded by wall material which is continuous with the wall of the intercellular mycelium. They lack the long narrow stalk or neck often associated with this organ in other host parasite systems, and there is considerable cytoplasmic continuity between the haustorium and the intercellular mycelium. They described haustoria as anucleate and ranging in shape from small globes to short straight or curved pegs with a slight constriction at the point of penetration through the cell wall, and varying in width from 0.7 to 1.0μm.

Because haustoria appear to lack nuclei, they suggest that they might be less active physiologically than the nucleate haustoria of other obligate parasites. However, Sivak and Shaw (1969) reported that nuclei were present in haustoria in tissues of the clone, Green Mountain. Haustoria in the leaf tissue when observed using the electron microscope appear to be surrounded by an unknown material which has been given the term "encapsulation" by Ehrlich and Ehrlich (1966). This term was first used by the same authors (1963) to describe similar material surrounding the haustoria of Puccinia graminis in wheat. They suggested that the term "encapsulation" should be used to designate "any simple or complex structure between the plasma membranes of the host and parasite which is neither typical host or fungal cell wall and the term "sheath" used by Blackwell (1949, 1953) and earlier workers should be reserved for those portions of the haustorial complex which are clearly developed from the host cell wall, whether present merely as a collar around the penetration peg or as an envelope completely surrounding it". Ehrlich and Ehrlich,(1966) attributed the failure of Blackwell, to see the thickening upon haustoria in leaf tissue, to the fact that the encapsulation may have been below the limit of resolution of the light microscope or that the treatment used in clearing the leaf may have destroyed the structure.

Opinions regarding this extrahaustorial material are divided. Ehrlich and Ehrlich (1963, 1966) thought it was secreted by the fungus while Peyton and Bowen (1963), Berlin and Bowen (1964) concluded that it was secreted by the host.

Relation between tissue colonization and haustorial formation and tissue resistance

All the work described above is purely descriptive and few attempts have been made to determine whether any aspect of tissue resistance may be related to haustorial formation or haustorial type.

A number of factors have been implicated in haustorial formation, some of which may be associated with resistance. These factors are:-

a) The age of the host

b) The physiological condition of the host, particularly the level of available nutrients.

c) The effect of physical barriers on hyphal growth between cells

d) The physiological condition of the fungus (Blackwell, 1953; Fraymouth, 1956)

e) Reduced aeration

f) Surface tension effects (Brown, 1936)

According to Blackwell (1953) the occurrence of haustoria varies between clones and between different parts of the same clone. They were frequent in the tubers of Majestic, Arran Banner and Up-to-Date; occasional in Epicure and Gladstone; rare in Golden Wonder, but not found at all in the tubers of Arran Pilot and Doon Star. They were found occasionally in the leaves of King Edward and Majestic; rarely in Arran Banner and Gladstone, but not in Arran Bard, Arran Pilot, Arran Peak and Golden Wonder. Thus the frequency of occurrence of haustoria is not obviously related to tissue resistance. Blackwell did attribute their occurrence to the influence of the host cell and considered that some cells permitted penetration by haustoria more readily than others. She stated that the presence of haustoria may be taken as an indication of susceptibility. On the other hand Gausuzzaman (1966) did not observe any appreciable differences in

the forms of haustoria occurring in the cortex and medulla of the same tuber or in tubers of different clones, so he concluded that no relation could be drawn between haustorial production and tuber resistance.

Thus no conclusion can yet be drawn regarding the relationship between the extent of tissue colonization or haustorial development and tissue resistance. Clearly this subject requires investigation.

The following investigation was, therefore, carried out in an attempt to determine whether any such relationship could exist.

General Methods

1 - Inoculation Procedure

Tuber slices 12 mm thick of the five clones were cut and inoculated as described on page 21 and incubated at 20°C for five days.

2 - Preparation of Sectioned Material for Microscope Examination

Blocks of tissue were removed from the infected slices, five days after inoculation as shown in the diagram (Fig. 10). Sections 72µm thick were cut from these blocks usinga Lab line/Hooker Plant microtome.

3 - Staining Techniques

In preliminary trials a number of stains were used to differentiate the hyphae and haustoria in the host tissues. These included:-

1 - Safranine-picroaniline blue (Gurr, 1965)
2 - Magdala red-fast green " "
3 - Iron haematoxylin (Sass, 1940)
4 - Delafield's haematoxylin " "
5 - Safranin-fast green " "
6 - Lactophenol cotton blue

Safranin-Picroaniline blue, Iron haematoxylin and Delafield's haematoxylin did not clearly differentiate both hyphae and haustoria. Magdala red-fast green and Safranin-fast green stained the hyphae red while the host tissue stained green. The hyphae were easy to observe but the stains did not differentiate the haustoria clearly within the cells. Lactophenol cotton blue, using the procedure described below, proved to give the best results with both hyphae and haustoria staining deep blue, while the host tissue stained green-blue. The only disadvantage of this stain is that it did not stain

hyphae and haustoria clearly in the periderm or between necrotic cells. However, lactophenol cotton blue was used according to the procedure detailed below, for all further investigations.

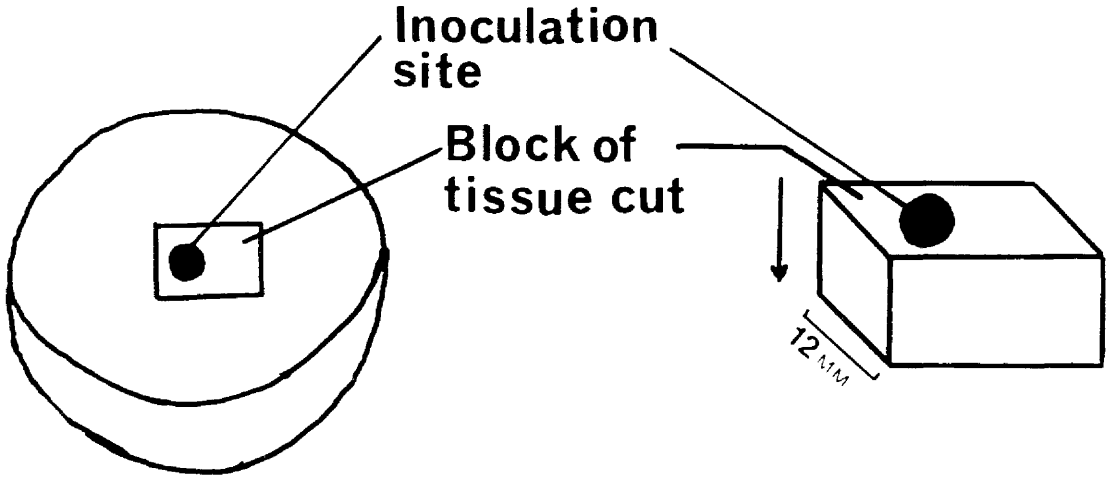
Sections were placed in one or two drops of stain on a clean glass slide and gently warmed, but not boiled, over a very low bunsen flame. The slide was removed from the flame and allowed to cool at the room temperature for several minutes. The process of alternately heating and cooling was repeated five to six times, care being taken not to allow the stain to boil. The sections were then cleared by repeatedly heating and cooling in clear lactophenol, again without boiling. They were then either mounted in clear lactophenol or dehydrated by passing successively through 70%, 90% and absolute alcohol followed by a mixture of absolute alcohol, xylene and clove oil (2:2:1) before finally clearing in pure xylol and mounting in Canada balsam.

Lactophenol cotton blue stain was prepared as the following:-

Phenol (pure crystals)	20 g.
Lactic acid	16 ml
Glycerol	31 ml
Distilled water	20 ml
Cotton blue	0.5 g.

The phenol was dissolved in water by warming gently, then the lactic acid and glycerol were added. Finally 0.5 g of cotton blue was added to the solution and shaken until it dissolved.

Figure 10 Diagrams illustrating the preparation of blocks of tissue for sectioning and plane of sectioning



TUBER SLICE

PLANE OF SECTIONING

Number of cells per unit volume of medullary tissue

In order to make comparisons between clones in the number of haustoria and number of haustoria containing cells it was necessary to determine whether or not the number of the cells per unit of the tissue of the different clones are similar.

The number of cells in five microscopic fields per section for total of eight sections taken from eight tubers from each clone were counted. The analysis of variance (Table 11 of the appendix) showed that the differences between clones were not significant indicating that cell sizes in these clones are similar. Thus the number of cells adjacent to a unit length of hyphae in the medullary tissue of these clones will be similar.

A - Hyphae and haustoria in tuber tissues

1 - Preliminary Observations

Hyphae in the tuber tissue of all clones were intercellular and aseptate with a diameter varying from 4µm to 11µm. Haustoria were frequent in all cultivars. Both hyphae and haustoria were most abundant near the inoculation site becoming less frequent and more scattered with increasing distance (Figure 11).

A range of haustorial types was found. The commonest type of haustorium in all clones was the simple unbranched structure. Occasionally branched forms having two approximately equal lobes (Figure 12) or two unequal lobes (Figure 13) or more than two lobes (Figure 14) were observed. In some cases the haustorium branched at the base near the cell wall (Figure 12) while in others it branched at a distance from the base (Figure 13). Some haustoria were narrower at the base than at the tip (Figure 15) while others had a swollen base (Figure 16). Other forms encountered were elongated and straight (Figures 13, 17, 18) or curved (Figures 12, 15, 17, 18) or spirally coiled near the tip (Figure 13) or pointed at the tip (Figures 12, 17, 18), or swollen at the tip (Figure 15).

For the purpose of further investigation the different haustorial types were classified arbitrarily into four types on the basis of their morphology. These are the same groups as defined by Gausuzzaman (1966).

1. Knob: Approximately spherical, 3-6µm length x 3-6µm diameter (Figures 18, 19)

2. Peg: Finger-shaped or conical structures, 6-15µm length x 3-5µm diameter (Figure 16)

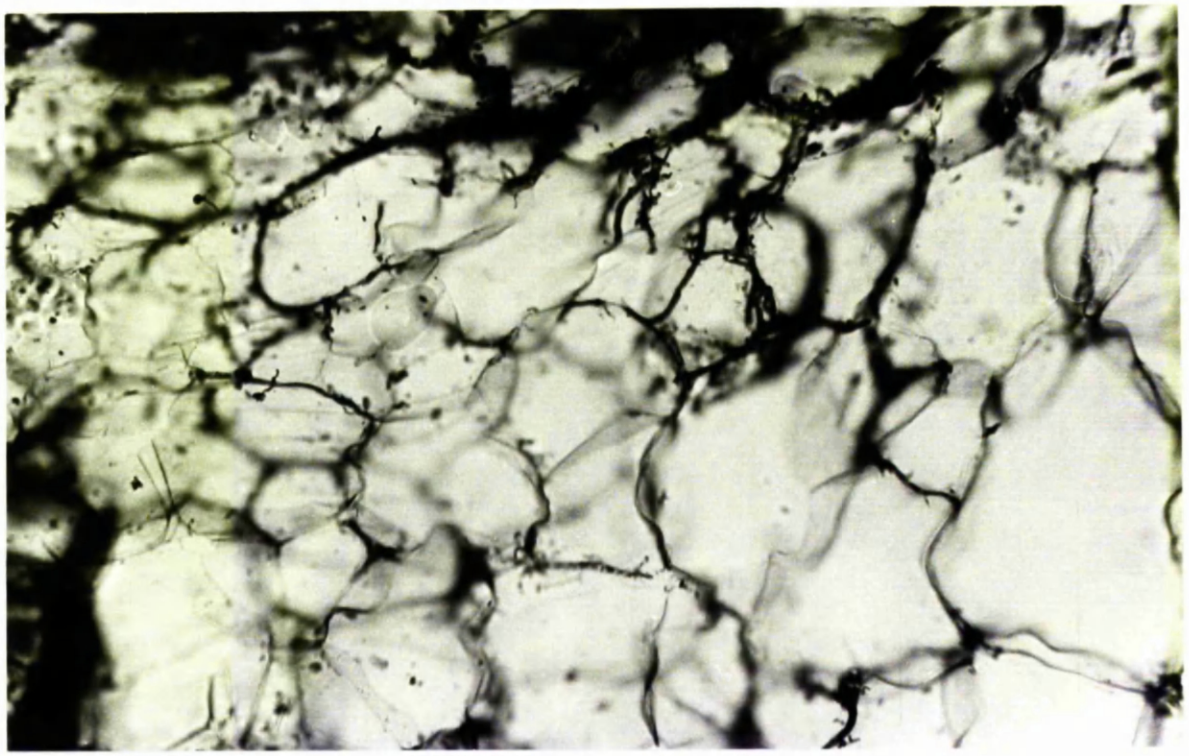
3. Filiform: Much longer slender structures, sometimes branched, 15-35µm length x 2-5µm diameter (Figures 12, 13, 14, 15, 17, 18)

4. Hook: Similar in shape to the filiform, except that they are unbranched and bent into a hook or crozier near the tip, 24-38µm x 2-4µm diameter (Figure 12).

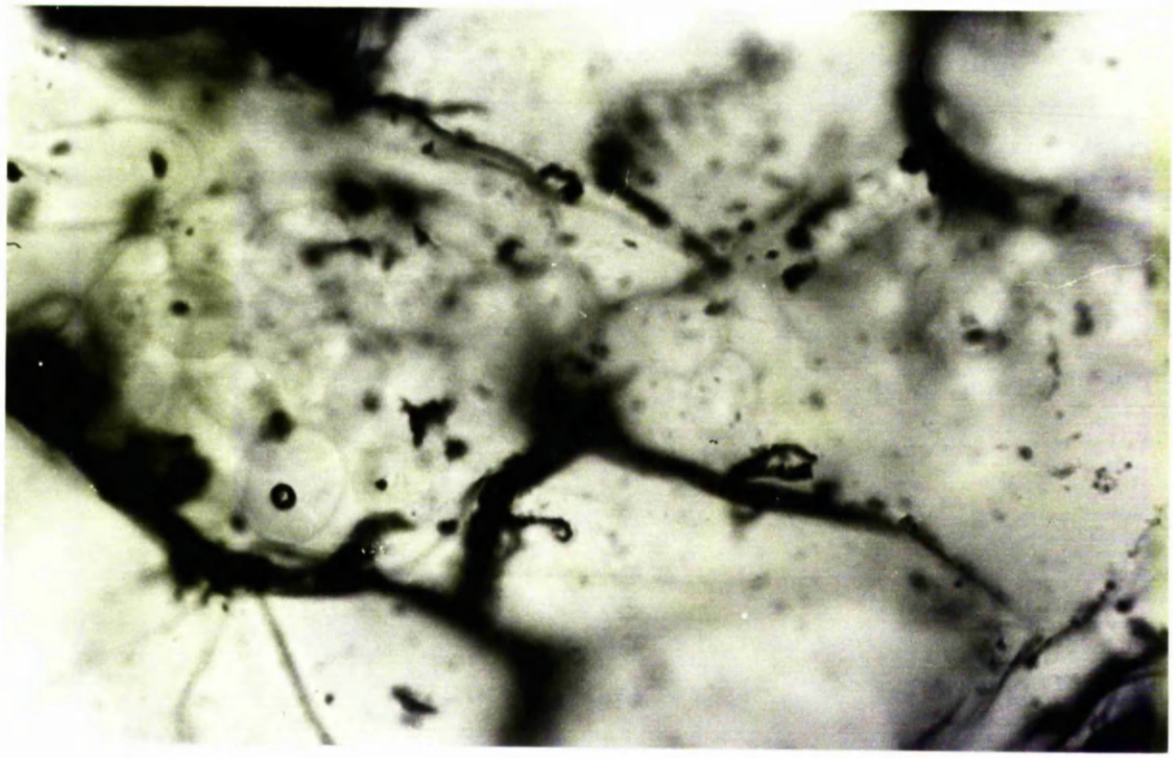
Haustoria were not evenly distributed along hyphae and present in all cells. Most cells adjacent to the hyphae did not contain any haustorium while others contained one, two, or more than two. They sometimes appeared to be produced along one side only of a length of hypha while in other cases they were produced along all sides at irregular intervals. No clearly sheathed haustoria or diverticula were observed.

Figure 11 Hyphae growing out from the inoculating site through the medullary tissue of a tuber of Craigs Alliance.

Figure 12 Hook type haustorium in one cell and filiform type haustorium branched to give approximately two equal lobes in the adjacent cell in the medullary tissue of a tuber of Craigs Alliance.



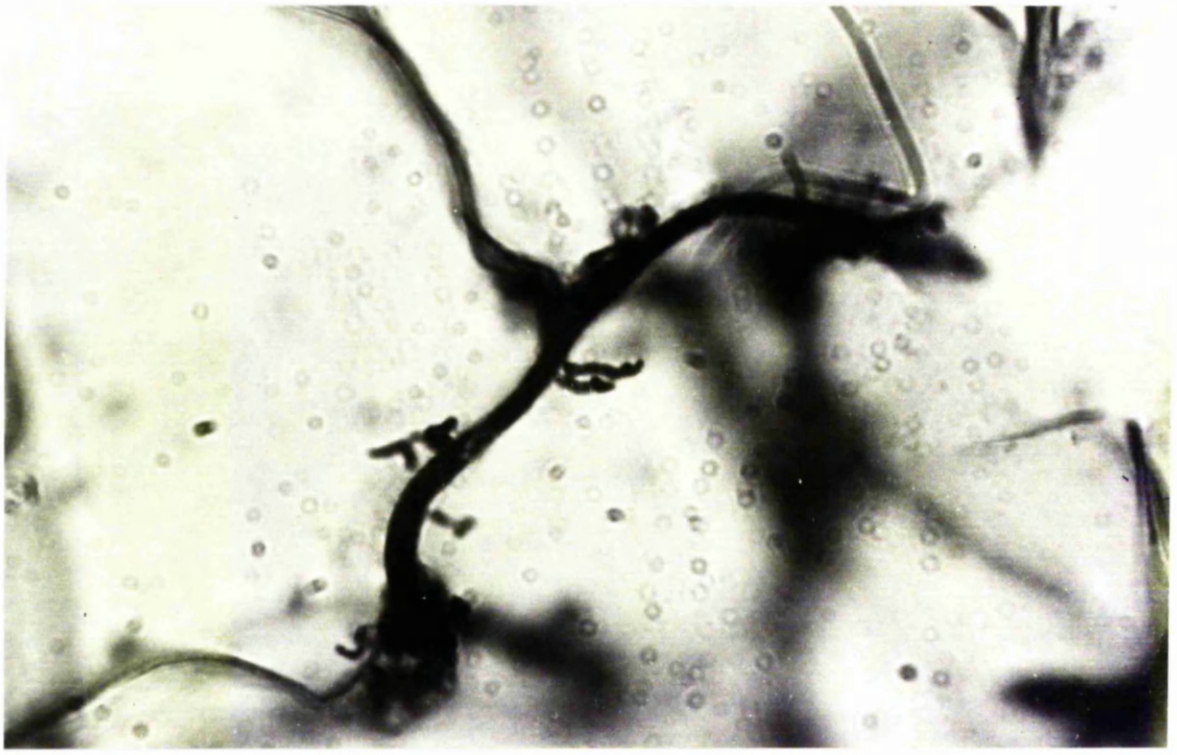
154 µm

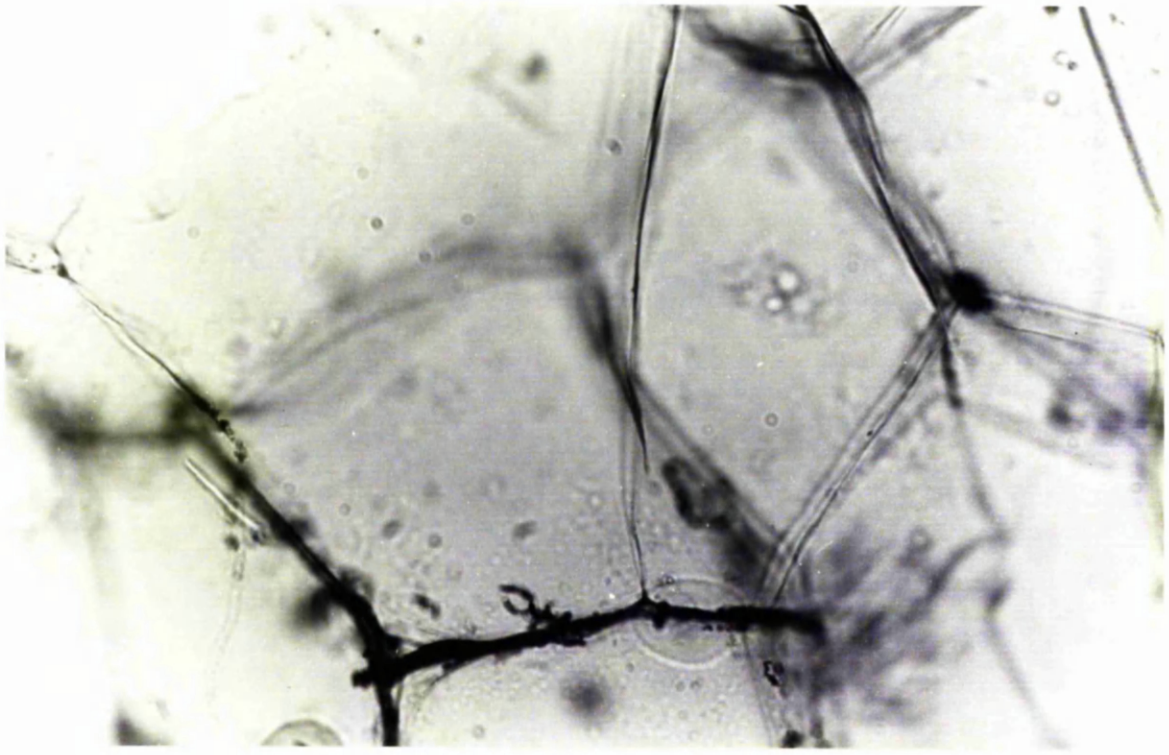


56 µm

Figure 13 Filiform type haustoria occurring singly and in pairs, two with spirally coiled tips and one branched with two unequal lobes in cells of medullary tissue of a tuber of Craigs Alliance.

Figure 14 Branched filiform haustorium having a number of lobes, in cell of medullary tissue of a tuber of 6003ab(33)

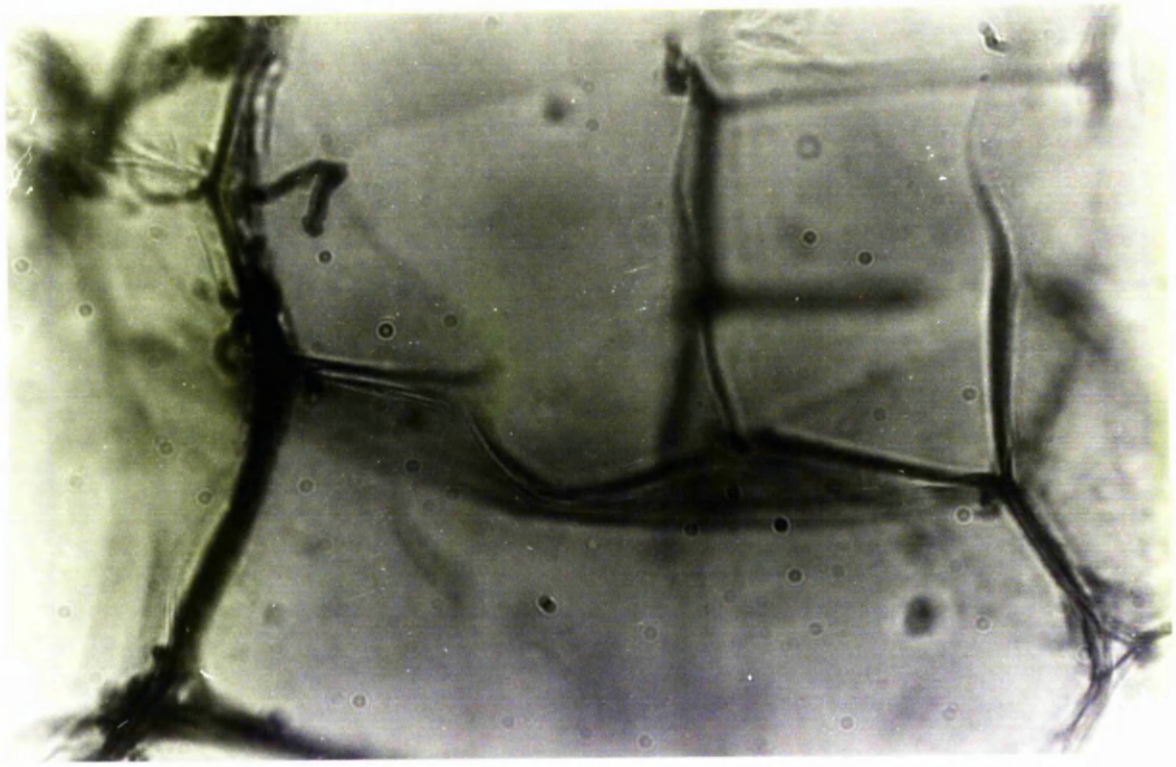


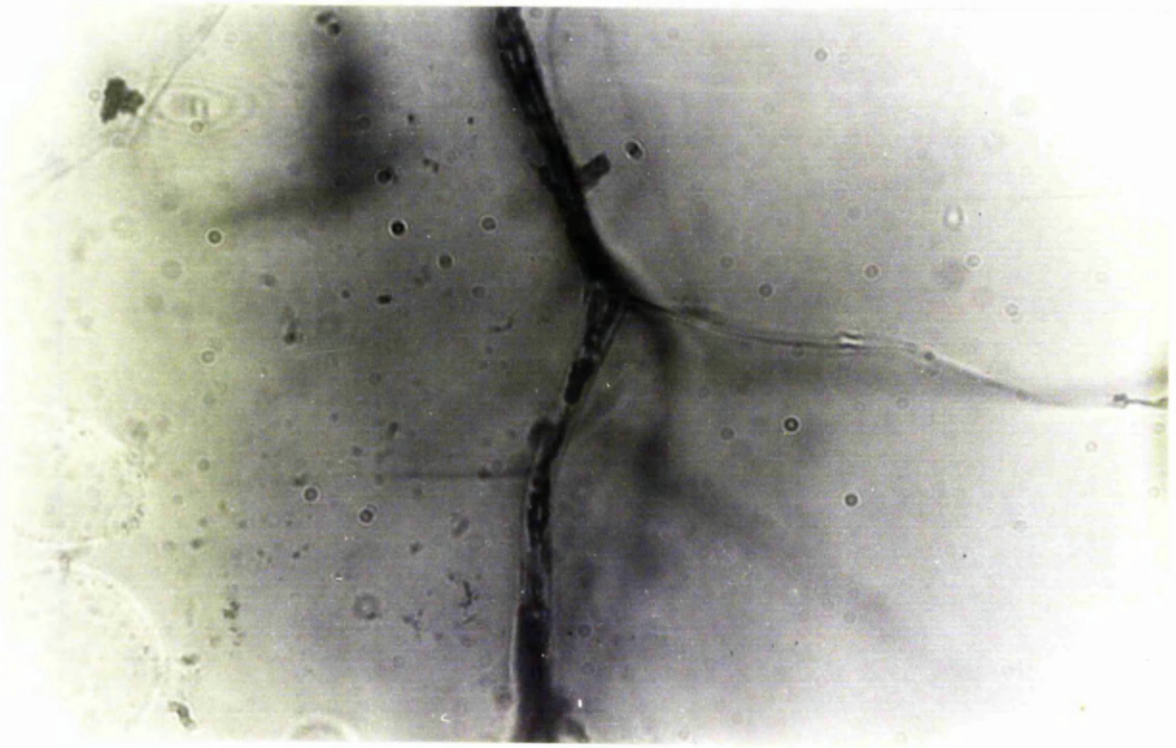


56 µm

Figure 15 Filiform type haustorium bending approximately at the middle in cell of medullary tissue of a tuber of 6003ab(33)

Figure 16 Peg type haustorium in medullary cell of medullary tissue of a tuber of Pentland Dell.

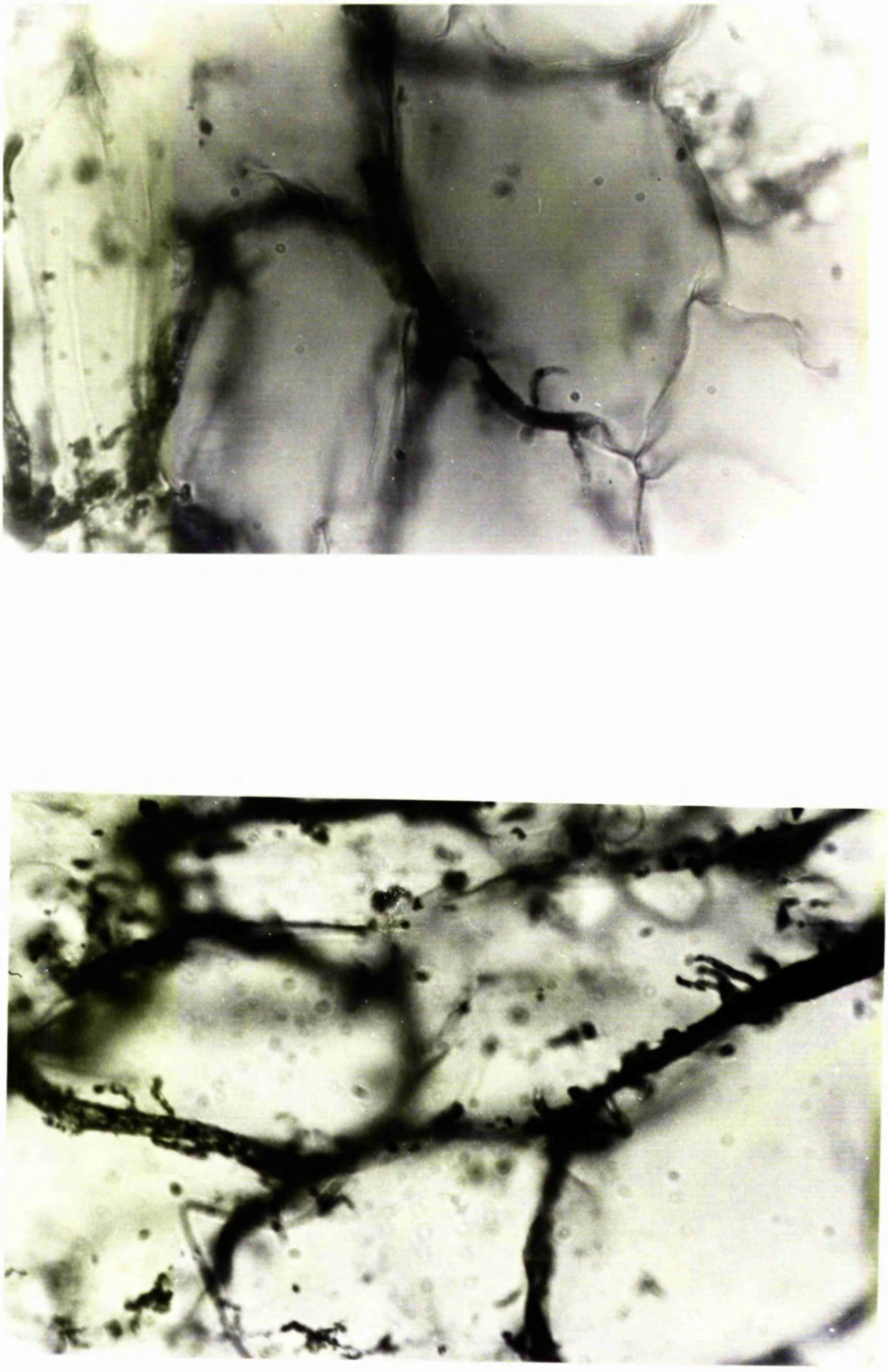


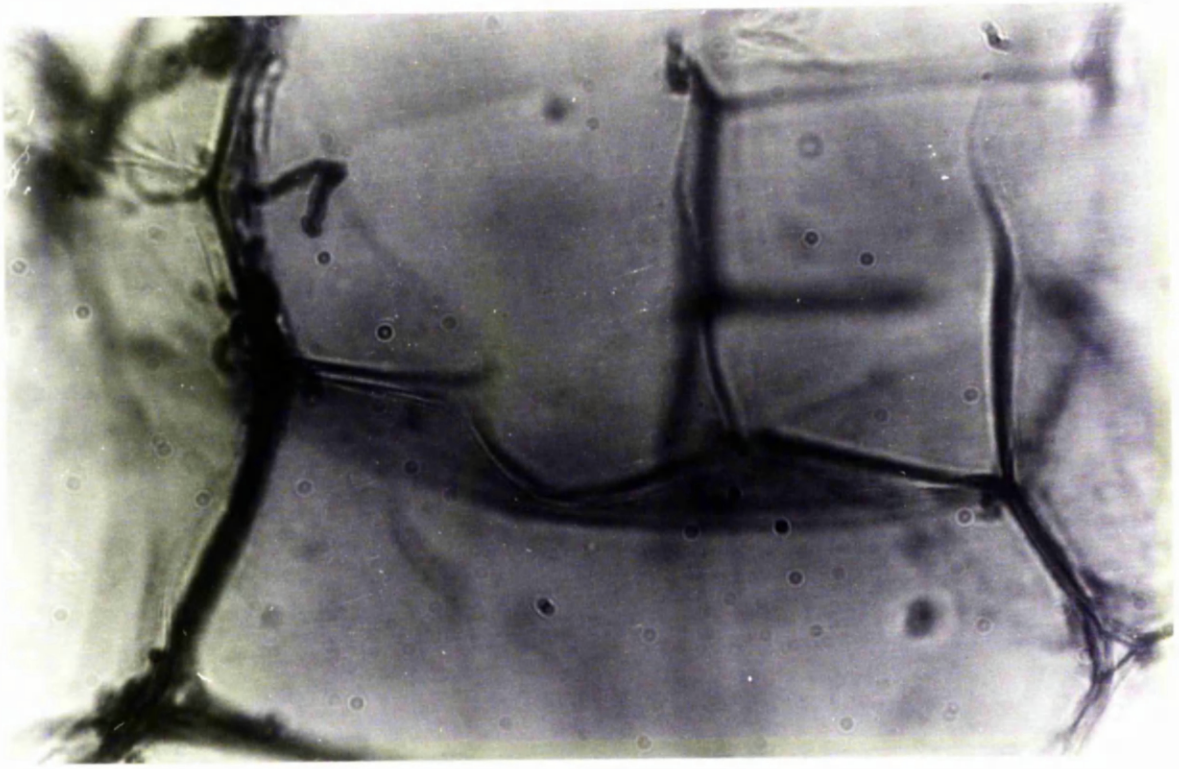


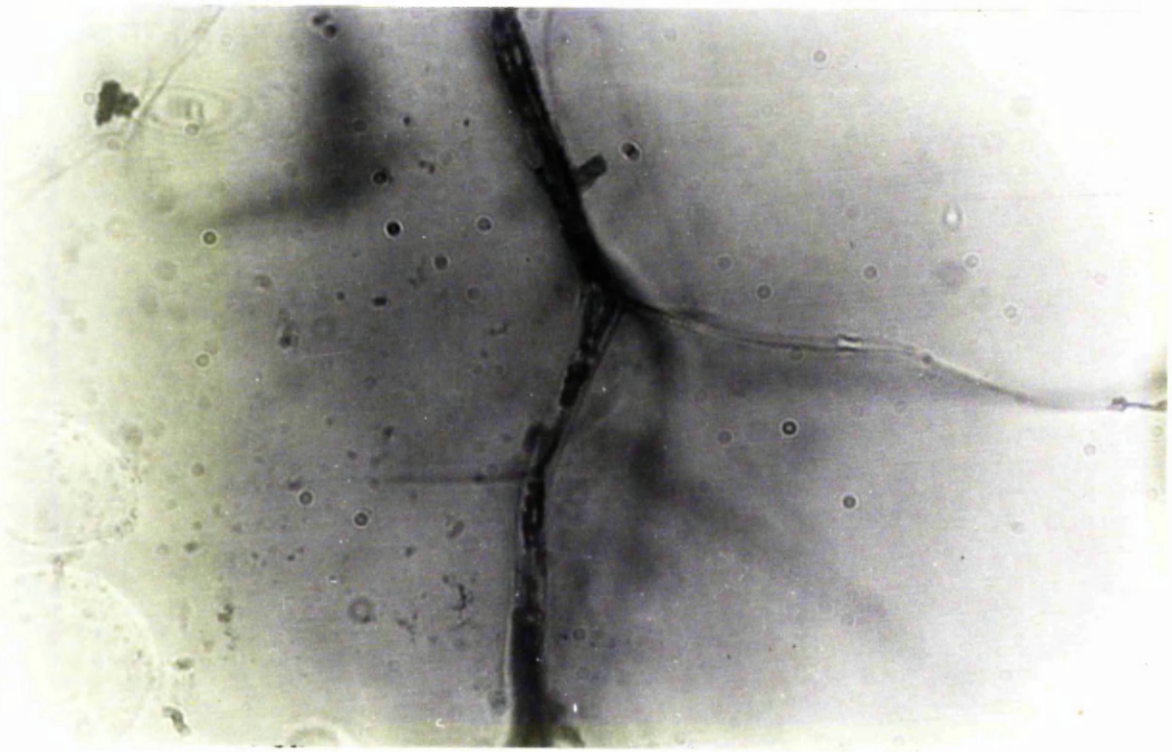
35 µm

Figure 17 Straight filiform haustorium in one cell and curved filiform of haustorium in the adjacent cell of medullary tissue of a tuber of Pentland Squire.

Figure 18 Cells of medullary tissue of a tuber of Craigs Alliance penetrated by several haustoria, some of them in groups.


56 µm





35 µm

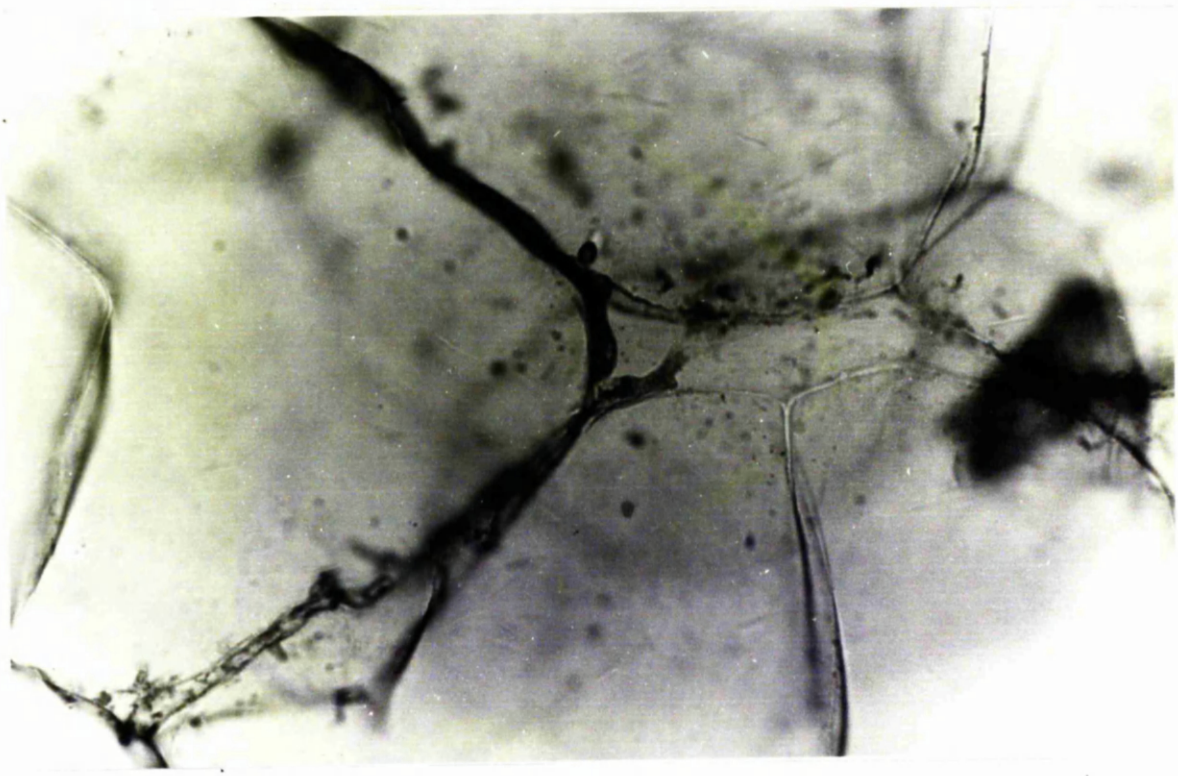


Figure 19 Knob type haustorium in cell of medullary tissue of a tuber of Pentland Squire.

2 - Quantitative assessments of tissue colonization

Methods

In a preliminary examination a number of sections were cut from each clone, but only five were selected at random for examination. Two microscopic fields situated adjacent to the necrotic tissue were examined from each section. Thus ten microscopic fields were examined from each clone. In each microscopic field the total number of hyphal segments, total hyphal length, total number of branches and total number of haustoria were recorded. Fewer sections were examined than might be desirable in view of the range of variation between the fields of view, but because measurements of total hyphal length were time consuming, it was impossible to do more.

Results and Discussion

The results are given in Tables 12a, 12b, 12c, 12d in the Appendix and the analyses of variance are given in Table 19. The analyses show that in no case are the differences between clones significant.

Although total hyphal length per unit volume of tissue would be the most exact measure of tissue colonization, measurements of total hyphal length were tedious to make and so correlation coefficients between total hyphal length and the other hyphal characters were calculated to investigate the possibility of using them as measures of tissue colonization. Correlation coefficients were also calculated between total number of hyphal segments and total number of branches, total number of hyphal segments and total number of haustoria, and total number of branches and total number of haustoria. The results are given in Table 20.

Total number of hyphal segments was correlated with total hyphal length in all clones and so was used to estimate the extent of tissue

colonization in all further experiments.

Total number of branches was correlated with total hyphal length in four out of the five clones, while total number of haustoria showed the least degree of correlation being correlated with total hyphal length in one clone only. The absence of a close correlation between number of haustoria and total hyphal length is interesting because it indicates differences between clones in the number of haustoria produced per unit length of hyphae (see Table 21). When the clones are ranked in order of decreasing ratio of total hyphal length to total number of haustoria the ranking is in agreement with the Pentlandfield Scale of race non-specific resistance. It is also in general agreement with the ranking based on the length of time required for growth phases I and III, except that 6003ab(33) has a higher ranking, while Pentland Raven has a lower ranking.

TABLE 19 Analyses of variances of hyphal growth characteristics in the five clones

Source of Variance	N	M.S.	V.R.	P
a - Total hyphal length	4	309.15	--	N.S.
Residual	45	1646.60		
b - Total number of hyphal segments	4	11.6	--	N.S.
Residual	45	12.8		
c - Total number of branches	4	17.3	1.1	N.S.
Residual	45	15.2		
d - Total number of haustoria	4	33.3	--	N,S.
Residual	45	38.3		

TABLE 20 Correlation coefficient between pairs of hyphal growth characteristics

Character pairs	Craigs Alliance	Pentland Squire	Pentland Dell	Pentland Raven	6003ab(33)
THL : TNH	0.92***	0.80**	0.93***	0.73*	0.84**
THL : TNB	0.87***	0.78**	0.73*	0.70*	0.68
THL : TNHaus.	0.54	0.62	0.74*	0.48	0.47
TNH : TNB	0.84**	0.63	0.81**	0.80**	0.88***
TNH : TNHaus.	0.59	0.60	0.72*	0.30	0.74**
TNB : TNHaus.	0.71*	0.07	0.84**	0.31	0.84**

*** Significant at P = 0.001
** " at P = 0.01
* " at P = 0.02

THL = Total hyphal length (μm)

TNH = Total number of hyphal segments

TNB = Total number of branches

TNHaus = Total number of haustoria

TABLE 21 Ratio between total hyphal length (THL) and total number of haustoria (TNHaus.) in relation to resistance

Clone	Ratio of THL : TNHaus. arranged in decreasing order	Tissue resistance to growth Phases I & III	Race non-specific resistance on Pentlandfield Scale
Pentland Raven	162.3µm : 1	2 - 3	2
Pentland Dell	113.8µm : 1	2 - 3	4
6003ab(33)	106.2µm : 1	1	1-2
Pentland Squire	84.0µm : 1	5	3
Craigs Alliance	66.5µm : 1	4	4^{+}

3 - Tuber tissue resistance to colonization

Introduction

The preliminary experiment showed that total number of hyphae was correlated with total hyphal length in all clones examined. Thus using total number of hyphae as a parameter of tissue colonization it was possible to obtain more data more rapidly and so be able to compare resistance to colonization of different regions within the tuber and between tubers of the different clones more easily.

Methods

Comparison between medullary tissue and cortical tissue, between medullary tissue at the rose and heel end of the tuber and between medullary tissue of large and small tubers within clones and between clones were carried out using sections as detailed below.

1. Between clones

Seven sections 72µm thick were cut from the medullary region of seven tubers of each of the clones, Craigs Alliance, Pentland Dell, and 6003ab(33) five days after inoculation. After staining, the numbers of hyphal segments in nine fields of view per section were recorded.

2. Between cortex and medulla within clones

Sections 72µm thick were cut from the cortex and medullary region of slices of Pentland Dell and Pentland Squire, again five days after inoculation. After staining the numbers of hyphal segments per section for five sections from the cortex and five sections from the medulla were recorded.

3. Between rose and heel end within clones

Sections 72µm thick were cut from the medullary tissue of the rose and heel ends of slices of Pentland Dell and Pentland Squire five days after inoculation. After staining the numbers of hyphal segments

per section for eight sections from the rose end and eight sections from the heel end were recorded.

4. Between small and large tubers within clones

Sections 72µm thick were cut from the medullary tissue of slices from small and large tubers of Pentland Dell five days after inoculation. After staining the numbers of hyphal segments per section for eight sections from the small tubers and eight sections from the large tubers were recorded.

Results and Discussion

The results are given in Tables 13, 14, 15, 16 in the Appendix.

Analyses of variance were carried out and the results revealed that in no cases were the differences significant (Tables 22, 23, 24, 25). Thus the medullary tissues of the three clones, Craigs Alliance, Pentland Dell and 6003ab(33) are equally susceptible to colonization. This result is in agreement with that obtained in the preliminary experiment.

The cortex and medullary tissue in each of the two clones, Pentland Dell and Pentland Squire was also colonized to the same extent, and so not only are the two clones equally susceptible to colonization, but their cortex and medullary tissues are also equally susceptible to colonization. The tissues at the rose end and heel end of the tuber in these two clones are also equally susceptible to colonization as also are the medullary tissues of small and large tubers of the clone Pentland Dell.

Thus there is no evidence to indicate that active factors accumulate to different levels in the tissues behind the hyphal front and affect the extent to which lateral branches develop to further colonize the tissues. The results, however, do not rule out the possibility that

active factors accumulate to the same extent in all clones, and so equally affect the extent to which lateral branches develop.

TABLE 22 Analysis of variance on the total number of hyphal segments in Craigs Alliance, Pentland Dell and 6003ab(33)

Source of Variance	N	M.S.	V.R.	P
Between clones	2	3	0.33	NS
Residual	18	9.1		
	20			

TABLE 23 Analysis of variance on the total number of hyphal segments in the cortex and medulla

Clone	Source of Variance	N	M.S.	V.R.	P
a - Pentland Dell	Between cortex and medulla	1	84	0.35	NS
	Residual	8	239		
b - Pentland Squire	Between cortex and medulla	1	36	0.14	NS
	Residual	8	265		

TABLE 24 Analysis of variance on the total number of hyphal segments in the heel and rose ends

Clone	Source of Variance	N	M.S.	V.R.	P
Pentland Squire	Between rose and heel ends	1	2	0.003	NS
	Residual	14	584		
Pentland Dell	Between rose and heel ends	1	5	0.04	NS
	Residual	14	155		

TABLE 25 Analysis of variance on the total number of hyphal segments in small and large tubers of Pentland Dell

Clone	Source of Variance	N	M.S.	V.R.	P
Pentland Dell	Between small and large tubers	1	60	0.17	NS
	Residual	14	347		

4 - Local variation in tuber tissue resistance to colonization

Introduction

Some observations made during the preliminary experiment indicated that tuber tissue was not always colonized uniformly. Hyphae appeared to be present in abundance in some parts of sections but absent from other parts of what appeared morphologically to be identical tissue.

A series of statistical analyses of the distribution of hyphal segments within sections from different tissue systems were thus made.

If the hyphal segments were distributed randomly then the frequency of occurrence of fields of view without hyphal segments or with different numbers of hyphal segments should show a Poisson distribution (Gilbert, 1973; Kershow, 1973). Non-randomness can thus be demonstrated by comparing the observed distribution with the expected Poisson distribution.

Methods

The analyses were carried out on the following statistics obtained from Craigs Alliance, Pentland Dell, Pentland Squire and 6003ab(33).

1. Pentland Dell Sections cut on five separate occasions were analysed.

A - Seven tubers, one section from the medulla in the middle of each tuber, nine fields recorded for each section = 63 fields

B - Ten tubers, one section from the medulla in the middle of each tuber, 36 fields recorded for each section = 360 fields

C - Five tubers, one section from both cortex and medulla in the middle of each tuber, 36 fields recorded for each section = 180 fields

D - Eight tubers, two sections from the rose and heel ends of each tuber, 36 fields recorded for each section = 288 fields

E - One section from the middle of each of eight small and eight large tubers, 36 fields recorded for each section = 288 fields

2. Pentland Squire Sections cut on two occasions analysed

A - Five tubers, one section from both cortex and medulla in the middle of each tuber, 36 fields recorded for each section = 180 fields

B - Eight tubers, one section from the rose and heel ends of each tuber, 36 fields recorded for each section = 288 fields

3. Craigs Alliance

Seven tubers, one section from the medulla in the middle of each tuber, nine fields recorded for each section = 63 fields

4. 6003ab(33)

Seven tubers, one section from the medulla in the middle of each tuber, nine fields recorded for each section = 63 fields

Results and Discussion

The results of analyses for non-randomness are given in the table (26). The hyphae in the cortex of Pentland Dell and Pentland Squire were found to be distributed randomly, but they were distributed non-randomly in the medulla at the centre of the tuber and at the rose and heel ends of the tuber in Pentland Squire and also in most cases in Pentland Dell. Hyphae were also non-randomly distributed in the central medulla of Craigs Alliance but not in 6003ab(33). These results in general indicate that the medullary tissue of the clones Craigs Alliance, Pentland Dell and Pentland Squire is not uniformly susceptible, and some pockets of tissue appear to be more

resistant than others. This may be due to one or more of the following factors:-

1 - The nutrients required by the fungus are not distributed uniformly in these tissues and some pockets of tissue contain more nutrients than others.

2 - Inhibitory substances may be distributed in the same way as above.

3 - Tissue anatomy is not uniform and the intercellular spaces in some pockets of tissue are wider than in others. The hyphae may be physically restricted to regions of tissue with the larger intercellular spaces. Microscopic observations did not indicate that this was a likely possibility.

TABLE 26 Analyses for non-random distribution of hyphae in tuber tissue of 6003ab(33), Pentland Dell, Pentland Squire and Craigs Alliance

Clone	Tissue	Number of individual hyphal segments per microscopic field	0	1	2	3	4	5	6	x^2	P
6003ab(33)	Central medulla	Observed frequency	31	16	10	4	2				
						16					
		Expected frequency	25.9	23	10.3	3.1	0.7			3.4	NS
						14.1					
		Differences	5.1	7		1.9					
Pentland Squire	Cortex	Observed frequency	42	57	52	23	6				
		Expected frequency	45.4	62.3	50	20.7	7.3			1.27	NS
		Difference	3.4	5.3	2	2.3	1.3				
	Central medulla	Observed frequency	62	43	43	23	9				
		Expected frequency	49.2	63.9	41.6	18.1	5.9			13.09	< 0.01
		Difference	13.8	20.9	1.4	4.9	3.1				
	Medulla at rose end	Observed frequency	116	61	54	35	20	2			
							22				
		Expected frequency	81.7	103.3	65.2	27.4	8.6	2.1		47.6	< 0.001
							10.7				
		Difference	34.3	42.3	11.2	7.6	11.3				
	Medulla at heel end/										

TABLE 26 (Cont.)

Clone	Tissue	Number of individual hyphal segments per microscopic field	0	1	2	3	4	5	6	x^2	P
	Medulla at heel end	Observed frequency	114	68	58	27	14	5	2		
								21			
		Expected frequency	83.1	103.3	64.2	26.6	8.3	2.1	0.4	33.8	< 0.001
								10.8			
		Difference	30.9	35.3	6.2	0.4		10.2			
Pentland Dell	Cortex	Observed frequency	53	57	46	19	3	2			
								5			
		Expected frequency	50.7	64.1	40.8	17.1	5.4	1.4		2.24	NS
								6.8			
		Difference	2.3	7.1	5.2	1.9		1.8			
	Medulla Exp. 1	Observed frequency	26	21	12	3	1				
						16					
		Expected frequency	25.9	23.1	10.2	3.0	0.8			0.48	NS
						14					
		Difference	0.1	2.1		2.0					
	Medulla Exp. 2	Observed frequency	94	154	83	24	5				
		Expected frequency	115.6	131.8	75.2	28.6	8.2			10.4	< 0.01
		Difference	21.6	22.2	7.8	4.6	3.2				
	Medulla Exp. 3	Observed frequency	58	58	48	14	2				
						16					
		Expected frequency	58.2	66.3	37.4	14.4	4			4.24	NS
						18.1					
		Difference	0.2	8.3	10.6	2.1					
	Central medulla of small tuber/										

TABLE 26 (Cont.)

Clone	Tissue	Number of individual hyphal segments per microscopic field	0	1	2	3	4	5	6	x^2	P
	Central medulla of small tuber	Observed frequency	109	84	61	29	5				
		Expected frequency	97.5	106	57.6	20.9	5.7			9.35	< 0.01
		Difference	11.5	22	3.4	8.1	0.7				
	Central medulla of large tuber	Observed frequency	83	93	84	25	3				
		Expected frequency	86.4	104.6	63.3	25.6	7.7			11.07	< 0.01
		Difference	3.4	11.6	20.7	0.6	4.7				
	Medulla at rose end	Observed frequency	115	84	45	29	11	4			
							15				
		Expected frequency	106	106	53	17.7	4.4	0.9		31.1	< 0.001
							5.3				
		Difference	9	22	8	11.3	9.7				
	Medulla at heel end	Observed frequency	125	46	73	34	9	1			
							10				
		Expected frequency	95.9	105.5	58	21.3	5.9	1.3		54.9	< 0.001
							7.2				
		Difference	29.1	59.5	15	12.7	2.8				
Craigs Alliance	Central medulla	Observed frequency	30	11	17	3	1	1			
						5					
		Expected frequency	23.2	23.2	11.6	3.9	1.0	0.2		10.5	< 0.01
						5.1					
		Difference	6.8	11.8	5.4		0.1				

5 - The relation between haustorial development and tuber tissue resistance

Introduction

The results of the preliminary experiment showed that the number of haustoria was not correlated with total hyphal length indicating that the cells of some clones, e.g. Pentland Raven, were less readily penetrated than were the cells of other clones, e.g. Craigs Alliance. It was also noted that haustoria occurred singly or in groups of two or more in some cells but not in all cells in contact with the hyphae. This has already been noted by Blackwell (1953) and Gausuzzaman (1966). Blackwell has suggested that the host cells might differ in resistance to penetration by haustoria.

This section reports a more detailed examination of the distribution of haustoria in cells adjacent to hyphal segments with a view to determining any relationship between haustorial development and tissue resistance.

5.1 - Number and type of haustoria in relation to resistance

Results and Discussion

The numbers of each of the four types of haustoria, knob, peg, filiform and hook were counted in a total of six sections, one section from each of six tubers from each clone (Table 17 in the Appendix).

The percentage number of each type of haustorium in each clone is given in Table 27. The filiform type is the commonest and the hook type is the least frequent in all five clones. However, the percentage occurrence of all four types of haustoria is similar in all clones and thus haustorial types do not appear to be correlated with differences in the tissue resistance of these clones in relation to differences in the rate of penetration and establishment of growth in tuber slices and the establishment of visible sporulating mycelium

on the opposite surface (Growth Phases I and III). On the other hand the analysis of variance of the total numbers of haustoria (Table 28) shows that the differences between clones are significant ($P < 0.05$). This result although not in agreement with that obtained in the preliminary experiment, where no significant differences were found, is likely to be more reliable because it is based on more extensive data than that of the preliminary experiment. It is possibly of significance in this context that the clones rank in exactly the same order in both experiments with Craigs Alliance having the greatest number and Pentland Raven having the least number of haustoria per unit volume of tissue. This rank (Table 27) is in the same order as that for clones based on rate of lesion growth and also in agreement with the rank based on resistance to fungal growth phases I and III (see Table 18, p. 65).

5.2 The proportion of cells in contact with hyphae containing haustoria and the distribution of haustoria between these cells

Results and Discussion

The distribution of haustoria in the cells adjacent to hyphae in the tubers of the three clones Craigs Alliance, Pentland Dell, and 6003ab(33) was investigated. Counts were made of the number of cells in contact with hyphae without haustoria and with haustoria in six sections from six tubers from each of the three clones to determine the proportion of cells in each clone which contained haustoria. The results are given in Table 29. It can be seen from the Table that most cells in contact with hyphae in all three clones did not contain haustoria. It can also be seen that Craigs Alliance had the highest percentage of haustorial containing cells.

Counts were also made of the number of haustoria in each cell.

The results were analysed for non-random distributions using the methods described previously, and they are given in Table 30 and show that haustoria are not randomly distributed in all cells in contact with hyphae. There are at least two possible explanations for this. Firstly, cells may differ in their resistance to penetration by haustoria and only the more susceptible ones are penetrated. Alternatively, haustoria may be produced only at certain sites along the hypha and only cells near these sites are penetrated.

The high frequency of multiple penetrations can be accounted for by either hypothesis since a susceptible cell may stimulate the formation of more than one haustorium, or, sites at which haustoria are produced may be concentrated together at certain regions only on the hypha and not be distributed uniformly along its length. If we assume that haustoria are modified lateral branches, then there is no reason to believe from pure culture studies that the sites at which haustoria would be produced are not more or less uniformly distributed along the hyphae. Thus the non-random distribution is more likely to be determined by differences in host cell resistance, but the observations reported here alone, do not differentiate between the two possibilities.

TABLE 27 The percentage number of the different types of haustoria and the total number of haustoria in the five clones

Cultivar	Knob		Peg		Filiform		Hook		Totals
	T.N.	Perc.	T.N.	Perc.	T.N.	Perc.	T.N.	Perc.	
Pentland Raven	26	15	43	24	99	56	10	6	178
6003ab(33)	26	12	73	33	114	52	5	2	218
Pentland Dell	28	12	84	37	109	48	7	3	228
Pentland Squire	38	14	82	30	138	50	18	6	276
Craigs Alliance	45	10	143	33	228	52	22	5	438

TABLE 28 Analysis of variance on total numbers of haustoria in the five clones

S. of V.	S. of S.	D. of Fr.	M.S.	F	P
Bet. Clones	9,860	4	2465	3.3	< 0.05
Residual	18,805	25	752		
Total	28,665	29			

TABLE 29 Percentage number of cells with and without haustoria in contact with fungal hyphae in tuber tissue of 6003ab(33), Pentland Dell and Craigs Alliance

Clone	Cells without haustoria	Percentage	Cells with haustoria	Percentage	Total number of cells examined
6003ab(33)	415	84.3	77	15.7	492
Pentland Dell	428	85.4	73	14.6	501
Craigs Alliance	565	78.9	151	21.1	716

TABLE 30 Analyses for non-random distribution of haustoria in the cells of the medullary tissue of three clones, 6003ab(33), Pentland Dell and Craigs Alliance

Clone	Number of individual haustoria per cell	0	1	2	3	4	5	6	x^2	P
6003ab(33)	Observed frequency	415	50	21	5	1				
					27					
	Expected frequency	400.3	90		10.7				43.1	< 0.001
	Difference	14.7	40		16.3					
Pentland Dell	Observed frequency	428	46	20	4	1	1	1		
						27				
	Expected frequency	400.3	90	10.0	0.66	0.04	0.003	0.00005	48.2	< 0.001
						10.7				
	Difference	27.7	44			16.3				
Craigs Alliance	Observed frequency	565	60	50	21	9	5	1		
						36				
	Expected frequency	470.8	197.7	41.5	5.8	0.6	0.05	0.003	254.5	< 0.001
						–	6.45			
	Difference	94.2	137.7	13.5		29.55				

B - Hyphae and haustoria in stem tissues

Introduction

This study was carried out in order to compare the extent of tissue colonization in the stem with that which occurs in tuber tissue. Craigs Alliance was chosen for study because in preliminary trials its stems were found to be more easily infected than were the stems of the other clones. Time did not permit this comparison to be made for all clones.

Materials and Methods

Plants were grown in Levington compost in 12 inch pots under lights in the greenhouse. They were fed at weekly intervals with John Innes liquid feed.

Detached stems from seven plants were cut into 4 cm lengths using a flamed knife and the segments were put in sterile petri dishes lined with moist filter paper. The stem pieces were inoculated by placing discs of filter paper soaked in a zoospore suspension on one of the cut ends. Attempts to infect the stems through the cuticles were not very reliable. The inoculated pieces were incubated at 20°C.

Five days after inoculation sections 72µm thick were cut parallel to the cut surface approximately 5 mm from the inoculated end using the Lab-line/Hooker plant microtome. The sections were then stained with lactophenol cotton blue and mounted in clear lactophenol.

Results and Discussion

The hyphae were morphologically similar to those observed in tuber tissue. They were freely branched and variable in width with diameters varying from 5µm to 12µm. The number of hyphal segments from four fields of view in the cortex, four in the vascular region and four

in the medulla were counted in each section. The results are given in Table 18 in the Appendix. (Fig. 20 illustrates the three regions examined.)

The results clearly show that there are more hyphal segments in the medulla than in the cortex, while the vascular region contains the least. A statistical analysis (Table 31) shows that the differences were significant ($P < 0.01$) indicating that the medulla is much more susceptible than the cortex and the vascular tissue is most resistant to colonization. This result is in agreement with results obtained by Lapwood (1965c) and Cupsa (1974) for tuber tissue when they showed that the vascular ring normally prevents spread of infection which is established in the cortex, through into the medulla.

An analysis of the distribution of hyphal segments was also carried out, Table 32, and they were shown to be non-randomly distributed in both cortex and medulla ($P < 0.001$). Thus as in the tubers of this clone pockets of tissue exist within the stem which appear to be more resistant to invasion than others.

The four types of haustoria found in tuber tissue were also observed, but in stem tissue the filiform and knob types were the most frequent, in contrast to the tuber where the filiform and peg types were commonest (Table 33). All the haustoria observed in the stem appeared to be without a sheath.

The numbers of cells in contact with hyphae with and without haustoria are given in Table 34. However, a much greater percentage of the cells in contact with hyphae in stem tissues contained haustoria than in tuber tissue; 59.6% compared to 21.1% (See tables 29, 34).

An analysis of their distribution was carried out (Table 35) and thus is consistent with the results for the tuber tissue of all clones examined. This non-random distribution, as discussed earlier, could

be either due to the host cells differing in their resistance to penetration by haustoria or to the sites at which haustoria can develop along the hyphae not being uniformly distributed. These studies alone do not differentiate between the two possibilities.

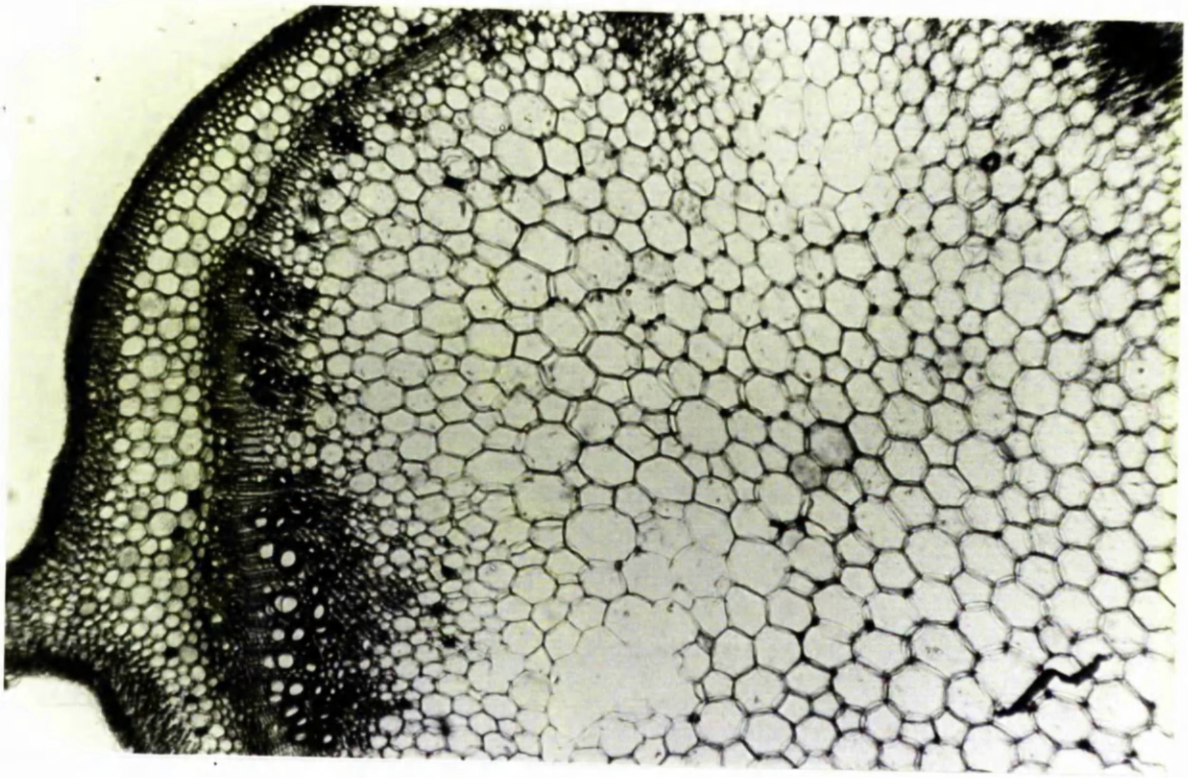


Figure 20 The three regions, cortex, vascular tissue and pith examined in stem sections of Craigs Alliance

TABLE 31 Analysis of variance on the total number of hyphal segments in the cortex, medulla and vascular bundle of the stem of Craigs Alliance

S. of V.	N	M.S.	F	P
Between Treatments	2	26.5	5.4	< 0.01
Residual	102	4.9		

TABLE 32 Analyses for non-random distribution of hyphae in the stem tissues of Craigs Alliance

		0	1	2	3	4	5	x^2	P
Cortex	Observed frequency	118	13	6	2	0	1		
					9				
	Expected frequency	108.3	27.8		3.9			15.4	< 0.001
	Difference	0.87	7.88		6.67				
Medulla	Observed frequency	67	26	16	14	17			
	Expected frequency	38.7	49.9	32.2	13.8	5.8		61.8	< 0.001
	Difference	28.3	23.9	16.2	0.2	11.2			

TABLE 33 Total numbers and percentage distribution of different types of haustoria in the cells of stem tissue of Craigs Alliance

Peg	Knob	Filiform	Hook
30	141	279	29
6.3%	29.4%	58.2%	6.1%

TABLE 34 Percentage number of cells with and without haustoria in contact with fungal hyphae in stem tissues of Craigs Alliance

Cells without haustoria	Percentage	Cells with haustoria	Percentage	Total Number of cells examined
110	40.4	162	59.6	272

TABLE 35 Analysis for non-random distribution of haustoria in the cells of the stem tissue of Craigs Alliance

Number of individual haustoria per cell	0	1	2	3	4	5	6	7	x^2	P
Observed frequency	110	23	43	42	26	9	4	15 (28)		
Expected frequency	40.7	77.3	73.5	46.5	22.1	8.5	2.7	0.7 (11.9)	191.7	<0.001
Difference	69.3	54.3	30.5	4.5	3.9	16.1				

C - Hyphae and haustoria in leaf tissue

Materials and Methods

The cultivar Craigs Alliance was again used.

Eight week old plants grown in 12 inch pots in the greenhouse were inoculated by spraying the leaves with a concentrated suspension of sporangia. The plants, covered with polythene bags for 18 hours to provide a high relative humidity, were incubated in a growth room at 20°C.

Detached leaves were inoculated with discs of filter paper soaked in a sporangial suspension before incubating in dishes lined with moist filter paper at 20°C for five days.

In preliminary trials six different methods for clearing and staining the infected leaf material were tried:-

A - Sectioned material

i) Fixed leaflets Leaflets were put in F.A.A. for 24 hours. After fixing, the leaflets were dehydrated using the tertiary butyl alcohol method described by Sass (1940), and then embedded in paraffin wax. Sections 18µm thick were cut on a Cambridge rocker microtome, and the sections in the wax ribbons were attached to glass slides using egg albumen. The slides were placed in xylene for 5 minutes to remove the wax and then passed through absolute alcohol and a series of alcohol water mixtures to rehydrate them before staining with lactophenol cotton blue as described on page 76 . The sections were dehydrated using alcohol water mixtures, finally absolute alcohol before clearing in pure xylene and mounting in Canada balsam.

ii) Fresh leaflets were embedded in 10% gelatine and sections 25µm

thick were cut with a freezing microtome. The sections were stained with lactophenol cotton blue and mounted in clear lactophenol.

B - Whole leaf mounts

i) Leaflets were cleared by immersion in a mixture of equal parts of chloral hydrate and pure phenol (crystals), and placed in 10% caustic potash solution for a few minutes. They were then washed in several changes of water before staining with aniline blue and finally clearing with lactic acid (Wild, 1947).

ii) Leaflets were killed with boiling water and decolorized with boiling 95% ethanol before clearing in a 1:1 mixture of phenol and chloral hydrate. The leaflets were then stained in boiling lactophenol cotton blue removing excess stain using several changes of boiling lactophenol before mounting in clear lactophenol. This is a slight modification of the method used by Miss Godwin as detailed by Blackwell (1953).

iii) Leaflets were cleared in Carnoy's solution (2 parts absolute ethanol and 1 part acetic acid), stained in a mixture of lactophenol and aniline blue and mounted in lactophenol (Ferris, 1955).

iv) Leaflets were immersed in alcoholic lactophenol cotton blue (2:1) brought to the boil and simmered for one minute after the leaflets sank. The solution was brought to the boil again for 30 seconds and then allowed to stand for 48 hours before removing the leaflets and rinsing in water. They were then placed in chloral hydrate (5 gm chloral hydrate to 2 ml water) for 30 - 50 minutes before mounting in 50% glycerine (Shipton & Brown, 1962).

Results and Discussion

The best method for getting clearly differentiated hyphae and

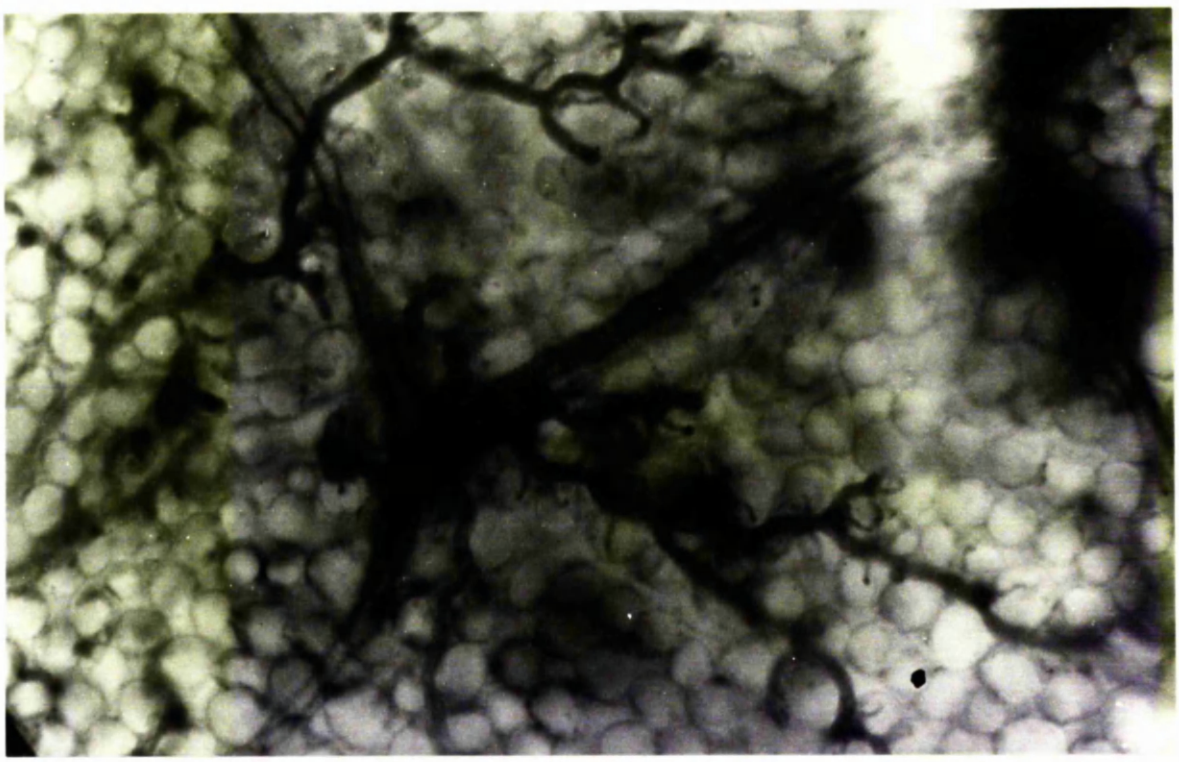
haustoria was the modified method of Miss Godwin (Blackwell, 1953).

The hyphae were aseptate and intercellular, branching frequently to form dichotomous branches (Figure 21). The hyphae in the leaves were generally narrower than in the tuber and stem with diameters varying between 3 and 8µm.

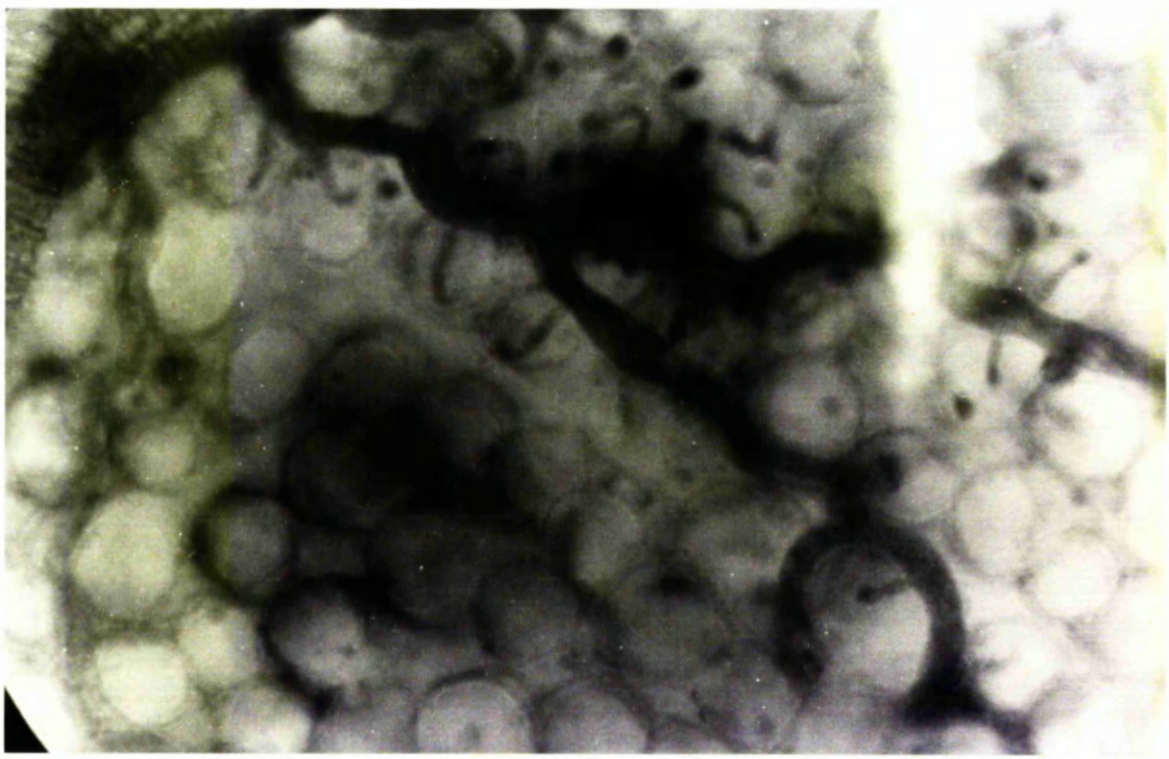
Haustoria were infrequently formed. Out of a total of 551 cells observed adjacent to hyphae only 5.6% contained haustoria, and never more than two haustoria per cell (Table 36). Sometimes they were formed when the hyphae branched but also along unbranched regions of hyphae as well (Figure 21). They were slender and shorter than those commonly observed in the tuber and stem tissues but because the cells were smaller they sometimes reached through to the opposite wall. Then its tip bent back giving a hook type haustorium (Figure 22). The only types of haustoria observed were straight or curved filiform types or hooked types (Figure 22) with the straight filiform type being most common (Table 37). The length of the filiform type varied from 9 - 20µm and the hook type from 10 - 20µm, but they were never more than 2.5µm in diameter and no haustoria with sheaths were found. No evidence was obtained to indicate why the haustoria were less frequent and why only two types of haustoria were present in leaf tissue. Thus it is not known whether this is because the cells of the leaf are more resistant or because it is more difficult to differentiate the other two types of haustoria in stained whole leaf preparations than it is in thin sections of stem and tuber tissues.

Figure 21 Dichotomously branched hyphae growing between the mesophyll cells of the leaf of Craigs Alliance. Some cells contain long unbranched haustoria.

Figure 22 Filiform and hook types of haustoria in mesophyll cells of the leaf of Craigs Alliance.



105 µm



35 µm

TABLE 36 Percentage number of cells with and without haustoria in contact with fungal hyphae in leaf tissues of Craigs Alliance

Cells without haustoria	Percentage	Cells with haustoria	Percentage	Total number of cells examined
520	94.4%	31	5.6%	551

TABLE 37 Total number and percentage distribution of the two types of haustoria in the cells of leaf tissue of Craigs Alliance

Filiform	Hook
67	16
80.7%	19.3%

General Conclusions

There were no significant differences in the total number of hyphal segments in the tuber tissues between the different clones or between the different regions of the tuber within each clone. Thus there is no evidence to indicate that active factors accumulate to different levels in the tissues behind the hyphal front in the different clones and affect the extent to which lateral branches develop and further colonize the tissues. On the other hand the medullary tissues in the tubers and stems of Craigs Alliance and in the tubers of Pentland Dell and Pentland Squire are not uniformly colonized. This indicates that uneven distribution of passive factors plays a role in tissue colonization.

Four types of haustoria have been observed in tuber and stem tissues, but they have the same percentage occurrence in the tubers of all clones, and thus there is no relation between haustorial type and resistance to penetration and the establishment of growth at the inoculated surface of the slice and the establishment of visible sporulating mycelium on the opposite surface (Growth Phases I and III). However, the total number of haustoria is correlated with resistance to growth phases I and III and to resistance to lesion growth, and thus factors affecting haustorial development may be important in the differences in race non-specific resistance between the clones. The non-random distribution of haustoria is consistent with the suggestion by Blackwell (1953) that host cells may differ in their resistance to penetration by haustoria.

It is not known why two of the types of haustoria were absent from the leaf tissues. It may be because the haustorial type is mainly influenced by the host cell and that the knob and peg types are not

induced to form in leaf tissues. It may also be that they are present but it is not possible to differentiate them in whole leaf mounts.

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APPENDIX

APPENDIX

Table 1 Growth of hyphae through tuber tissue slices of different thicknesses

Clone	Replicate	Slice thickness (mm) 7	12	17	22	27	33
Craigs Alliance	1	120*	180	240	276	288	324
	2	120	144	192	216	228	276
	3	156	186	204	216	234	264
	4	132	168	216	228	252	288
	5	120	156	210	234	252	300
	6	120	168	204	228	264	312
	Total	768	1002	1266	1398	1518	1764
	Mean	128	167	211	233	253	294
Pentland Dell	1	138	216	228	282	312	324
	2	162	180	204	216	234	288
	3	168	204	216	228	252	288
	4	132	186	228	240	288	312
	5	120	168	240	252	264	300
	6	144	192	204	234	252	294
	Total	864	1146	1320	1452	1602	1806
	Mean	144	191	220	242	267	301
Pentland Squire	1	132	168	204	240	276	312
	2	144	186	204	228	264	300
	3	120	156	180	210	240	276
	4	132	186	228	276	288	300
	5	162	186	228	252	276	312
	6	162	180	204	234	252	288
	Total	852	1062	1248	1440	1596	1788
	Mean	142	177	209	240	266	298
Pentland Raven	1	138	216	258	288	312	330
	2	168	186	216	234	276	324
	3	180	192	210	234	252	300
	4	138	168	240	288	300	312
	5	132	186	252	276	288	324
	6	168	210	216	228	252	300
	Total	924	1158	1392	1548	1680	1890
	Mean	154	193	232	258	280	315
6003ab(33)	1	192	228	264	300	324	360
	2	156	192	216	210	288	336
	3	168	204	240	252	276	312
	4	144	168	234	288	312	360
	5	168	186	216	240	288	312
	6	180	216	240	276	288	324
	Total	1008	1194	1410	1566	1776	2004
	Mean	168	199	235	261	296	334

*Time (hours) required for hyphae to grow through slice and to develop aerial sporulating mycelium on the opposite surface.

Table 2 Growth of hyphae through tuber tissue slices of different thicknesses

Clone	Replicate	Slice thickness (mm) 7	12	17	22	27	33
Craigs Alliance	1	120*	156	210	234	252	264
	2	156	186	204	216	234	264
	3	96	114	138	180	210	252
	4	96	108	132	168	192	240
	5	108	132	144	180	210	264
	6	96	114	144	156	180	228
	Total	672	810	972	1134	1278	1512
	Mean	112	135	162	189	213	252
Pentland Dell	1	114	138	162	180	204	288
	2	120	138	168	192	216	264
	3	120	138	168	192	216	264
	4	132	144	180	210	228	276
	5	132	144	162	204	228	252
	6	120	138	168	186	204	264
	Total	738	840	1008	1164	1296	1608
	Mean	123	140	168	194	216	268
Pentland Squire	1	96	120	144	180	210	264
	2	96	120	156	186	240	276
	3	120	132	162	186	210	264
	4	120	144	168	204	228	276
	5	132	144	180	192	210	288
	6	108	132	144	156	180	240
	Total	672	792	954	1104	1278	1608
	Mean	112	132	159	184	213	268
Pentland Raven	1	120	132	156	192	216	264
	2	132	144	162	180	210	264
	3	108	138	156	192	204	252
	4	120	144	156	186	210	264
	5	108	120	162	180	204	288
	6	114	138	168	192	216	300
	Total	702	816	960	1122	1260	1632
	Mean	117	136	160	187	210	272
6003ab(33)	1	120	144	180	192	216	288
	2	138	156	186	204	228	276
	3	132	144	180	204	252	300
	4	144	156	192	216	240	276
	5	120	138	186	192	210	288
	6	144	138	192	204	228	312
	Total	798	876	1116	1212	1374	1740
	Mean	133	146	186	202	229	290

*Time (hrs) required for hyphae to grow through slice and to develop aerial mycelium on the opposite surface.

Table 2a Joint regression analysis of variance of hyphal growth rate in tuber slices of the five clones

Source of Variation	N	M.S.	V.R.	P
Joint regression	1	73929.2		
Heterogeneity of regression	4	21.65	--	NS
Error	20	88.82		

Table 2b Analyses of differences between points of intercept of the regression lines of pairs of clones

Intercepts being compared	N	t	P
Craigs Alliance vs. 6003ab(33)	8	0.839	NS
Craigs Alliance vs. Pentland Squire	8	0.938	NS
Pentland Squire vs. 6003ab(33)	8	1.274	NS
Pentland Squire vs. Pentland Dell	8	1.160	NS
Pentland Squire vs. Pentland Raven	8	0.323	NS
Pentland Dell vs. Pentland Raven	8	0.671	NS
Pentland Dell vs. 6003ab(33)	8	0.342	NS

Table 3 Growth of hyphae through tuber tissue slices of different thicknesses

Clone	Replicate	Slice thickness (mm) 7	12	17	22	27	33
Craigs Alliance	1	108*	138	168	180	216	264
	2	168	180	192	216	240	288
	3	168	180	216	240	264	312
	4	138	168	204	228	252	300
	5	144	156	192	204	240	288
	6	132	152	180	192	288	276
	Total	864	974	1152	1260	1440	1728
	Mean	144	162	192	210	240	288
Pentland Dell	1	132	156	186	216	240	300
	2	120	144	180	204	234	288
	3	108	132	168	180	228	276
	4	132	156	192	228	258	300
	5	144	168	210	234	264	312
	6	156	180	216	234	288	324
	Total	792	936	1152	1296	1512	1800
	Mean	132	156	192	216	252	300
Pentland Squire	1	108	120	144	180	210	240
	2	96	108	132	168	204	252
	3	144	162	186	234	258	288
	4	120	132	156	192	240	276
	5	132	162	186	234	252	312
	6	120	144	168	216	240	288
	Total	720	828	972	1224	1404	1656
	Mean	120	138	162	204	234	276
6003ab(33)	1	180	204	234	264	288	300
	2	162	180	210	240	276	324
	3	180	204	228	264	288	312
	4	156	180	216	264	300	348
	5	132	162	180	240	264	330
	6	168	192	228	276	306	330
	Total	978	1122	1296	1548	1722	1944
	Mean	163	187	216	258	287	324

*Time (hrs) required for hyphae to grow through slice and to develop

Table 3a Joint regression analysis of variance of hyphal growth rate in tuber slices of the four clones

Source of Variance	N	M.S.	V.R.	P
Joint regression	1	68877.99		
Heterogeneity of regression	3	98.4	2.22	NS
Error	16	44.2		

Table 3b Analyses of differences between points of intercept of the regression lines of pairs of clones

Clones being compared	N	t	P
Craigs Alliance vs. 6003ab(33)	8	1.56	NS
Craigs Alliance vs. Pentland Squire	8	2.90	0.02
Pentland Squire vs. 6003ab(33)	8	5.14	< 0.001
Pentland Squire vs. Pentland Dell	8	1.46	NS
Pentland Dell vs. 6003ab(33)	8	4.24	< 0.01

Table 4i Effect of the wound periderm on hyphal growth

Clone	Replicate	Slice thickness 2.3 cm	3.3 cm	4.3 cm	5.3 cm
Craigs Alliance	1	252*	336	420	504
	2	240	312	372	456
	3	252	324	396	492
	4	264	312	396	468
	5	240	324	396	492
	6	252	330	360	480
	Mean	250	323	390	482
Pentland Dell	1	258	348	432	468
	2	240	312	408	522
	3	282	324	396	492
	4	252	312	402	480
	5	258	324	408	492
	6	258	324	408	540
	Mean	258	324	409	499
Pentland Squire	1	234	336	402	480
	2	234	288	360	450
	3	240	312	384	456
	4	252	300	372	492
	5	240	300	378	468
	6	240	306	378	468
	Mean	240	307	379	469
6003ab(33)	1	288	354	444	540
	2	252	330	408	480
	3	282	348	420	498
	4	264	336	408	528
	5	282	354	420	504
	6	288	312	420	558
	Mean	276	339	420	518

*Time (hours) required for hyphae to grow through slice and develop aerial sporulating mycelium on the opposite surface.

Table 4ii Effect of the wound periderm on hyphal growth

Clone	Replicate	Slice thickness 2.3 cm	3.3 cm	4.3 cm	5.3 cm
Craigs Alliance	1	210*	252	300	348
	2	228	264	312	360
	3	216	252	306	354
	4	228	276	330	372
	5	234	288	348	384
	6	222	300	318	360
	Mean	223	272	319	363
Pentland Dell	1	216	264	306	354
	2	234	276	324	360
	3	240	288	336	384
	4	234	282	330	384
	5	240	288	336	396
	6	234	282	300	408
	Mean	233	280	322	381
Pentland Squire	1	192	240	288	324
	2	216	252	300	354
	3	216	264	312	360
	4	234	276	324	372
	5	216	240	300	348
	6	234	252	306	354
	Mean	218	254	305	352
6003ab(33)	1	240	282	330	384
	2	240	276	324	372
	3	240	288	336	396
	4	252	300	346	408
	5	252	306	354	420
	6	240	264	336	450
	Mean	244	286	338	405

*Time (hours) required for hyphae to grow through slice and to develop aerial sporulating mycelium on the oppcsite surface.

Table 5 Effect of temperature on hyphal growth through tuber tissue

Temperature	Replicate	Slice thickness (mm) 7	12	17	22	27
15°C	1	120*	156	192	234	276
	2	138	168	204	240	288
	3	120	168	204	258	300
	4	144	180	210	264	306
	5	162	186	216	288	336
	6	156	180	216	276	312
	Total	840	1038	1242	1560	1818
	Mean	140	173	207	260	303
18°C	1	96	120	156	192	240
	2	108	156	186	216	276
	3	138	168	216	264	288
	4	144	144	180	240	282
	5	120	132	192	216	252
	6	114	132	156	204	264
	Total	720	852	1086	1332	1602
	Mean	120	142	181	222	267
20°C	1	96	108	138	180	216
	2	120	138	168	216	240
	3	120	144	168	204	234
	4	120	132	162	192	234
	5	108	144	186	210	258
	6	114	144	168	192	216
	Total	678	810	990	1194	1398
	Mean	113	135	165	199	233
22°C	1	90	108	180	216	258
	2	120	156	192	234	276
	3	96	132	168	210	252
	4	114	156	180	228	264
	5	138	144	180	216	258
	6	114	138	168	204	240
	Total	672	834	1068	1308	1548
	Mean	112	139	178	218	258
25°C	1	120	144	180	228	264
	2	144	168	204	264	288
	3	132	168	192	234	300
	4	138	180	270	240	276
	5	138	162	270	252	300
	6	144	168	204	258	288
	Total	816	990	1200	1476	1716
	Mean	136	165	200	246	286

*Time (hours) required for hyphae to grow through slice and to develop aerial sporulating mycelium on the opposite surface

Table 6 Hyphal growth on media

Medium	Days after inoculation	Temperature °C 15	18	20	21	22	25
French Bean Agar	5	36*	46	54	57	52	36
	6	45	55	65	68	62	44
	7	51	63	75	79	72	53
Pea Sucrose Agar	5	34	44	51	52	51	35
	6	41	53	61	64	58	43
	7	48	60	70	73	71	51
Complete Medium	5	23	29	32	34	30	24
	6	28	34	40	43	38	30
	7	33	43	50	51	47	36
Minimal Medium	5	19	23	25	26	24	19
	6	23	29	32	34	31	21
	7	27	34	40	42	39	28

*Colony diameter (mm)

Each figure is the mean of five replicates

Table 7 Comparison of growth in rose and heel ends of the tuber

Clone	Replicate	Rose end Slice thickness (mm) 7	Rose end Slice thickness (mm) 12	Heel end Slice thickness (mm) 7	Heel end Slice thickness (mm) 12
Pentland Dell	1	144*	168	132	162
	2	168	192	156	186
	3	162	162	138	156
	4	156	180	156	180
	5	15[illegible]	180	168	186
	6	156	168	132	180
	7	120	156	120	168
	8	132	156	120	168
	9	162	180	168	210
	10	144	168	144	168
	Total	1500	1710	1434	1764
	Mean	150	171	143.4	176.4
Pentland Squire	1	156	180	120	156
	2	132	168	132	144
	3	120	156	144	168
	4	132	144	156	168
	5	144	180	168	192
	6	168	186	162	192
	7	144	156	132	144
	8	120	162	144	168
	9	132	168	120	144
	10	132	168	144	180
	Total	1380	1668	1422	1656
	Mean	138	166.8	142.2	165.6
6003ab(33)	1	132	168	162	192
	2	144	168	156	168
	3	162	210	162	192
	4	168	204	168	216
	5	190	216	138	204
	6	152	216	168	192
	7	192	216	180	204
	8	156	180	138	168
	9	180	216	158	180
	10	180	216	180	204
		1646	2010	1608	1920
		165	201	161	192

*Time (hours) required for hyphae to grow through slice and to develop aerial sporulating mycelium on the opposite surface.

Table 8 Comparison of growth in slices from small and large tubers

Clone	Replicate	Slices from small tuber Slice thickness (mm) 7	12	17	Slices from large tuber Slice thickness (mm) 7	12	17
Pentland Dell	1	114	162	204	114	144	180
	2	120	156	180	114	138	168
	3	120	162	192	96	120	168
	4	90	144	204	132	162	192
	5	108	144	180	96	144	192
	Total	552	768	960	552	708	900
	Mean	110	154	192	110	142	180
Pentland Squire	1	114	144	180	90	132	180
	2	114	138	180	114	138	180
	3	90	120	168	120	144	192
	4	96	114	156	90	120	168
	5	120	138	180	108	144	168
	Total	534	654	864	522	678	888
	Mean	106	131	173	104	136	178

*Time (hours) required for hyphae to grow through slices and to develop aerial sporulating mycelium on the opposite surface.

Table 9 Comparison of growth in cortex and medulla

Clone		Replicate	Cortex Slice thickness (mm) 7	12	17	Medulla Slice thickness (mm) 7	12	17
Pentland Dell	Expt. 1	1	96*	138	180	114	144	180
		2	90	120	162	114	138	180
		3	96	138	180	90	120	168
		4	80	114	144	96	114	156
		5	96	120	156	96	138	180
		Total	458	630	822	510	654	864
		Mean	92	126	164	102	131	173
	Expt. 2	1	72	114	156	120	156	192
		2	108	144	180	96	114	168
		3	96	132	168	114	138	180
		4	96	120	156	90	144	168
		5	108	138	180	96	144	168
		Total	480	648	840	516	696	876
		Mean	96	130	168	103	139	175
Pentland Squire	Expt. 1	1	72	108	168	72	96	168
		2	90	120	162	96	120	162
		3	84	120	156	84	114	144
		4	72	132	144	72	120	138
		5	96	132	168	96	144	168
		Total	414	612	798	420	594	780
		Mean	83	122	160	84	119	156
	Expt. 2/							

Table 9 (Cont.)

Clone	Replicate	Cortex Slice thickness (mm) 7	12	17	Medulla Slice thickness (mm) 7	12	17
Pentland Squire Expt. 2	1	72	108	144	96	132	162
	2	84	120	156	84	120	162
	3	90	120	162	96	120	156
	4	96	132	168	120	144	186
	5	84	120	168	90	120	168
	Total	426	600	798	486	636	834
	Mean	85	120	160	97	127	167

*Time (hours) required for hyphae to grow through slice and to develop aerial sporulating mycelium on the opposite surface.

Table 10 Comparison of lesion development in tubers from different clones

a- 5 days after inoculation

Clone	Tuber	Rose end			Heel end		
		Necrosis	Fluorescence	Total	Necrosis	Fluorescence	Total
Craigs Alliance	1	9*	7*	16	4	8	12
	2	8	4	12	11	9	20
	3	11	8	19	9	5	14
	4	5	8	13	7	7	14
	5	12	7	19	7	10	17
	Total	45	34	79	38	39	77
	Mean	9.0	6.8	15.8	7.6	7.8	15.4
Pentland Dell	1	8	6	14	6	6	12
	2	6	7	13	7	8	15
	3	7	5	12	3	5	8
	4	3	3	6	4	6	10
	5	4	6	10	9	5	14
	Total	28	27	55	29	30	59
	Mean	5.6	5.4	11.0	5.8	6.0	11.8
Pentland Squire	1	9	8	17	4	5	9
	2	7	8	15	7	7	14
	3	8	5	13	5	5	10
	4	6	7	13	10	9	19
	5	9	5	14	6	4	10
	Total	39	33	72	32	30	62
	Mean	7.8	6.6	14.4	6.4	6.0	12.4

6003ab(33)/

Table 10 (Cont.)

Clone	Tuber	Rose end			Heel end		
		Necrosis	Fluorescence	Total	Necrosis	Fluorescence	Total
6003ab(33)	1	7	4	11	4	6	10
	2	4	5	9	3	3	6
	3	3	4	7	4	5	9
	4	5	7	12	3	4	7
	5	5	6	11	5	7	12
	Total	24	26	50	19	25	44
	Mean	4.8	5.2	10.0	3.8	5.0	8.8
b- 15 days after inoculation							
Craigs Alliance	1	24	19	43	27	27	54
	2	33	24	57	32	19	51
	3	27	27	54	23	20	43
	4	30	21	51	25	23	48
	5	23	23	46	33	16	49
	Total	137	114	151	140	105	245
	Mean	27.4	22.8	50.2	28	21	49
Pentland Dell	1	29	15	44	27	20	47
	2	23	19	42	21	20	41
	3	15	20	35	25	14	39
	4	24	17	41	25	19	44
	5	21	16	37	20	14	34
	Total	112	87	199	118	87	205
	Mean	22.4	17.4	39.8	23.6	17.4	41

Pentland Squire/

Table 10 (Cont.)

Clone	Tuber	Rose end			Heel end		
		Necrosis	Fluorescence	Total	Necrosis	Fluorescence	Total
Pentland Squire	1	25	28	53	28	26	54
	2	21	25	46	25	18	43
	3	25	24	49	22	19	41
	4	28	19	47	25	15	40
	5	23	19	42	21	20	41
	Total	112	115	237	121	98	219
	Mean	24.4	23.0	47.4	24.2	19.6	43.8
6003ab(33)	1	22	17	39	19	9	28
	2	22	14	36	21	15	36
	3	24	13	37	19	8	27
	4	18	9	27	23	13	36
	5	21	11	32	17	16	33
	Total	107	64	171	99	61	160
	Mean	21.4	12.8	34.2	19.8	12.2	32

*Width of necrotic or fluorescent tissue in mm.

TABLE 11 Analysis of variance on number of cells per unit volume of medullary tissue between the five clones

Source of Variance	N	M.S.	V.R.	P
Number of cells between clones	4	7.7	--	NS
Residual	195	21.76		

TABLE 12a Total hyphal length (μm) in two fields of view per section from ten sections of each of the five clones

Craigs Alliance	Pentland Dell	Pentland Squire	Pentland Raven	6003ab(33)
450	900	600	480	240
570	540	130	00	500
520	1960	830	430	1090
1240	140	850	660	230
700	360	750	710	00
00	00	840	500	800
210	310	1050	510	640
950	490	00	00	730
80	430	580	280	550
00	220	00	650	00
4720	5350	5630	4220	4780

TABLE 12b Total number of hyphal segments in two fields of view per section from ten sections of each of the five clones

Craigs Alliance	Pentland Dell	Pentland Squire	Pentland Raven	6003ab(33)
3	4	4	3	1
3	2	1	0	4
3	5	4	1	7
4	1	5	2	1
4	2	3	2	0
0	0	1	1	2
1	1	4	1	3
4	2	0	0	2
1	1	3	2	3
0	1	0	3	0
23	19	25	15	23

TABLE 12c Total number of branches in two fields of view per section from ten sections of each of the five clones

Craigs Alliance	Pentland Dell	Pentland Squire	Pentland Raven	6003ab(33)
3	9	9	6	1
5	4	3	0	12
3	12	13	0	11
8	1	11	5	5
5	12	7	5	0
0	0	12	6	3
0	4	6	4	8
3	4	0	0	5
2	4	2	6	6
0	1	0	6	0
29	51	63	38	51

TABLE 12d Total number of haustoria in two fields of view per section from ten sections of each of the five clones

Craigs Alliance	Pentland Dell	Pentland Squire	Pentland Raven	6003ab(33)
11	4	9	3	2
28	0	0	0	6
1	17	0	5	9
14	1	10	0	7
9	12	6	2	0
0	0	3	4	2
0	5	25	5	10
7	6	0	0	4
1	0	14	3	5
0	2	0	4	0
71	47	67	26	45

TABLE 13 Total number of hyphal segments in nine fields of view per section from seven sections of each of three clones, Craigs Alliance, Pentland Dell and 6003ab(33)

	Craigs Alliance	Pentland Dell	6003ab(33)
1.	13	6	10
2.	9	5	8
3.	7	7	4
4.	6	10	10
5.	8	9	7
6.	12	14	3
7.	8	6	12
Ex	63	57	54

TABLE 14 Total number of hyphal segments per section from eight sections from the medulla of each rose and heel ends of the tubers of two clones, Pentland Dell and Pentland Squire

	Pentland Dell		Pentland Squire	
Section	Rose End	Heel End	Rose End	Heel End
1.	57	55	70	88
2.	16	34	63	45
3.	47	44	54	38
4.	48	59	84	55
5.	37	36	46	24
6.	40	39	16	47
7.	35	19	17	23
8.	45	48	13	38
Ex	325	334	363	358

TABLE 15 Total number of hyphal segments per section from eight sections from the central medulla of each small and large tubers of Pentland Dell

Section	Small Size	Large Size
1.	81	33
2.	53	38
3.	32	45
4.	45	76
5.	9	40
6.	23	50
7.	33	37
8.	39	27
	315	346

TABLE 16 Total number of hyphal segments per section from five sections of each of the cortex and central medulla of two clones, Pentland Dell and Pentland Squire

Section	Pentland Dell		Pentland Squire	
	Cortex	Medulla	Cortex	Medulla
1.	67	48	74	62
2.	26	46	60	54
3.	42	30	31	38
4.	34	54	61	45
5.	64	26	28	36
Ex	233	204	254	235

TABLE 17 Total number of each of the four types of haustoria per section from six sections of each of the five clones

K = Knob, P = Peg, F = Filiform, H = Hook, T = Total

Section	Pentland Raven					6003ab(33)					Pentland Dell					Pentland Squire					Craigs Alliance				
	K	P	F	H	T	K	P	F	H	T	K	P	F	H	T	K	P	F	H	T	K	P	F	H	T
1.	6	14	18	2	40	11	19	27	2	59	3	19	28	3	53	14	25	51	9	99	12	26	48	5	91
2.	4	8	42	6	60	8	9	10	0	27	7	17	14	0	38	2	2	2	0	6	7	37	62	10	116
3.	7	6	11	0	24	2	14	18	1	35	8	11	8	1	28	4	12	9	1	26	7	33	57	4	101
4.	1	1	9	1	12	0	11	14	0	25	6	7	7	0	20	5	25	54	6	90	2	12	14	0	28
5.	2	9	10	1	22	2	20	45	0	67	2	18	25	3	48	6	13	11	2	32	10	22	31	3	66
6.	6	5	9	0	20	3	0	0	2	5	2	12	27	0	41	7	5	11	0	23	7	13	16	0	36
	26	43	99	10	178	26	73	114	5	218	28	84	109	7	228	38	82	138	18	276	45	143	228	22	438

TABLE 18 Total number of hyphal segments from four fields of view from each tissue region per section in stem of Craigs Alliance

Stem	Section	Cortex	Vascular	Medulla
1	1	6	1	10
	2	3	0	7
	3	3	0	13
	4	1	0	12
	5	2	1	8
2	1	4	2	13
	2	0	0	6
	3	0	0	5
	4	1	1	2
	5	1	1	4
3	1	0	0	3
	2	0	0	4
	3	0	0	0
	4	2	0	11
	5	3	0	5
4	1	1	0	12
	2	0	0	3
	3	1	0	3
	4	1	0	1
	5	0	0	7
5	1	0	0	2
	2	1	1	7
	3	1	0	5
	4	0	0	3
	5	0	0	1
6	1	2	0	7
	2	0	0	5
	3	0	0	2
	4	0	0	1
	5	0	0	0
7	1	0	0	4
	2	0	0	7
	3	1	0	4
	4	2	0	2
	5	0	0	1
		36	7	180