Factors influencing the resistance of potato cultivars to the gangrene pathogen, *Phoma exigua* Desm. var. *foveata* (Foister) Boerema

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by

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I hereby declare that this thesis has been composed entirely by myself and that all the work described herein was carried out by myself alone, except where otherwise stated in the acknowledgements.

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CONTENTS

ABSTRACT

This study was concerned with factors affecting the development of potato gangrene caused by Phoma exigua var. foveata, with particular reference to the assessment of cultivar resistance to the disease. The factors considered were isolate pathogenicity, tuber inoculum level, tuber damage, method of inoculation and assessment, incubation temperature and tuber tissue and damage resistance. In addition the influence of isolate and cultivar on the transmission of inoculum from seed to progeny tubers was assessed.

The importance of using isolates of high pathogenicity to detect differences in resistance among cultivars was emphasised. Variation in pathogenicity among isolates was associated with isolate source and history, but there was no evidence of consistent cultivar x isolate interactions of an order to suggest physiological specialisation in this pathogen. In considering the relative contribution of isolate and cultivar to variation in disease development the contribution of isolate was much less than that of cultivar. The relative resistances of cultivars depended to some extent on inoculation technique and assessment method. This indicated the existence of two major components of tuber resistance, namely damage and tissue resistance. There was also evidence that the

relative susceptibilities of cultivars could differ for the tuber cortex and medulla: thus some cultivars may have lesions with a small surface area but an appreciable depth of penetration whereas others show wide, shallow rots. The actual level of gangrene depended upon the interaction between cultivar, tuber damage and tuber inoculum density. High inoculum doses coupled with inoculation techniques which allowed the expression of cultivar damage resistance were the most useful in discriminating among cultivars. However, with some assessment methods cultivar differences were obscured at high inoculum densities. Incubation temperature also influenced the course of disease development. At higher incubation temperatures (10°C) lesions were arrested in all cultivars tested whereas at lower temperatures (4°C) cultivars showed differences in tissue resistance reflected in the degree of lesion retardation but rot development continued in all cultivars. In attempts to gain further evidence of tissue resistance factors gamma irradiation studies were carried out. Irradiation of tubers reduced their resistance to gangrene to an extent dependent upon the irradiation dose and the delay, after irradiation, between wounding and inoculation. There was evidence that wound periderm formation was of minor importance in tissue resistance and that irradiation-induced susceptibility

was not associated with tuber cell death. Transmission of P. exigua var. foveata from seed to daughter tubers was shown to be affected by isolate and cultivar but further work is required in this area.

CHAPTER 1

General Introduction

1.1 INTRODUCTION

Potato gangrene is recognised as one of the major storage disease problems in the UK and since it was first identified (Alcock & Foister, 1936) it has generated considerable research interest. Nevertheless, there is still a need for a fuller knowledge of the variation in pathogenicity of the causal fungus and of the components of resistance to infection of the host in order to provide a better basis for breeding for resistance. The purpose of the present study was to investigate further certain aspects relating to fungal pathogenicity and host resistance and also methods of screening cultivars for resistance. Various factors which contribute to disease development, ie inoculum level, tuber damage and tuber tissue susceptibility were considered. The influence of exposure of tubers to gamma irradiation was also considered as an approach to a fuller understanding of tissue resistance.

1.2 LITERATURE REVIEW

1.2.1 Potato gangrene

In the United Kingdom the fungus Phoma exigua Desm. var. foveata (Foister) Boerema is the main cause of potato gangrene, while the ubiquitous soil-borne P. exigua Desm. var. exigua is occasionally associated with the disease (Logan, 1967; Todd & Adam, 1967; Khan

& Logan, 1968; Malcolmson & Gray, 1968b; Fox & Dashwood, 1972). Two synonyms for P. exigua var. foveata have been used: Phoma foveata Foister and Phoma solanicola Prill. and Del. f. foveata (Foister) Malcolmson (Boerema & Howeler, 1967).

In general var. foveata is more pathogenic on potato tubers than var. exigua (Todd & Adam, 1967; Anon., 1977), although in certain circumstances var. exigua has an equal, or greater, effect. Malcolmson & Gray (1968c) demonstrated that different potato cultivars may respond differentially to the two varieties of the fungus. They found that whereas var. foveata produced larger rots than var. exigua on Arran Pilot and Golden Wonder, var. exigua caused more severe rotting in Kerr's Pink, Doon Star and Catriona. Malcolmson & Gray also found that tuber maturity influences the relative effects of the two pathogens. They demonstrated that var. foveata is more pathogenic than var. exigua if tested on immature Doon Star tubers, but the opposite is true if the test is repeated using tubers of the same cultivar which have been stored for several months.

P. exigua var. foveata has a narrower host range than var. exigua: var. foveata is pathogenic only on potatoes whereas var. exigua infects a wide variety of plants (Boerema & Howeler, 1967).

P. exigua var. foveata and var. exigua cannot be distinguished morphologically (Malcolmson & Gray, 1968c).

They are identified on the basis of a biochemical difference: only var. foveata isolates produce yellowish brown, anthraquinone pigments (Logan & Khan, 1969). There are three methods which are used to test for these pigments. The Ammonia Test (Logan & Khan, 1969) consists of exposing cultures to ammonia vapour. The anthraquinone pigments of the var. foveata isolates react with the vapour to give a characteristic pinkish-red colour to the mycelium within seconds. In the Thiophanate-methyl Test (Tichelaar, 1974) isolates are grown on malt agar containing 25 ppm of the fungicide thiophanate-methyl. The chemical accelerates the production of yellow anthraquinone crystals in cultures of var. foveata. These cultures are easily distinguished from var. exigua cultures which remain black. The third test involves thin layer chromatography. Bick & Rhee (1966) demonstrated that one of the major anthraquinone derivatives produced by var. foveata is chrysophanol. Isolates of P. exigua var. foveata can be distinguished from var. exigua isolates by chromatographing an extract of the fungus against a chrysophanol standard (Mosch & Mooi, 1975). A great advantage of this method is that since rotted tissue can also be assayed, and therefore isolation of the fungus is not necessary, the method is very quick.

The optimum temperatures for *in vitro* growth and spore germination are lower for var. *foveata* than var. *exigua* (see Table 1.1).

Table 1.1 The optimum temperatures for *in vitro* growth and spore germination of *P. exigua* var. *foveata* and var. *exigua*
(From data of Logan & Khan, 1969)

	Phoma exigua var. *foveata*	*Phoma exigua* var. *exigua*
Optimum growth temperature	20-22 °C	24-26 °C
Temperature above which growth does not occur	24 °C	28 °C
Optimum temperature for spore germination	22 °C	25 °C

Logan & O'Neill (1970) found that isolates of var. *foveata* could be placed into two groups depending on whether or not they produced an antibiotic termed antibiotic 'E'.

Although Malcolmson (1958) and Todd & Adam (1967) established that *P. exigua* var. *foveata* can infect sound tubers through eyes and lenticels, the fungus is primarily a wound pathogen: most gangrene infection occurs when contaminated tubers are injured at harvest or during subsequent handling. Potato gangrene is a storage disease: symptoms are generally not evident at harvest but develop during storage of the crop.

Several sources and pathways of contamination of potato tubers by P. exigua var. foveata have been demonstrated. Their importance depends largely on whether the tubers are free from the pathogen or are already contaminated. The sources and pathways will therefore be placed in two groups: those important in the recontamination of P. exigua var. foveata-free nuclear seed stocks and those responsible for increasing the inoculum level on already contaminated stocks.

There are several ways in which foveata-free stocks can be contaminated. Carnegie, Adam & Symonds (1978) found that dry soil and dust in potato stores was infested with P. exigua var. foveata. The storage of foveata-free tubers in such buildings could lead to stock contamination. Whenever tubers are handled there is a risk of contamination since they will be in contact with machinery which may be coated with P. exigua var. foveata-infested soil: Copeland (1982) has indicated that pathogen-free stocks could become contaminated by being planted, harvested or graded using machinery which is coated with soil infested with P. exigua var. foveata. Field soil is another possible source of P. exigua var. foveata. Although it has been shown that the level of inoculum declines steadily after potatoes are harvested from a field, and can be difficult to detect even only two months after harvest (Todd & Adam, 1967), P. exigua var. foveata has been isolated from fields which have

not grown potatoes for five years (Khan & Logan, 1968) and seven years (Malcolmson & Gray, 1968b). In these cases it is possible that the pathogen was maintained either on potato groundkeepers, or on weeds or other crop species which can act as symptomless carriers (Fox & Dashwood, 1969; 1972; Adam & Todd, 1974). Another possible source of inoculum for the contamination of foveata-free stocks is air-borne propagules of P. exigua var. foveata in aerosols produced by rain (Carnegie, 1980).

For stocks already contaminated with the gangrene fungus the most important source of gangrene inoculum is the seed tuber (Fox & Dashwood, 1979; Adams, 1980b). The fungus can be borne on the seed tuber in a gangrene lesion, or it can colonise the tuber periderm without producing a lesion (Todd & Adam, 1967) or be carried as surface contamination (Adams, 1980b). It is unlikely that the soil is an important source of P. exigua var. foveata when there is inoculum already on the seed. The level of P. exigua var. foveata in a stock can increase by the following methods. Seed-borne P. exigua var. foveata can infect and colonise the growing stems produced by that seed (Adams, 1980b; 1983). When the haulm senesces, or is desiccated, large numbers of pycnidia are produced on the stems. Pycnospores released from these pycnidia can be washed down into the soil by rain to contaminate the daughter tubers (Khan & Logan,

1968; Logan, 1970; Adams, 1980b). Spread of the pathogen in the field from stem pycnidia can be achieved by splash dispersal (Entwistle, 1972; Logan, 1976). Seed-borne inoculum can contaminate daughter tubers more directly when P. exigua var. foveata is released from the rotting mother tuber. Copeland (1982) has shown that spread from daughter tuber to daughter tuber can occur when potatoes are mechanically handled.

The first indication that a tuber is infected with gangrene is the presence of small, dark, depressed areas on the tuber surface. These areas slowly enlarge to give characteristic 'thumb mark' lesions. Pycnidia can be produced on the lesion surface. The area of the surface lesion does not always accurately indicate the extent of internal rotting. A lesion which covers most of the tuber surface may be associated with an internal rot which is little more than two or three millimetres deep. Alternatively, the internal rot can be very extensive when the external lesion is small. In gangrene infected tubers there is a distinct boundary between rotted and healthy tissue. Cavities frequently develop within the lesion. They are lined with grey, dark brown or black mycelium and pycnidia are produced on the inner surface (Todd, 1963; Boyd, 1972; Anon., 1973).

Crop yields are not necessarily reduced by planting gangrene infected seed, in spite of the facts that crop

emergence can be delayed by as much as three weeks at low soil temperatures (Malcolmson & Gray, 1968b), blanking sometimes occurs and the plants produced are more prone to infection by Erwinia carotovora pv. atroseptica, the blackleg bacterium (Griffith, Hide, Hirst & Stedman, 1974). Yields are reduced only when the incidence of disease in the seed is very high (Hirst, Hide, Griffith & Stedman, 1970). Griffith et al. (1974) demonstrated that yields were significantly reduced only when the proportion of tubers with lesions in the stock was greater than 60%. Similarly, to reduce tuber yield by 20% the severity of infection had to be such that on average 40% of each tuber's surface area was diseased.

Gangrene incidence and severity can be reduced by minimising daughter tuber inoculum, reducing the extent of tuber damage and/or ensuring the storage environment is not conducive to gangrene development.

Since the seed tuber is the most important source of inoculum (Logan, 1969; Fox & Dashwood, 1979), the seed planted should be as free of P. exigua var. foveata as possible. It is not sufficient simply to ensure that the seed planted is free of lesions, since it has been demonstrated that the level of daughter tuber contamination can be as great when the seed planted is merely contaminated as when the seed is diseased (Griffith et al., 1974; Adams, 1980b). Attempts have been made by

the Department of Agriculture and Fisheries for Scotland to minimise seed tuber contamination by introducing P. exigua var. foveata-free nuclear stocks derived from stem cuttings or by micropropagation. However, these seed stocks can rapidly become contaminated during multiplication by VTSC growers (Carnegie, Adam, MacDonald & Cameron, 1981).

Early haulm destruction and early harvest reduces daughter tuber contamination by restricting inoculum build-up (Fox & Dashwood, 1972; Boyd, Lang & Witney, 1979). It is reported that the method used to destroy the haulm can influence gangrene incidence. When the haulm was pulled and carted off, instead of being burnt down, gangrene incidence was greatly reduced. The reduction was considered to be due to the removal of an important inoculum source (Logan, 1969; 1970).

Disinfectant fungicides applied to the harvested crop reduce gangrene development in storage. Thiabendazole (TBZ) generally gives effective control of gangrene (Copeland & Logan, 1975) as well as controlling skin spot (Polyscytalum pustulans) and dry rot (Fusarium solani var. coeruleum). It is safe for use on ware as well as seed. TBZ, in the form of a 2% mist (Logan, Copeland & Little, 1975), should be applied as soon as possible after harvest for effective control. The penetration of TBZ is not good, so the tubers must be clean when treated. The fumigant 2-amino butane (sec-butylamine) gives good

control of gangrene as well as skin spot but does not control dry rot (Boyd, 1976). It can be used on seed and ware. Potatoes to be treated must be mature and dry. The fumigant should be applied about one week after lifting to allow wounds to heal, as 2-amino butane can accentuate wound damage. Tubers need not be cleaned prior to treatment, as the presence of soil on the tubers does not impede the gas from reaching the gangrene inoculum (Boyd, Lang & Witney, 1979). The chemical sprout inhibitor tecnazene (TCNB), which is applied as a dust, gives some control of gangrene if applied at the recommended rate (Boyd, Lang & Witney, 1979). Disinfection of the seed fraction of the newly harvested crop with methoxy ethyl mercuric chloride (MEMC) was discontinued in Scotland in 1976 (Boyd, 1976) despite giving very good control of gangrene in storage and, unlike TBZ or 2-amino butane, on progeny tubers (Logan, Copeland & Little, 1975; Hide & Cayley, 1978). MEMC treatment was phased out because of the toxicity hazard of mercury.

Gangrene incidence can be considerably reduced by minimising tuber injury. Where wounding is severe a change to a more damage resistant cultivar should be considered. Good cultivation can reduce damage. For example, minimising clod formation and reducing field stoniness helps to avoid excessive wounding. Crops should be desiccated and harvested early when soil

temperatures are still relatively high since warm tubers are less susceptible to damage (Boyd, 1972). The amount of damage inflicted on potatoes during dressing varies with the type of grader. Rotating chain or rubber spool type graders cause less damage than those incorporating a reciprocating riddle (Griffith, 1969). The temperature of a stock to be graded should be raised to approximately 7 °C two to three days before dressing (Anon., 1978).

Gangrene development in storage is reduced by curing (Malcolmson & Gray, 1968a; Boyd, 1972). Curing consists of subjecting the newly harvested potatoes to a high temperature, eg about 15 °C, and high humidity, 95% RH, for about two weeks prior to storing the crop at a lower temperature and humidity. Curing reduces gangrene development by enhancing rapid wound healing.

1.2.2 Resistance of potato tubers to Phoma exigua var. foveata infection

No potato cultivar has tubers which are completely resistant to gangrene. However, cultivars differ markedly in their degree of susceptibility. There are reports that gangrene incidence tends to be higher in early than in maincrop cultivars, but Malcolmson (1958) found that susceptibility was not correlated with earliness in tests on 39 cultivars.

As P. exigua var. foveata is primarily a wound pathogen the resistance of a cultivar to gangrene is greatly influenced by the resistance of its tubers to

damage. Since different types of wound differ in their susceptibility to infection (Adams, 1980a), it is the level of resistance to the wound types which particularly predispose tubers to gangrene which has the greatest influence on resistance. Cultivars differ greatly in their susceptibility to damage (Blight & Hamilton, 1974) but the damage resistance ranking order of cultivars varies with damage type (Jellis & Haslam, 1980). Wounds which incorporate crushing of the tuber tissue are more susceptible to infection by P. exigua var. foveata than cuts, splits or scuffs (Griffith, 1970; Bak Henriksen, 1975b; Jellis & Howard, 1975; Adams, 1980a). There are two components of resistance to tuber damage, namely, resistance to wound number and resistance to wound severity. Differences in wound size are important, since severe wounds are more susceptible to gangrene than slight wounds of the same type. Adams (1980a) confirmed that it is a genuine difference in susceptibility and that larger wounds do not simply trap more inoculum. Furthermore, deeper wounds which expose the tuber's more susceptible medullary tissue are more likely to become infected than more superficial wounds which only penetrate the more resistant cortical tissue (Pietkiewicz & Jellis, 1975).

Although the basis of cultivar differences in susceptibility to damage is not fully established,

cultivar susceptibilities have been linked with specific characters which are genetically determined. Cultivars with large tubers are generally more susceptible to damage (Blight & Hamilton, 1974). However, Jellis & Haslam (1980) found that varietal differences in susceptibility to wounds which exposed the medulla were not accounted for by tuber size alone. Tuber shape also affects susceptibility to damage. Hughes (1980) stated that tubers with small radii of curvature tend to damage more easily than those with large radii of curvature. Blight & Hamilton (1974) found that, although low skin hardness is generally associated with high damage, the susceptibility of the tuber to mechanical damage appears to be affected by other characters. Umaerus (1975), from a study with 54 clones, reported that those with tubers of high elasticity, as measured by the rebound pendulum, were more resistant to shatter cracks and splits than those with less elastic tissues. Wellving (1976) also found that cultivars with tuber tissue of low elasticity more often sustained wounds severe enough to allow infection by P. exigua var. foveata than did cultivars with a higher elasticity.

Cultivars differ in the resistance of their tuber tissue to infection and colonisation by P. exigua var. foveata (Malcolmson, 1958; Jellis, 1975; Pietkiewicz & Jellis, 1975; Wellving, 1976). Wellving found that resistance to infection was closely related to resistance

to colonisation in cultivars. Walker & Wade (1976) demonstrated that there were three phases of tissue resistance following infection by P. exigua var. foveata: the retardation of lesion development, its arrest and lesion rejection. It was found that tuber incubation temperature influenced which of the phases operated. At 2°C none operated whereas at 6°C only lesion retardation occurred but at 10°C lesion arrest followed lesion retardation.

The physiological, or biochemical, processes of the tuber responsible for cultivar differences in tissue resistance to P. exigua var. foveata are unknown. This is because insufficient work on gangrene resistance mechanisms has been carried out. What can be concluded from studies so far is that none of the proposed resistance mechanisms appear to be solely responsible for resistance.

The role of wound healing in gangrene resistance has been investigated because healed wounds were shown to be less susceptible to infection by P. exigua var. foveata than fresh ones (Adams & Griffith, 1978). Wound healing has three overlapping stages, suberisation, lignification and wound periderm formation.

Suberisation is the deposition of the water impermeable substance suberin in cell walls. Walker & Wade (1978) found that suberisation occurred in advance

of the infecting hyphae of P. exigua. They concluded that suberisation is partly responsible for lesion retardation but that it cannot bring about lesion arrest because it does not provide an impenetrable barrier to hyphal growth. The authors considered that suberisation retards lesion development by reducing the supply of nutrients from the host to the pathogen and by restricting the penetration of toxins and hydrolytic enzymes into the host tissue.

Lignification is the deposition of lignin in cell walls. It has been proposed that lignification prevents infection, or arrests lesion development, by making cell walls resistant to degradation by pathogen-produced hydrolytic enzymes (Friend, 1973). Walker & Wade (1978) regarded lignification as an important component of tissue resistance to gangrene because hyphae of P. exigua never effectively penetrated the lignified zone, which appeared coincidentally with lesion arrest.

The role of wound periderm formation in gangrene resistance is questionable since Walker & Wade (1978) found an interval of several days between lesion arrest and the development of a wound periderm at 10°C.

The role of phenolic compounds in the resistance of tubers to gangrene has been investigated. The phenolic compound present in the highest concentration in potato tubers is chlorogenic acid (CGA) (Hasegawa,

Johnson & Gould, 1966). When tubers are wounded the concentration of CGA in their tissue increases (Wellving, 1976). Although Wellving (1976) demonstrated that the acid inhibited the growth of P. exigua var. foveata in vitro, there was no relationship between gangrene resistance and the CGA content of tubers for cultivars, either before or after inoculation. Gans (1978), on the other hand, found that of the nine cultivars he tested the two which produced the most CGA in response to wounding were also the most resistant to colonisation by P. exigua var. foveata after inoculation. Two roles have been proposed for CGA in gangrene resistance. The first suggests that it is oxidised to its quinone form. It has been demonstrated that the quinone forms of phenols are more inhibitory to pathogen growth than the phenols themselves (Kosuge, 1969). Furthermore, Patil & Dimond (1967) found that the polygalacturonase of Verticillium albo-atrum was inhibited by the oxidation product of CGA but not by CGA itself. Wellving (1976) proposed that CGA acts in gangrene resistance as a lignin precursor. Friend, Reynolds & Aveyard (1973) found that CGA added to potato discs was converted to lignin.

No evidence has been found to demonstrate that phytoalexins play a major role in gangrene resistance. Although the phytoalexins rishitin, phytuberin, solavetivone and desacetylphytuberin have been shown to

be produced by tubers inoculated with P. exigua var. foveata, cultivar resistance to gangrene was not related to the levels of phytoalexin production (Price, Howard & Coxon, 1976). Furthermore, Walker & Wade (1978) found that the in vivo concentrations of rishitin induced by P. exigua var. exigua and var. foveata were not sufficient to inhibit fungal growth in vitro. They concluded that tuber resistance to gangrene was not solely due to the production of rishitin or phytuberin.

1.2.3 Screening for gangrene resistance and factors affecting the resistance of tubers to infection

Jellis (1978) found that the relative susceptibility of cultivars to infection of the medulla tissue by P. exigua var. foveata was affected by the site at which the tubers were grown. Wellving (1976) demonstrated that the resistance ranking order of cultivars was greatly influenced by the method used to inoculate the tubers, since different resistance characteristics were assessed by the various methods used. Pietkiewicz & Jellis (1975) observed that the ranking order of cultivars could be different for cortical inoculations compared with inoculations of the medulla.

The evidence is conflicting as to whether the resistance ranking order of cultivars is affected by the temperature at which the inoculated tubers are incubated.

Wellving (1976) observed no difference in ranking order at 3°C and 10°C. However, Seppänen (1982) observed differences at 6°C, 12°C and 18°C.

There are reports that the relative susceptibilities of cultivars can vary with different isolates of P. exigua var. foveata. Jellis (1978) found that, with one isolate, the normally resistant cultivar Maris Piper was sometimes more susceptible than the generally susceptible Pentland Crown. Rogers & Killick (1974) also reported a cultivar x isolate interaction. Of three cultivars tested, Pentland Falcon was the most susceptible to three isolates but the most resistant to one other. This interaction occurred only for lesion length, not breadth or depth.

Since the susceptibility of tubers to gangrene is related to their susceptibility to damage, as well as to infection, then factors which influence resistance to injury require to be considered in addition to those which affect tissue resistance to infection.

The availability of nutrients to the growing crop can influence the resistance of the tubers to gangrene. High nitrogen applications increase the resistance of tuber tissue to infection (MacKenzie, 1968; Paterson & Gray, 1972) but, since the susceptibility to damage can also be increased, the net effect of nitrogen on resistance is not clear. Olsson (1984) found that tubers grown in field plots which had extra magnesium were more resistant to gangrene than tubers from control plots.

Tuber age influences susceptibility to both damage and infection by P. exigua var. foveata. Immature tubers are more susceptible to mechanical damage than mature ones (Anon., 1978). However, no information was found indicating whether immature tubers were more susceptible to the specific types of damage which predispose them to infection. It is generally believed that tubers are less susceptible to external damage at grading time than at harvest. This is thought to be because tubers which have been stored for several months are less turgid. However, although the resistance of tubers to infection increases during the growing season to a maximum around harvest time it then decreases during storage (Malcolmson, 1958; Dowley, 1976; Fox & Dashwood, 1977).

Tuber temperature has a significant effect on resistance to damage. Tubers graded at 2°C were more susceptible to damage than those graded at 7°C or 12°C, according to Lang (1981).

The temperature at which tubers are stored, both prior to or after inoculation, markedly influences susceptibility to infection by P. exigua var. foveata. Adams & Griffith (1983) found that tubers were more susceptible to lesion development following point inoculation if stored at 20°C compared with 2°C for 1 month prior to inoculation. Tuber resistance, assessed in terms of lesion number, is reduced the lower the incubation

temperature after inoculation (Malcolmson, 1958; Malcolmson & Gray, 1968b; Langton, 1972; Adams & Griffith, 1983). The effect of post-inoculation temperature on lesion size is less clear. Some researchers have demonstrated that larger rots are produced at low temperatures, ie 4-5°C (Malcolmson, 1958; Khan, 1967; Wellving, 1976). Others have found that larger lesions were obtained at higher temperatures, ie 10-12°C (Seppänen, 1980). Adams & Griffith (1983) found that rot size increased with increasing temperature over the range 2-10°C then remained constant or declined between 10°C and 20°C. The effect of post-inoculation temperature on lesion size has been shown to depend on the age of the tubers (Langton, 1972). Langton found that tubers were more susceptible at 5°C than 10°C if inoculation was carried out soon after harvest. However, if tubers were inoculated 8 months after harvest the converse was true. Seppänen (1980) demonstrated that the temperature at which tuber susceptibility was greatest could depend on the cultivar. For example, Saturna was most susceptible at 6°C whereas Sabina's optimum temperature for lesion development was observed to be 16°C. Walker & Wade (1978) reported that at high incubation temperatures (14°C to 18°C) lesion development was most rapid initially but lesion arrest occurred sooner than at lower temperatures. At 2°C or

6 °C the rate of lesion development was much slower, but there was no lesion arrest and lesions were ultimately larger than at the higher temperatures.

Reports of the effect of relative humidity on gangrene development are contradictory. Bak Henriksen (1975a) and Wellving (1976) found that gangrene was increased by high relative humidity after inoculation. However, Malcolmson (1958) found that the opposite was true.

The site of tuber inoculation can influence resistance. Pietkiewicz & Jellis (1975) found that, although the rose and heel ends of the tuber were equally susceptible to infection, the heel end was significantly more susceptible to colonisation than the rose end. Tuber cortical tissue is more resistant to infection and colonisation by P. exigua var. foveata than the medulla (Jellis, 1975).

1.2.4 The effect of gamma irradiation of tubers on their resistance to Phoma exigua var. foveata

It has been reported that the treatment of potato tubers with gamma irradiation increases their susceptibility to gangrene (Dr L.J. Turkensteen, personal communication). There are also reports that exposing potatoes to gamma irradiation increases their susceptibility to other fungal and bacterial tuber pathogens. Schreiber & Highlands (1958) observed that irradiated tubers had a greater

extent of deterioration in storage than did non-irradiated samples. Tuber rots were associated with infection by Fusarium spp. and Phoma tuberosa (Phoma exigua var. exigua, Boerema & Howeler, 1967). Bintje tubers treated with 8 krads of gamma irradiation were 10 times more susceptible to infection by Fusarium solani var. coeruleum than untreated tubers (Skou & Henriksen, 1964). Hooker & Duncan (1959) found that the susceptibility of tubers to infection by Fusarium sambucinum was greater in gamma irradiated tubers than in the controls. Furthermore, the rate of colonisation of irradiated tissue by the pathogen was greater than that of untreated tissue. Both resistance to infection and to colonisation declined with increasing irradiation dose over the range 5 to 135 kreps. Similar results were also obtained with Erwinia carotovora.

The increased susceptibility of tubers to infection brought about by gamma irradiation treatment is temporary. Hooker & Duncan (1959) demonstrated that, although wounds in irradiated tubers take longer to heal sufficiently in order to resist infection by Fusarium sambucinum, tubers do eventually become resistant. The higher the dose of irradiation to which tubers were exposed the longer the healing period.

Gamma irradiation affects many of the biochemical and physiological processes in the potato tuber, some of which are thought to be involved in the resistance of

tubers to gangrene. Wound periderm formation is prevented in tubers which have been exposed to even very low doses of gamma irradiation (Brownell, Gustafson, Nehemias, Isleib & Hooker, 1957; Isleib, 1957). The inhibitory effect of gamma irradiation on wound periderm formation is long term. For example, Brownell et al. (1957) found the formation of wound periderm to be prevented when tubers were wounded 50 days after exposure to 15 kreps. Unlike wound periderm formation, suberisation is only delayed in irradiated potatoes: ultimately there is no difference in the degree of suberisation in treated and control tubers (Isleib, 1957; Skou & Henriksen, 1964; Thomas & Delincee, 1979). Brownell et al. (1957) found that suberisation was delayed by 36 hours in tubers exposed to 15 kreps of gamma irradiation then wounded and allowed to heal at 26°C. The length of the delay in suberisation was not related to the gamma irradiation dose, since it was as long in tubers treated with 15 kreps as in those exposed to 200 kreps.

The increased susceptibility to infection of irradiated tuber tissue may be due to the irradiation-induced alteration of the pectic compounds in the cell walls and middle lamellae of the tuber. Such an effect may render the pectic compounds more susceptible to degradation by the pectolytic enzymes of the pathogen. Kertesz, Morgan, Tuttle & Lavin (1956) reported that

solutions of pectin were depolymerised by gamma irradiation treatment. Deshpande (1965), in Skou (1979), found that the enzymatic degradation of pectin was enhanced after irradiation treatment. There is no information on the effect of gamma irradiation on the condition of pectic compounds in potato cell walls. However, Roberts & Proctor (1955) observed that the pectin content of tuber cell walls decreased as the dose of high energy cathode rays to which the tubers were exposed was increased.

Gamma irradiation may increase tuber susceptibility to infection by increasing tissue permeability. That increased tissue permeability makes plant tissue more susceptible to infection is suggested by the fact that, in several different host-pathogen interactions, increases in host tissue permeability occur in advance of colonisation of the tissue by the pathogen (Wheeler & Hanchey, 1968). There are no reports of gamma irradiation increasing potato tissue permeability but Skou (1963) found that carrot tissue permeability increased with increasing dose of gamma irradiation.

1.3 DETAILS OF STANDARD METHODS USED

In the majority of experiments in this thesis standard inoculation techniques were employed. Details of these techniques are given below to avoid unnecessary repetition throughout the thesis.

The sources and designations of the isolates of P. exigua var. foveata used are given in Table 2.1.

All potatoes were grown at the SCRI farm, 'The Murrays', Midlothian, and were harvested on the same day in each year. Tubers were stored in a frost-free potato store until required. Prior to inoculation tubers were washed and surface sterilized by dipping for 10 minutes in a Sodium hypochlorite solution which contained 0.05% available chlorine.

In some experiments tuber quarters were used. These were obtained by first cutting each potato longitudinally and then cutting each half transversely. The quarters inoculated with each treatment were each obtained from a different tuber.

For the point inoculation method a plug, 7 mm in diameter and 3 mm deep, was removed from the tuber with a flamed cork borer. The inoculum inserted into this wound was either a known volume of pycnospore suspension or a disc of mycelium. The pycnospore suspensions were produced from 4 to 6 week old malt extract agar cultures which had been incubated at 18°C. Approximately 10 mls of sterile distilled water were added to each culture. The concentration of pycnospores in the resulting suspension was checked using a haemocytometer and then the suspension was diluted to the required concentration. Each potato was inoculated

with 20 µl of suspension, usually containing 2000 pycnospores. In order to assess the pathogenicity of isolates which did not produce a sufficient number of pycnospores in axenic culture, discs of mycelium were used as a standard inoculum. To produce the mycelium free of agar the isolates were grown on 76 mm diameter, 0.45 µ pore size membrane filters placed over malt extract agar in Petri dishes (Jellis, 1975). Tubers were inoculated with 5 mm diameter discs of mycelium, cut from the margin of a 6 day old culture with a cork borer. After inoculation of the tuber a coverslip was fixed over the site, using soft paraffin wax, to prevent dehydration of the inoculum. This coverslip was removed after approximately one week of the incubation period. Inoculated tubers were incubated at 4°C and 100% relative humidity.

Tubers were generally inoculated during the winter storage season, ie between October and the following April. The month of inoculation for each experiment is given in Appendix 1.1. In all the storage experiments treatments were randomised within replicate blocks. The number of blocks varied between two and four depending on the experiment.

Lesion development, following point inoculation, was generally assessed by measuring, after cutting the

lesion through the point of inoculation, the diameter of rot at the tuber surface and its maximum depth. A mean lesion radius was calculated using the formula:

mean lesion radius = (diameter/2 + depth)/2

Where the pattern of results obtained for lesion diameter and depth was the same the results for mean lesion radius only are presented in the thesis.

The exposure of tubers to gamma irradiation was carried out at the Scottish Universities' Research and Reactor Centre, East Kilbride. Batches of 20 tubers were irradiated at a time using a Cobalt 60 source.

CHAPTER 2

Variation in pathogenicity in *Phoma exigua* var. *foveata*

2.1 INTRODUCTION

At present it is generally assumed that variation in pathogenicity among isolates of Phoma exigua var. foveata is small and therefore of little importance. For example, in the model proposed by Adams & Griffith (1983) to predict storage losses due to gangrene the inputs include tuber inoculum level, tuber susceptibility, storage temperature, wound type and wound severity, but the influence of isolate on gangrene development is not considered. The main objective of this section of the thesis was to examine the influence of variation among isolates of P. exigua var. foveata on gangrene development.

The first two experiments were designed to assess the extent of variation in pathogenicity among isolates of the pathogen. The isolates used in experiment 1 were obtained from a culture collection. It was decided to use recent field isolates in the second experiment as any variation among culture collection isolates may not be a realistic estimate of natural variation in the pathogen: it has been reported that pathogens may suffer a reduction in pathogenicity if they are maintained axenically over prolonged periods (Boyd, 1952).

The importance of pathogenicity variation in P. exigua var. foveata depends on its distribution. The aim of subsections 2.2.3 and 2.2.4 was to examine this distribution. Experiment 3 was intended to indicate

whether isolates from the same crop varied in pathogenicity and, if so, to compare the magnitude of this variation with the extent of differences between crops. Another objective of this experiment was to investigate whether isolate pathogenicity was related to the geographical location of its source. Carnegie, Adam, MacDonald & Cameron (1981) have shown that seed stocks in northern Scotland generally have a greater potential for gangrene development than those in Angus, Perth, Fife and Kinross. However, they have not established whether the greater potential is due to higher tuber inoculum levels, to differences in the tuber susceptibility of the stocks or to variation in the pathogen. The experiment therefore aimed to assess the role of pathogen variation as a possible contributory factor to gangrene potential differences among stocks. Experiments 4 and 5 (section 2.2.4) were designed to investigate whether there was variation in pathogenicity within isolates of P. exigua var. foveata and, if so, to compare it with that occurring among isolates.

The experiments in 2.2.5 were carried out to examine further the possibility of changes in pathogenic behaviour following axenic culture (Boyd, 1952) and to check to what extent differences demonstrated among isolates of P. exigua var. foveata might be due to the differential reduction in pathogenicity of isolates in culture.

The experiments in this section may be summarised under the following headings.

Variation in pathogenicity in Phoma exigua var. foveata:-

2.2.1 culture collection isolates (Experiment 1),

2.2.2 field isolates (Experiment 2).

Distribution of variation in pathogenicity in Phoma exigua var. foveata:-

2.2.3 the relationship between isolate pathogenicity and isolate source (Experiment 3),

2.2.4 variation within isolates (Experiments 4 & 5).

The stability of isolate variation:-

2.2.5 differential reduction in isolate pathogenicity following axenic culture (Experiments 6 & 7).

2.2 MATERIALS AND METHODS

2.2.1 Experiment 1

In this experiment the pathogenicities of eight isolates, designated A, B, C, D, E, F, G and I were assessed. Two inoculation methods were employed: a point inoculation technique and a whole tuber infection method. The same inoculum was used for both inoculation methods. The isolates were cultured on a wheatgerm and sand medium (5 parts wheatgerm, 100 parts sand and 15 parts water) for 4 weeks prior to use.

Table 2.1 Experimental isolates of Phoma exigua var. foveata and their sources

Isolate	Isolated from	Region	Date	Supplied by
Culture collection				
A	-	-	-	DANI, Belfast
B	-	-	-	SCRI, Edinburgh
C	-	-	-	DAFS, Edinburgh
D	-	-	-	DANI, Belfast
E	-	-	-	SCRI, Dundee
F	-	-	-	Rothamsted
G	-	-	-	DANI, Belfast
H	-	-	-	SCRI, Edinburgh
I	-	-	-	DAFS, Edinburgh
Field				
J	Air	Lanark	Aug 1981	DAFS, Edinburgh
K	"	East Craigs	"	"
L	"	"	"	"
M	Pentland Crown tuber	Ross-shire	Sept 1981	"
N	"	Aberdeenshire	"	"
O	Desiree tuber	Nairn	"	"
P	"	Kincardine	"	"
Q	"	Aberdeenshire	"	"
R	Maris Peer tuber	"	"	"
S	Air	Angus	"	"
T	"	"	"	"
U	"	"	"	"
V	"	"	"	"
W	"	"	"	"
A11-A17	Desiree tuber	Aberdeen	Mar 1983	DAFS, Edinburgh[*]
A21-A27	"	"	"	"
A31-A38	"	"	"	"
A61-A67	"	"	"	"
F11-F17	"	Fife	"	"
F31-F37	"	"	"	"
F71-F77	"	"	"	"
F91-F98	"	"	"	"

Isolates A-I were kindly provided by Dr R.L. Wastie, SCRI, Edinburgh and isolates J-W by Dr S.F. Carnegie, DAFS, Edinburgh.

[*] Isolates were taken by the author from infected tubers kindly supplied by Dr S.F. Carnegie, DAFS, Edinburgh

The point inoculation technique consisted of filling one wound per tuber with inoculum. Twelve tubers of each of the cultivars Maris Piper, Pentland Crown, Pentland Envoy, Pentland Ivory and Pentland Javelin were inoculated with each of the eight isolates. In order to estimate the effect of any natural contamination of tubers 24 potatoes of each cultivar were wounded only. After 12 weeks' incubation the surface diameter and maximum depth of each lesion was measured.

With the whole tuber inoculation method 16 potatoes (four replicates each of four tubers) of the five cultivars were inoculated with each isolate by rolling them in a mixture of one part wheatgerm/sand culture to two parts sterilized sand. To estimate the extent of natural contamination 16 tubers of each cultivar were rolled in sterilized sand. After 14.5 weeks' incubation gangrene levels were calculated, using a modification of the common scab assessment method described by McKee (1963). In this method the fraction of the tuber surface area which was diseased was determined and this fraction converted to a whole number (Table 2.2). The surface lesion index (SLI) is the mean value for the 16 tubers of each treatment.

The whole tuber inoculation method part of this experiment was a repeat of an experiment carried out a year previously as part of a BSc Honours Degree project (Bain, 1980).

Table 2.2 Scale to convert diseased fraction of tuber surface area values to whole numbers

Fraction	0	<1/16	1/16-1/8	1/8-1/4	1/4-1/2	>1/2
Whole number	0	1	3	6	12	24

(based on McKee, 1963)

By comparing the results for the 2 years the stability of isolate relative pathogenicities over time was assessed. The experimental methods were the same in both years except that in the first year, the inoculum was cultured for 2 weeks prior to use and 20 tubers (four replicates of five tubers) of each cultivar were inoculated with each isolate.

2.2.2 Experiment 2

The 14 field isolates used, designated J to W, were sampled in Autumn 1981 from a variety of locations in Scotland. These isolates were confirmed to be P. exigua var. foveata by checking for anthraquinone pigment production in malt extract agar cultures. The two culture collection isolates included in the experiment for comparison with the field isolates were A and C.

Isolate pathogenicity was assessed using both the whole tuber inoculation procedure and a point inoculation method. The whole tuber assessment method was the same as that used in experiment 1 except that 20 Pentland Envoy potatoes (four replicates of five tubers) were inoculated with 5 week-old inoculum of each isolate. Gangrene development was assessed as in 2.2.1, after 6 weeks' incubation. For the point inoculation assessment 20 Pentland Javelin tuber halves were infected with discs of mycelium. After 6 weeks' incubation the surface diameter and maximum depth of each lesion was measured.

2.2.3 Experiment 3

The variation among isolates from eight crops of FS grade Desiree was investigated. Four of the crops were grown in Fife and four in Aberdeenshire. Seven isolates were taken from each of the eight stocks. Each isolate was derived from one gangrene lesion on a tuber which had been selected at random from the stored crop. All except one of the isolates, A34, were confirmed as *P. exigua* var. *foveata* by checking for anthraquinone production in malt extract agar cultures.

Isolate pathogenicity was assessed by point inoculating Pentland Envoy tuber quarters with discs of mycelium. After 4.5 weeks' incubation the surface diameter and maximum depth of each lesion was measured.

2.2.4 Experiment 4

In this preliminary experiment variation in pathogenicity within isolates was investigated using several isolates derived from the original isolates B and C. The derived isolates were obtained by the following procedure. A spore suspension was made from a culture of each isolate and its concentration adjusted so that a 20 µl drop of the suspension should contain on average only one pycnospore. Single drops of the suspension were then plated out to give isolates derived from one, or a very small number of, pycnospores. Nine derived isolates of B and 11 of C were used.

Pathogenicity was assessed by point inoculating 20 Pentland Falcon tuber quarters with 1000 pycnospores of each derived isolate in 20 µl water. After 8 weeks' incubation the lesion diameter and depth for each quarter was measured.

Experiment 5

Single spore isolates were made from the two isolates A21 and F16. Samples of 10 µl of a pycnospore suspension, with a concentration of approximately 3×10^3 pycnospores per ml, were streaked on to plates of filtered water agar. After incubation at 18°C for 2 days, germinated pycnospores were cut from the agar, using a cutter mounted on a microscope objective, and transferred to a malt extract agar plate. Lesions produced by ten single spore isolates of A21, thirteen of F16, plus the two parent isolates were assessed following point inoculation of 20 Pentland Envoy tuber quarters with mycelial discs of each isolate. The surface diameter and maximum depth of each lesion was measured after 4.5 weeks' incubation.

2.2.5 Experiment 6

In this experiment two isolates were used: isolate B, which had been cultured axenically for many years was compared with a more recent isolate, N. In order to provide three replicate cultures per isolate for each of the two subculturing treatments, namely repeated or minimal subculturing, six cultures

were produced from one original culture of each isolate. The pathogenicity of these twelve cultures was assessed to check that there was no variation in rotting ability among the six cultures from each parent isolate at the start of the experiment. Twenty tubers of the cultivar Corrie were point inoculated with a pycnospore suspension of each culture. After 10.5 weeks' incubation the surface diameter and maximum depth of each lesion was measured.

The subculturing treatment consisted of incubating three replicate cultures of each isolate (B/4, B/5, B/6 and N/4, N/5, N/6) at 18°C and subculturing weekly, a total of 17 times on malt extract agar. The other three cultures were incubated at 4°C and were subcultured once during the experiment. Subculturing involved transferring a 7 mm diameter plug of mycelium and agar from the culture margin to a fresh plate. At the end of the subculturing treatments pathogenicity was again assessed. Twenty Pentland Falcon tuber quarters were point inoculated with mycelial discs from each culture. It was necessary to use mycelial discs in place of a pycnospore suspension since some of the N cultures no longer produced a sufficient number of pycnospores. After 4.5 weeks' incubation the surface diameter and maximum depth of each lesion was measured. The pathogenicity of N/4, N/5 and N/6, after repeated subculturing, was reassessed in order to confirm

the loss of rotting ability of N/6 compared with the others, observed at the first assessment. Twenty Pentland Envoy quarters were inoculated with mycelial discs of each culture. The surface diameter and maximum depth of each lesion was assessed after 4.5 weeks' incubation.

To assess whether repeated subculturing had had any effect on *in vitro* growth of *Phoma exigua* var. *foveata* the six cultures of N were grown at 18°C on malt extract agar membrane filter plates. Culture diameter was measured after 9 days. The production of anthraquinone pigments in these cultures was used to confirm them as *P. exigua* var. *foveata*.

Experiment 7

In this experiment four field isolates, designated A63, A38, F98 and A23 and known to be equally pathogenic, were used. Each isolate was sub-cultured twice weekly, using the method outlined in the previous experiment, a total of 15 times. The original cultures were maintained at 4°C throughout the subculturing phase of the experiment. The pathogenicity of these, and cultures produced from repeated subculturing, was assessed at the end of the subculturing treatment by inoculating Pentland Envoy tuber quarters with mycelial discs. After 4 weeks' incubation the surface diameter and maximum depth of each lesion was measured.

2.3 RESULTS

2.3.1 Experiment 1

An investigation of variation in pathogenicity in Phoma exigua var. foveata using culture collection isolates.

From the analyses of variance of the point inoculation method data (Appendix 2.1) both isolate and cultivar were found to have a significant effect on lesion diameter, lesion depth and mean lesion radius. Furthermore, there was a significant interaction between cultivar and isolate for all three criteria. For the whole tuber inoculation method, also, both isolate and cultivar had a significant effect on disease level with a significant interaction between isolate and cultivar (Appendix 2.2).

Figure 2.1 shows, for both the point and whole tuber inoculation methods, the disease level caused by each isolate averaged for the five cultivars. There was considerable variation among the isolates. The ranking orders of isolates were the same for both methods of inoculation with one exception: when the point inoculation method was used isolate G produced larger gangrene lesions than isolate B, whereas B caused more disease than G with the whole tuber inoculation method.

The results for the six isolates tested in two separate years, using the whole tuber inoculation method (Fig 2.2), indicate that the ranking orders were more or

Figure 2.1 The effect of isolate of P. exigua var. foveata on gangrene development using two inoculation methods (mean for five cultivars)

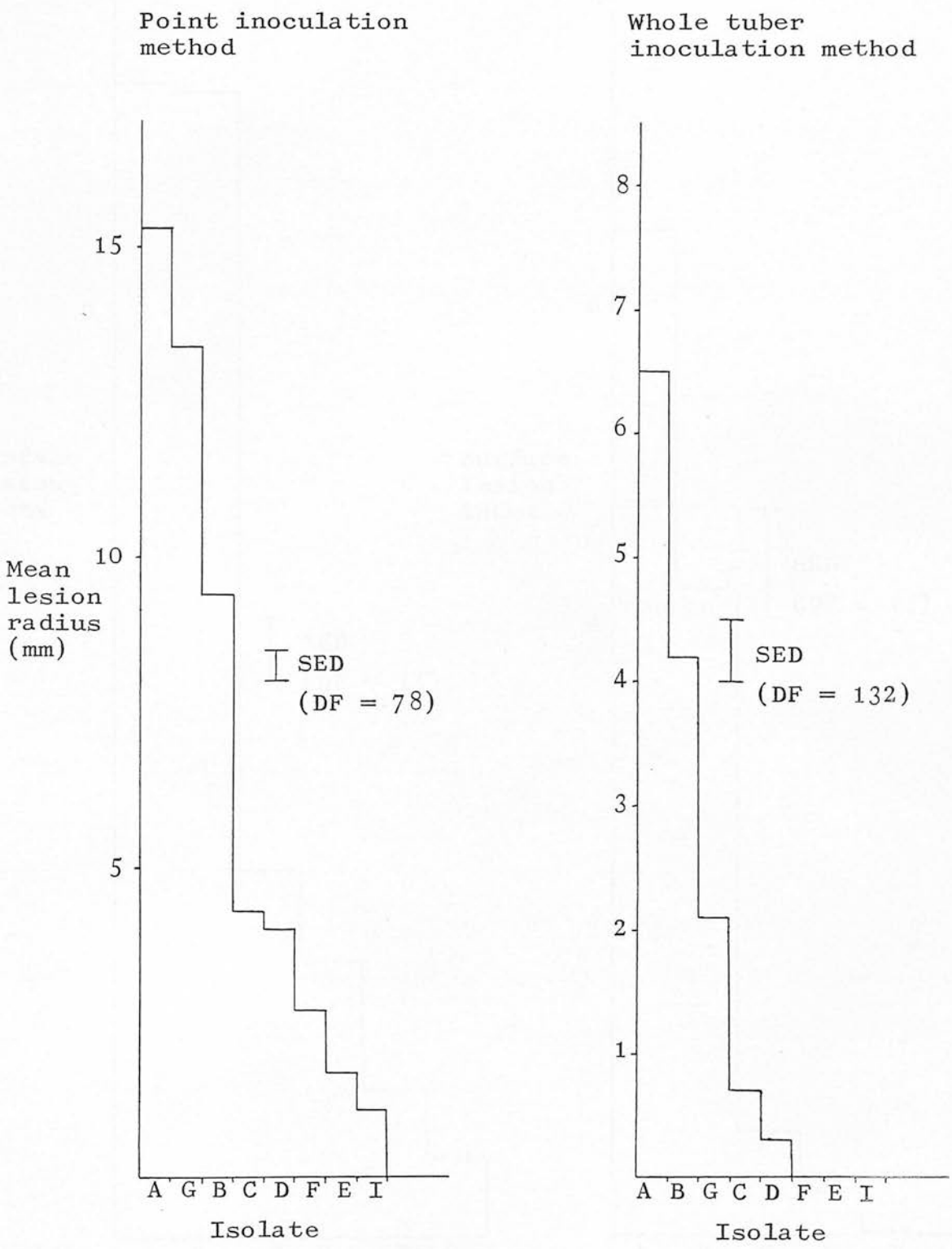
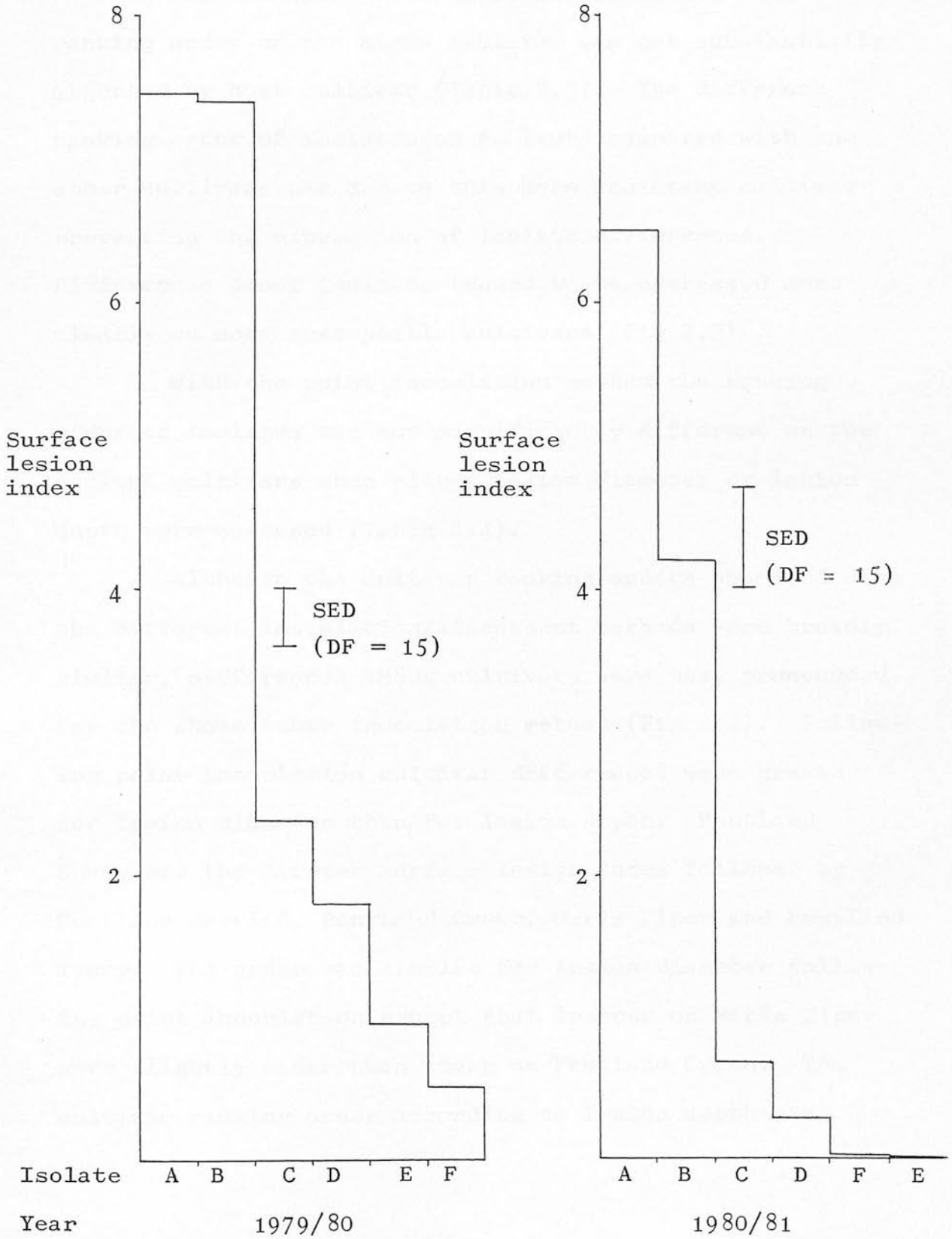


Figure 2.2 The effect of isolate of P. exigua var. foveata on gangrene development assessed in 2 years using the whole tuber inoculation method (mean of five cultivars)



less similar, although a significant difference between A and B was evident only in 1980/81.

For the whole tuber inoculation method the ranking order of the eight isolates was not substantially affected by host cultivar (Table 2.3). The different ranking order of isolates on P. Ivory compared with the other cultivars was due to this more resistant cultivar preventing the expression of isolate differences. Differences among isolates tended to be expressed more clearly on more susceptible cultivars (Fig 2.3).

With the point inoculation method the ranking order of isolates was not significantly different on the various cultivars when either lesion diameter or lesion depth were assessed (Table 2.4).

Although the cultivar ranking orders obtained with the different inoculation/assessment methods were broadly similar, differences among cultivars were most pronounced for the whole tuber inoculation method (Fig 2.4). Following point inoculation cultivar differences were greater for lesion diameter than for lesion depth. Pentland Envoy had the largest surface lesion index followed by Pentland Javelin, Pentland Crown, Maris Piper and Pentland Ivory. The order was similar for lesion diameter following point inoculation except that lesions on Maris Piper were slightly wider than those on Pentland Crown. The cultivar ranking order according to lesion depth was

Table 2.3 The effect of cultivar on isolate ranking order, for surface lesion index, using the whole tuber inoculation method

Cultivar	Isolate							
	A	B	G	C	D	F	E	I
P. Envoy	1	2	3	4	5	7	7	7
P. Javelin	1	2	3	4	5	6	7	8
P. Crown	1	2	3	4	5	7	7	7
M. Piper	1	2	3	4	5.5	5.5	7.5	7.5
P. Ivory	1	5.5	5.5	7	5.5	5.5	5.5	5.5
Mean rank	1	2.7	3.5	3.6	5.2	6.2	6.8	7.0

Kendall coefficient of concordance (W) = 0.767 ($P<0.01$)

Figure 2.3 The effect of cultivar on the expression of pathogenicity differences among P. exigua var. foveata isolates

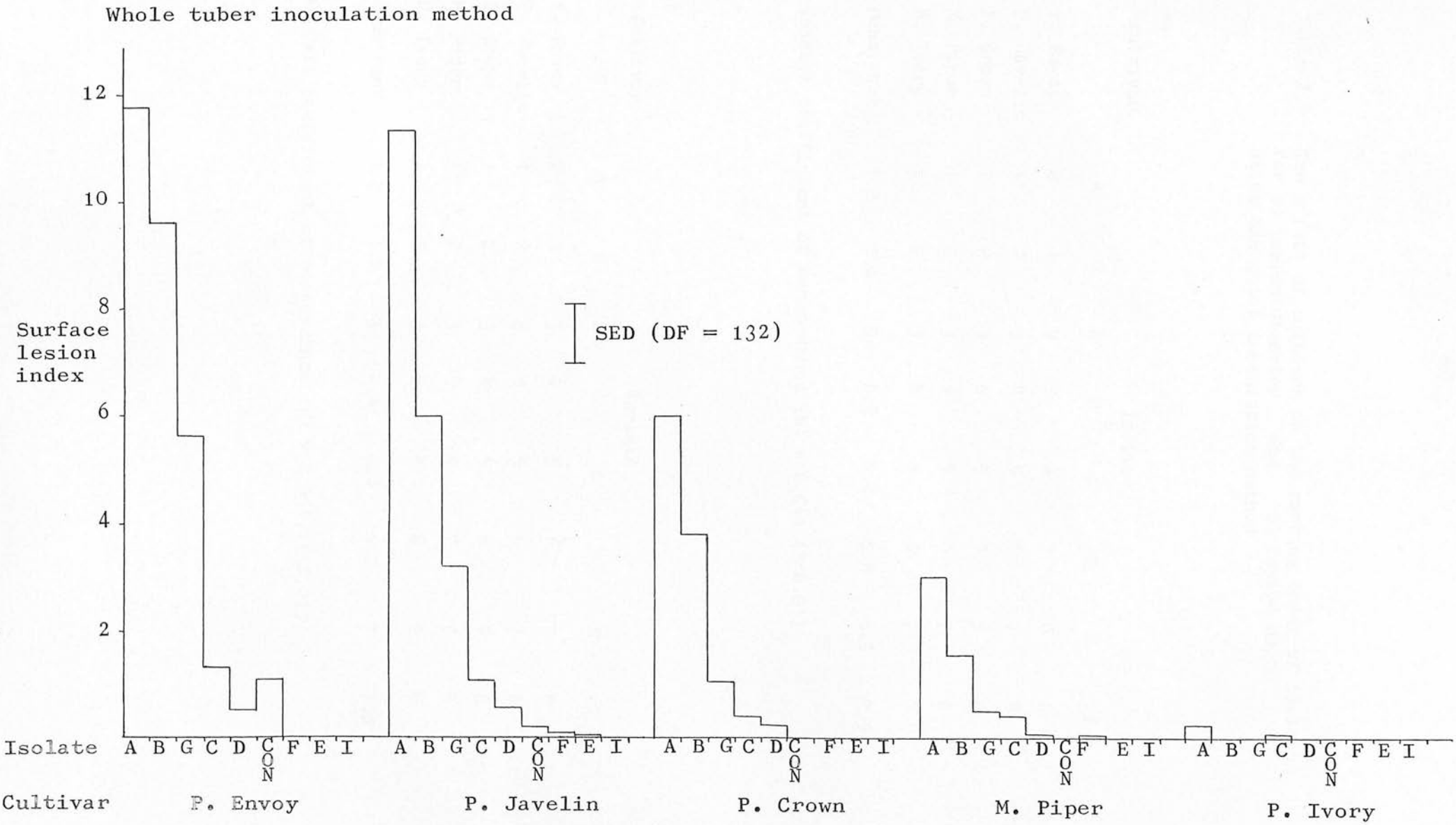


Table 2.4 The effect of cultivar on the ranking order of isolates for a) lesion diameter and b) lesion depth using the point inoculation method

a.

Cultivar	Isolate							
	A	G	B	D	C	F	E	I
P. Envoy	2	1	3	5	4	7	6	8
P. Javelin	1	2	3	4	5	6	7	8
P. Crown	1	2	3	6	5	4	7	8
M. Piper	1	2	3	4	5	6	7	8
P. Ivory	1	2	3	4	5	6	7.5	7.5
Mean rank	1.2	1.8	3	4.6	4.8	5.8	6.9	7.9

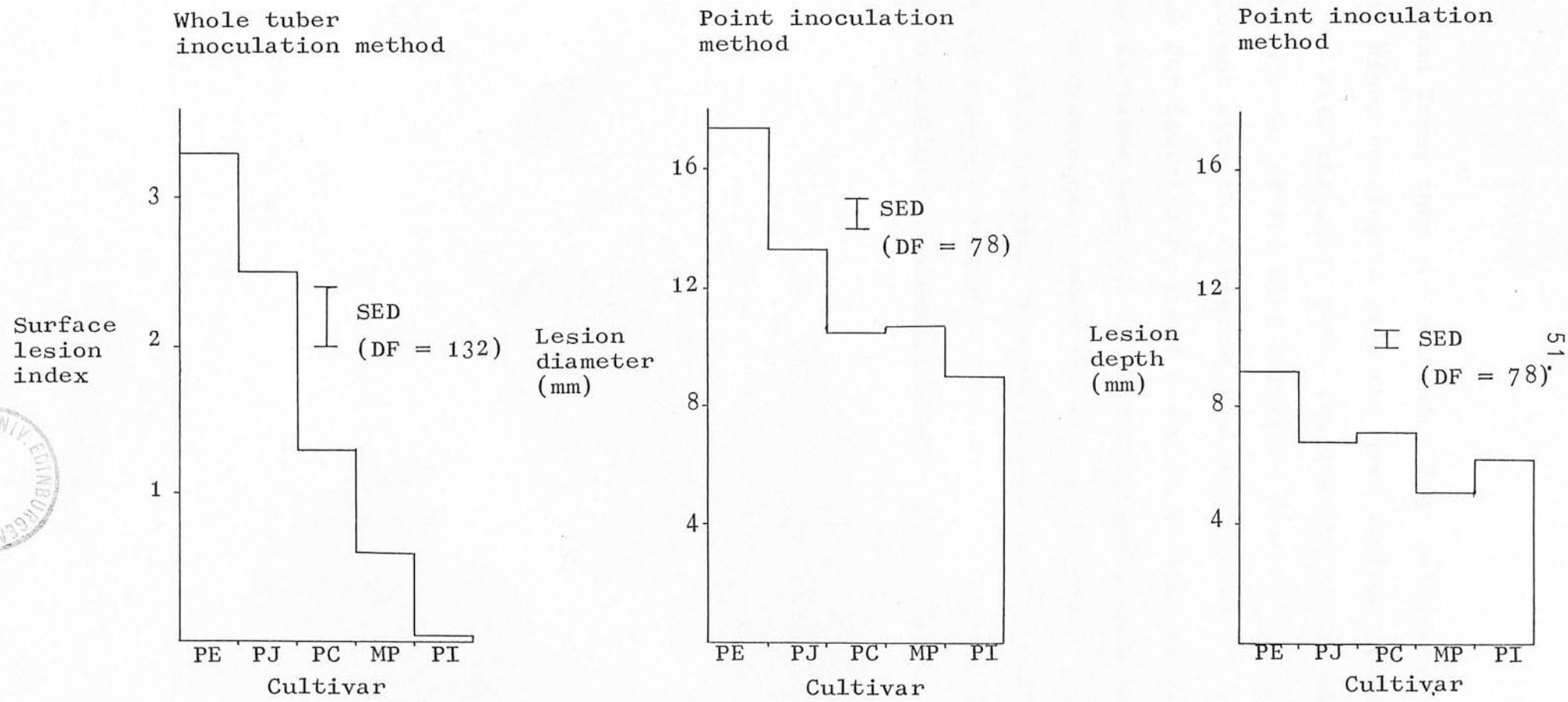
Kendall coefficient of concordance (W) = 0.941 ($P<0.01$)

b.

Cultivar	Isolate							
	A	G	B	C	D	F	E	I
P. Envoy	2	1	3	4	5	6	7	8
P. Javelin	1	2	3	4	5	6	7	8
P. Crown	1	2	3	4	5	6	7	8
M. Piper	1	2	3	5	4	7	6	8
P. Ivory	1	2	3	5	4	6	8	7
Mean rank	1.2	1.8	3	4.4	4.6	6.2	7	7.8

Kendall coefficient of concordance (W) = 0.964 ($P<0.01$)

Figure 2.4 The effect of inoculation and assessment methods on gangrene development in different cultivars (mean of eight isolates)



Cultivar key

PE Pentland Envoy
PJ Pentland Javelin
PC Pentland Crown
MP Maris Piper
PI Pentland Ivory

Pentland Envoy then the remaining four cultivars with Maris Piper tending to give the most shallow lesions.

With the whole tuber inoculation method the ranking order of the five cultivars was not significantly different with the different isolates (Table 2.5). The ranks for isolates F, E and I should be ignored since these isolates were not sufficiently pathogenic to be able to distinguish among the five cultivars.

With the point inoculation method the cultivar ranking orders were similar for those isolates which were able to distinguish among cultivars (Table 2.6).

2.3.2 Experiment 2

An investigation of variation in pathogenicity in *P. exigua* var. *foveata* using field isolates.

In the analyses of variance of the results (Appendices 2.3 & 2.4) a significant effect of isolate on gangrene development was found for both inoculation methods.

The field isolates used in this study showed a wide range of variation in the level of gangrene they produced on Pentland Envoy when the whole tuber inoculation method was used (Fig 2.5): this range was considerably greater than among the culture collection isolates used in experiment 1. All except one of the field isolates had a SLI greater than A, the most pathogenic of the old isolates.

Table 2.5 The effect of isolate on the ranking order of five cultivars for surface lesion index, using the whole tuber inoculation method

Isolate	Cultivar				
	P. Javelin	P. Envoy	P. Crown	M. Piper	P. Ivory
A	2	1	3	4	5
B	2	1	3	4	5
G	2	1	3	4	5
C	2	1	3	4	5
D	1	2	3	4	5
F	1	4	4	2	4
E	1	3.5	3.5	3.5	3.5
I	3	3	3	3	3
Mean rank	1.8	2.1	3.2	3.6	4.4

Kendall coefficient of concordance (W) = 0.486 ($P<0.01$)

Table 2.6 The effect of isolate on the ranking order of cultivars using the point inoculation method

Lesion diameter

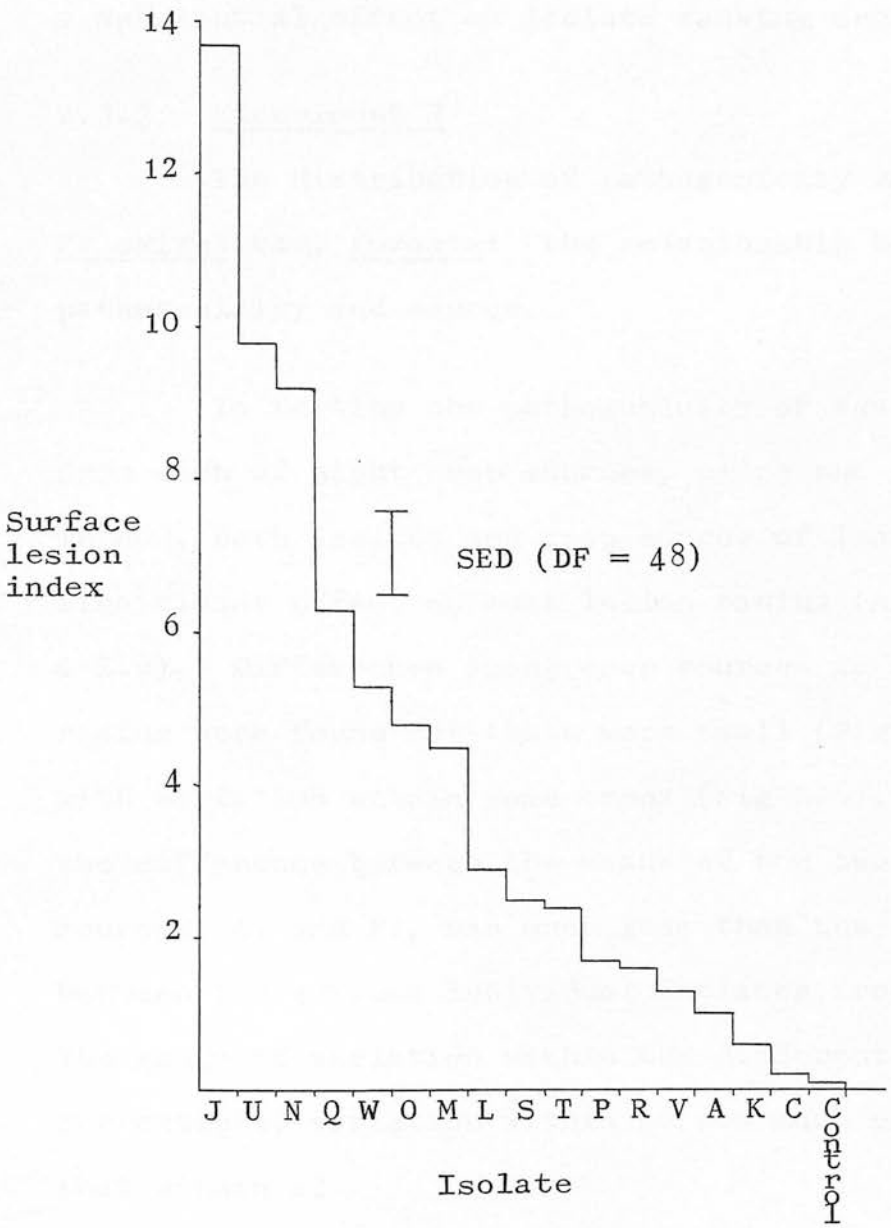
Isolate	Cultivar				
	P. Envoy	P. Javelin	P. Crown	M. Piper	P. Ivory
B	1	2	3	5	4
A	1	2	3	4	5
G	1	2	3	4	5
Mean rank	1	2	3	4.3	4.7

Lesion depth

Isolate	Cultivar				
	P. Envoy	P. Javelin	P. Crown	P. Ivory	M. Piper
B	1	3	2	4	5
A	1	4	2	3	5
G	1	2	4	3	5
C	1	2	3	5	4
Mean rank	1	2.7	2.7	3.7	4.7

The Kendall coefficient of concordance (W) for lesion diameter was 0.956 ($P<0.01$) and for lesion depth was 0.775 ($P<0.01$)

Figure 2.5 The effect of isolate of P. exigua var. foveata on gangrene development on Pentland Envoy using the whole tuber inoculation method



With the point inoculation method using Pentland Javelin the range of variation among isolates (Fig 2.6) was less than with the whole tuber inoculation method and the isolate ranking orders from the two inoculation methods differed (Figs 2.5 & 2.6). The method of assessing lesion size, after point inoculation, also had a substantial effect on isolate ranking order (Fig 2.6).

2.3.3 Experiment 3

The distribution of pathogenicity variation in P. exigua var. foveata: the relationship between isolate pathogenicity and source.

In testing the pathogenicity of seven isolates from each of eight crop sources, using the point inoculation method, both isolate and crop source of isolate had a significant effect on mean lesion radius (Appendices 2.5 & 2.6). Differences among crop sources in mean lesion radius were found but these were small (Fig 2.7) compared with variation within some crops (Fig 2.8). For example, the difference between the means of the two extreme crop sources, A1 and F1, was much less than the difference between the extreme individual isolates from crop A6. The range of variation within the different crops varied: for example, variation within A6 was much greater than that within A2.

There was no obvious relationship between the geographical location of the crops (A isolates from

Figure 2.6 The effect of isolate of P. exigua var. foveata on gangrene lesion size on Pentland Javelin using a point inoculation method

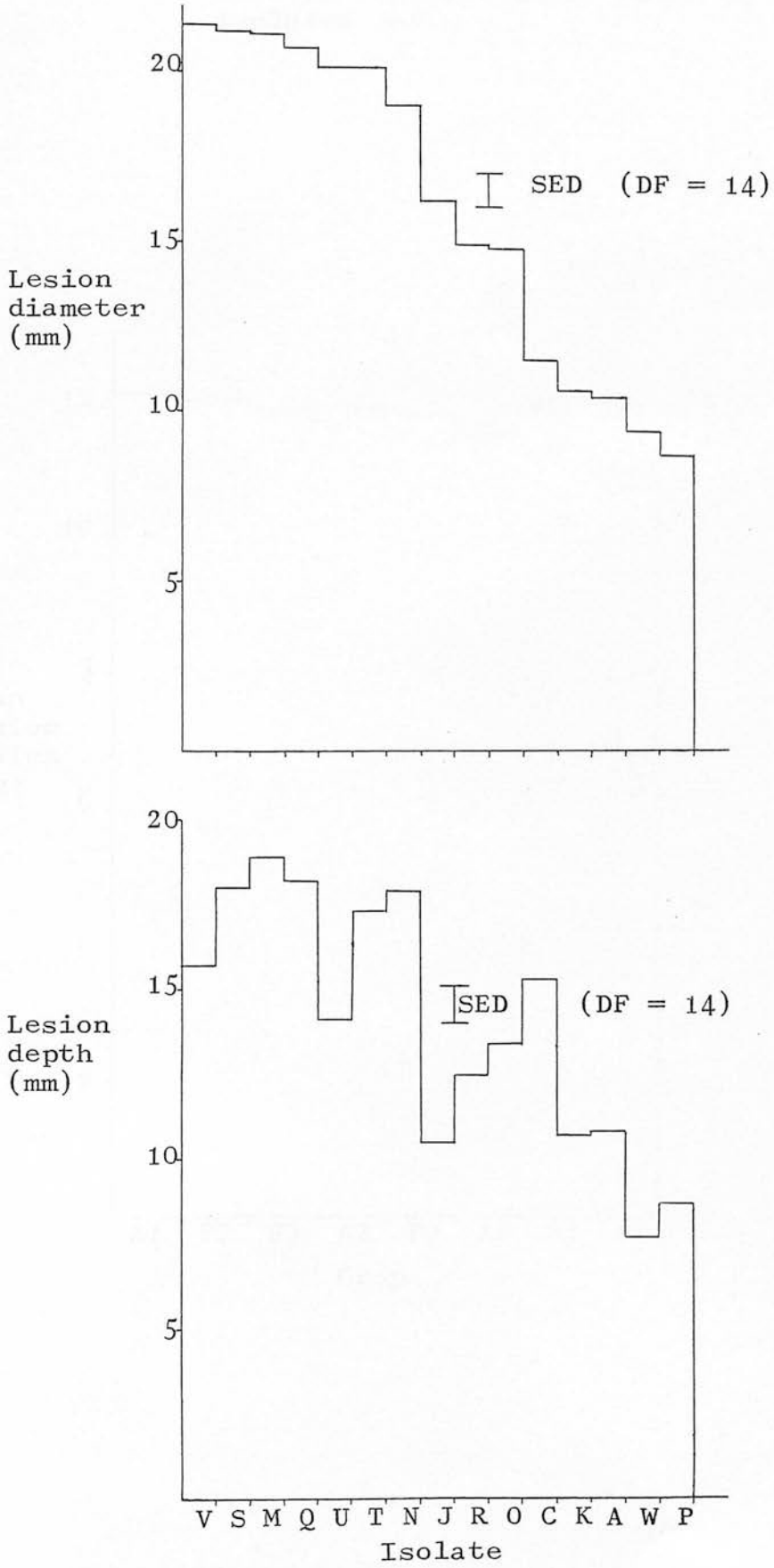


Figure 2.7 The effect of crop source of isolates of *P. exigua* var. *foveata* on gangrene lesion size on Pentland Envoy (mean of seven isolates per crop)

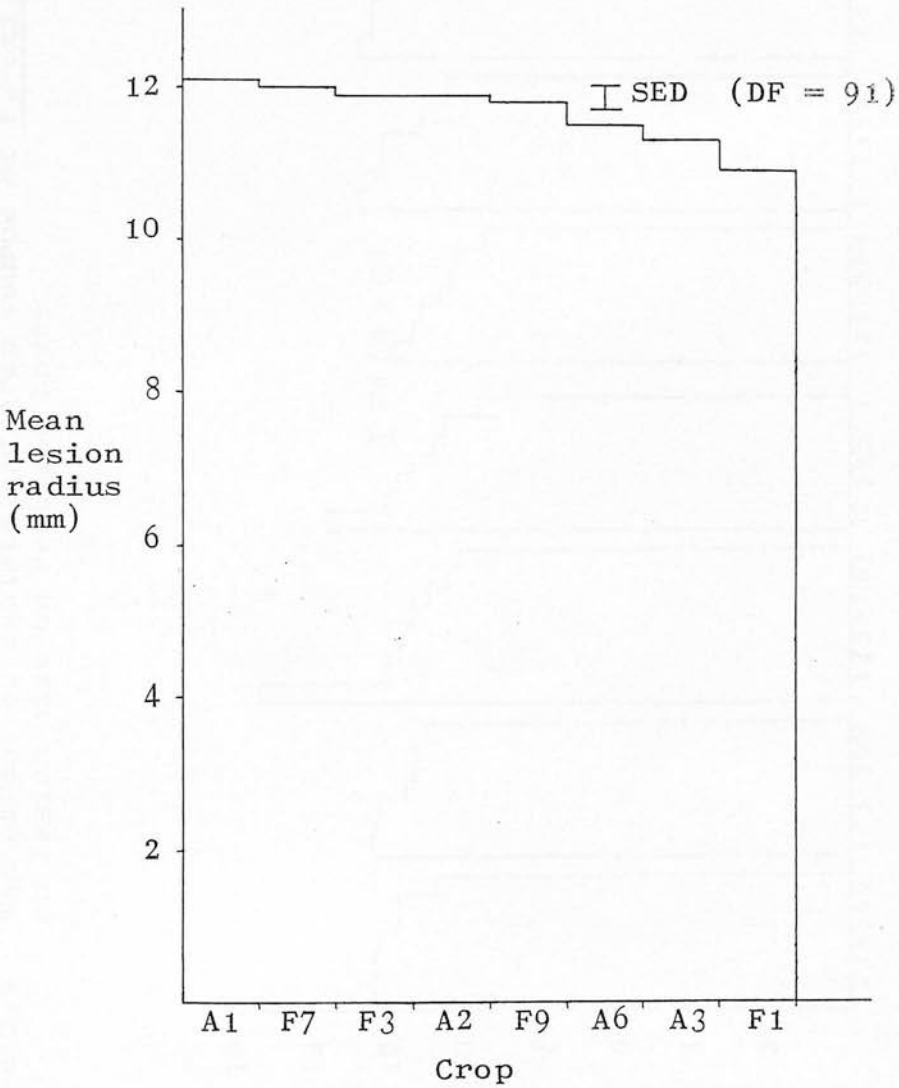
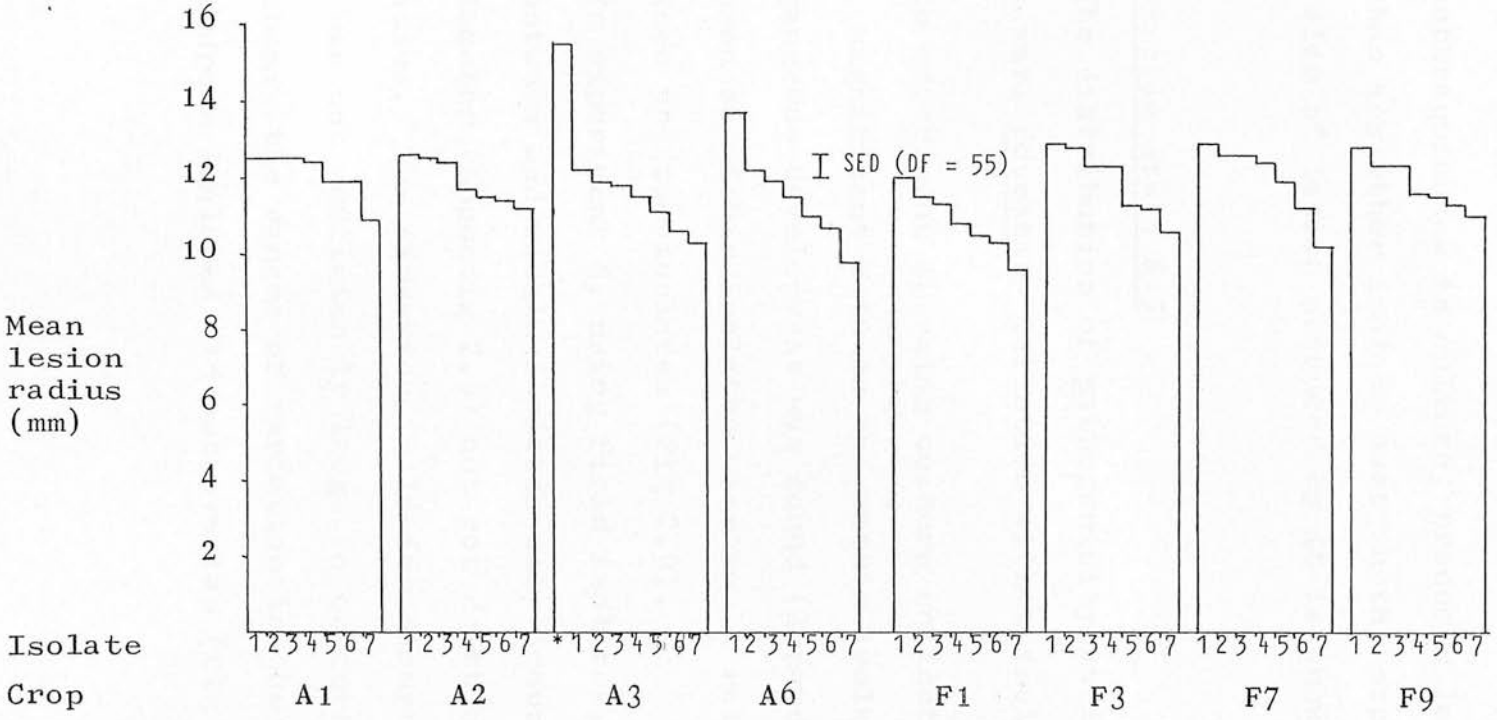


Figure 2.8 The effect of isolate and crop source of P. exigua var. foveata on lesion size on Pentland Envoy



*is isolate A34 which did not produce anthraquinones

Aberdeenshire, F from Fife) and the average size of lesion produced by the isolates from them (Fig 2.7).

One of the isolates taken from crop A3, but not used in the main investigation because it did not produce anthraquinones in culture, produced larger lesions than any other isolate used in the experiment. The mean size of lesion produced by it is shown in Fig 2.8.

2.3.4 Experiments 4 & 5

The distribution of pathogenicity variation in P. exigua var. foveata: variation within isolates.

In experiment 4, using culture collection isolates, significant between and within isolate variation in gangrene development was found (Appendix 2.7), but differences within isolates were small relative to that between the two isolates (Fig 2.9).

In experiment 5, using field isolates, significant effects between and within isolates only occurred for lesion diameter (Appendix 2.8) not rot depth or mean lesion radius. The variation in lesion diameter within isolates was not sufficiently large to obscure differences between them: the ranges of variation in the two sets of single spore isolates did not overlap (Fig 2.10).

Figure 2.9 Variation in lesion size between and within two isolates of P. exigua var. foveata using the point inoculation method (cv. Pentland Falcon)

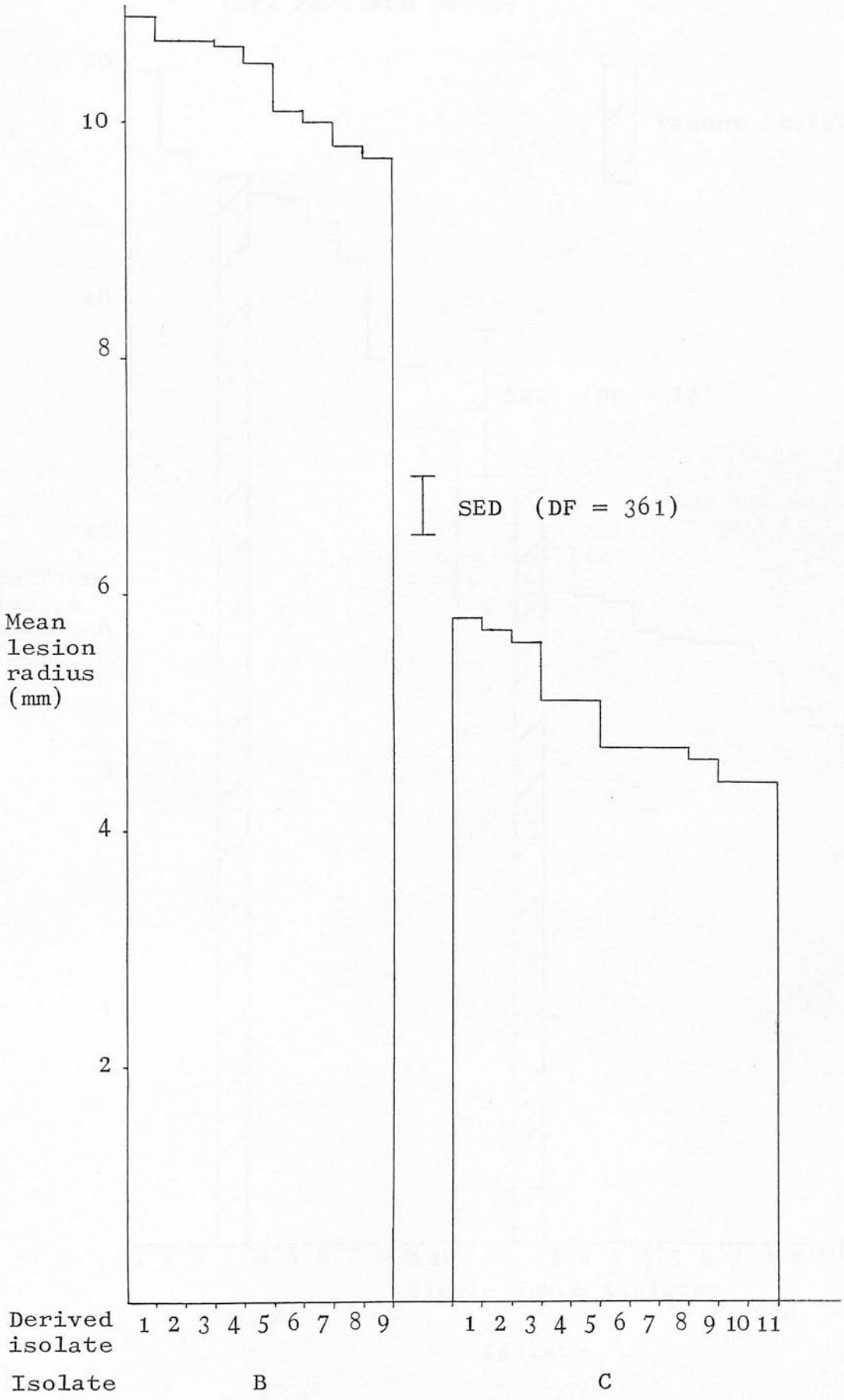
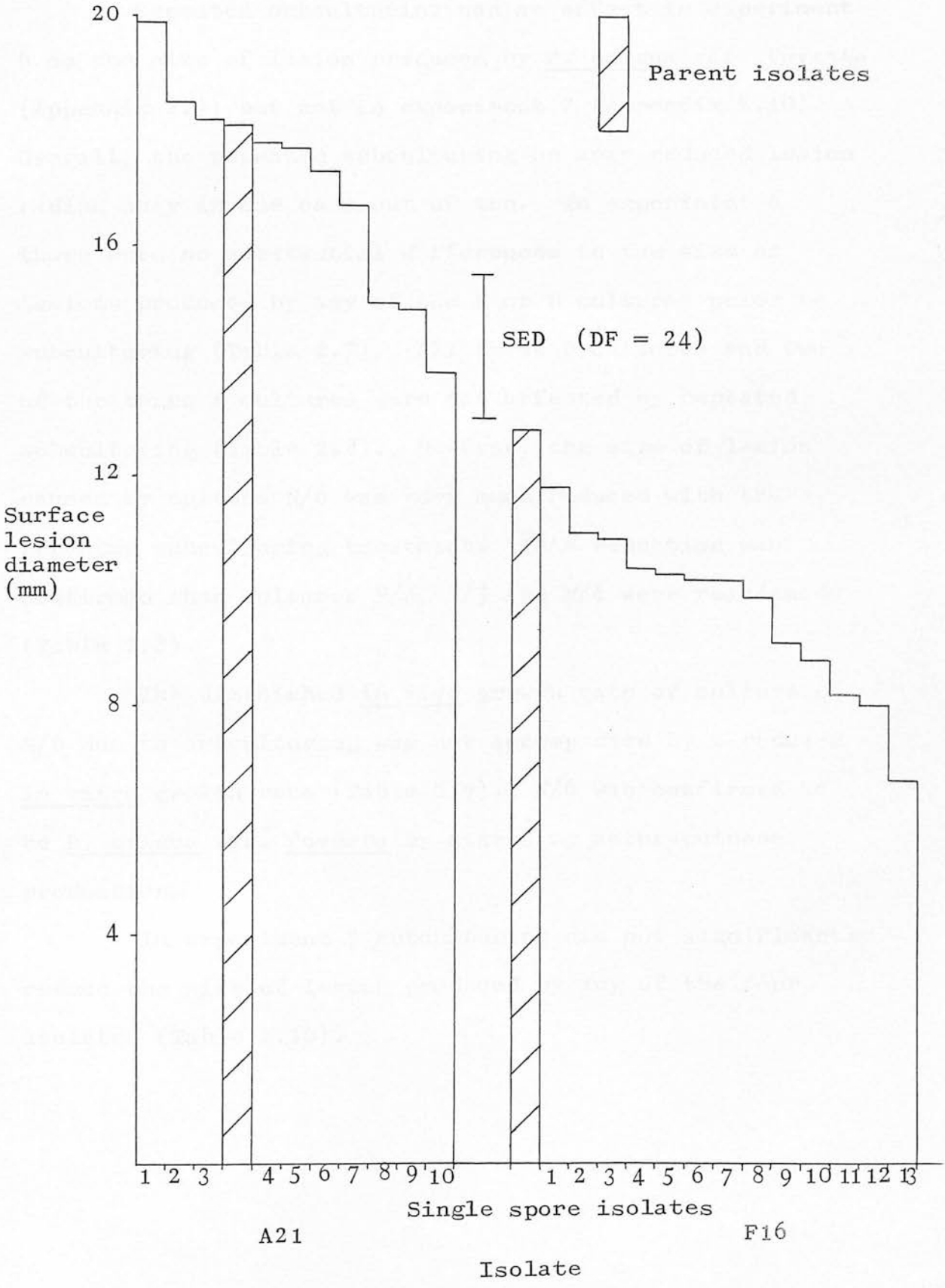


Figure 2.10 Variation in lesion diameter between and within two field isolates of P. exigua var. foveata using the point inoculation method (cv. Pentland Envoy)



2.3.5 Experiments 6 & 7

The stability of isolate variation.

Repeated subculturing had an effect in experiment 6 on the size of lesion produced by P. exigua var. foveata (Appendix 2.9) but not in experiment 7 (Appendix 2.10). Overall, the repeated subculturing on agar reduced lesion radius only in one case out of ten. In experiment 6 there were no substantial differences in the size of lesions produced by any of the N or B cultures prior to subculturing (Table 2.7). All three B cultures and two of the three N cultures were not affected by repeated subculturing (Table 2.8). However, the size of lesion caused by culture N/6 was very much reduced with the repeated subculturing treatment. This reduction was confirmed when cultures N/4, N/5 and N/6 were reassessed (Table 2.8).

The diminished in vivo growth rate of culture N/6 due to subculturing was not accompanied by a reduced in vitro growth rate (Table 2.9). N/6 was confirmed to be P. exigua var. foveata by assessing anthraquinone production.

In experiment 7 subculturing did not significantly reduce the size of lesion produced by any of the four isolates (Table 2.10).

Table 2.7 The size of lesions produced by the cultures prior to subculturing treatment

	Isolate/Culture						
	B/1	B/2	B/3	B/4	B/5	B/6	SED
Mean lesion radius (mm)	11.1	10.5	11.0	9.9	9.2	11.0	1.20 (DF=15)
	N/1	N/2	N/3	N/4	N/5	N/6	SED
Mean lesion radius (mm)	13.6	16.2	16.2	14.7	17.1	15.5	1.34 (DF=15)

Table 2.8 The effect of repeated subculturing on agar on the size of lesion produced by P. exigua var. foveata isolates. Mean lesion radius (mm)

Subcultured once		Subcultured 17 times		
Isolate/culture		Isolate/culture	Assessment 1	2
B/1	12.6	B/4	13.1	-
B/2	13.4	B/5	12.7	-
B/3	12.8	B/6	13.3	-
N/1	14.5	N/4	15.9	9.8
N/2	14.6	N/5	14.2	10.9
N/3	13.7	N/6	7.0	2.6
		SED	0.92 (DF=11)	0.96 (DF=2)

Table 2.9 The effect of repeated subculturing on the in vitro growth rates of the isolate N cultures

	N/1	N/2	N/3	N/4	N/5	N/6	SED
Culture diameter (mm)	56.7	61.3	66.0	59.7	56.7	64.7	2.61 (DF=10)

Table 2.10 The effect of repeated subculturing on agar on the size of lesion produced by *P. exigua* var. *foveata*

Isolate	Mean lesion radius (mm)	
	Subcultured once	Repeated subculturing
A23	7.0	6.6
A38	7.7	7.3
A63	7.7	7.2
F98	7.6	7.4
SED		0.49 (DF=7)

2.4 DISCUSSION

It can be concluded from the results of this section that there is considerable variation in pathogenicity among isolates of Phoma exigua var. foveata. In one experiment the most pathogenic isolate had a lesion diameter twice as large as the least pathogenic (Fig 2.6). It should be remembered that lesions are three dimensional. If it is assumed, for the sake of argument, that a lesion is hemispherical in shape then the volume of the lesion produced by the most pathogenic isolate was 13 times that of the least pathogenic. The existence of substantial variation among isolates has practical implications. First of all it means that methods of assessing P. exigua var. foveata incidence in soil, or on tubers, which involve the inoculation of tuber material and the assessment of the resulting lesions, may be inappropriate if there is pathogenicity variation in the P. exigua var. foveata sampled. Furthermore, the accuracy of models which have been developed to predict the level of gangrene likely to develop in storage from various factors at store loading time (Adams & Griffith, 1983) may be improved by taking account of the pathogenicity of the P. exigua var. foveata on the harvested tubers, although its influence is possibly small compared with factors already included in the model. The importance of variation in

pathogenicity in P. exigua var. foveata depends on the distribution of that variation. In an investigation comparing between and within crop variation the greatest variation occurred among isolates taken from the same crop. The variation between crops in P. exigua var. foveata pathogenicity, although significant, was smaller. However, variation within isolates was small in comparison with that observed between them. The large variation in isolate pathogenicity within crops means that in order to determine accurately the gangrene potential of tuber-borne inoculum for a particular stock the pathogenicity of P. exigua var. foveata from a representative sample of tubers has to be determined. This section provides some evidence suggesting that the greater gangrene potential of seed stocks grown in Aberdeenshire compared with those grown in Fife (Carnegie et al., 1981) is not due to the P. exigua var. foveata on the Aberdeenshire stocks being more pathogenic. The greater gangrene potential of Aberdeenshire stocks is therefore probably due to tubers being more heavily contaminated with P. exigua var. foveata or the tubers being more susceptible to infection.

It is unlikely that the differences in field isolate pathogenicity demonstrated in this section were due to differential reduction in isolate pathogenicity during culture of the isolates on agar, since it was demonstrated that isolates only infrequently suffered a

reduction in pathogenicity as a result of repeated subculturing over several weeks. However, it was found that the field isolates tested in this section were generally more pathogenic than the culture collection isolates. The reduced rotting ability of the collection isolates may have been due to them being cultured axenically over a period of many years. It was observed that a few of the culture collection isolates which at the start of the 3 year programme were able to rot tubers were no longer pathogenic after a further period in culture.

The results of this section emphasised the importance of using isolates with a high level of pathogenicity and thus of using recent field isolates. The use of culture collection isolates failed to demonstrate the general levels of pathogenicity of the fungus and the full range of variation in pathogenicity among isolates. Moreover, those isolates with a low pathogenicity were less able to demonstrate varied cultivar responses. This aspect is considered further in the next section.

CHAPTER 3

The influence of cultivar and isolate on gangrene development

3.1 INTRODUCTION

The resistance ranking order of cultivars to gangrene has been reported to be influenced by the isolate of Phoma exigua var. foveata used to inoculate the tubers (Rogers & Killick, 1974; Jellis, 1978, 1981). Thus the relative susceptibilities of cultivars in practice may not correspond to those determined during screening by breeders if the cultivars are exposed to different isolates. The primary purpose of the experiments in this section was to examine cultivar x isolate interactions using a range of cultivars and isolates. The relative importance of isolate and cultivar in influencing variation in gangrene development was also considered. The effects of the history of an isolate and the method of gangrene assessment were also taken into account.

Two experiments were carried out: in the first eight isolates were tested on 10 cultivars and in the second two isolates were used with 7 cultivars.

3.2 MATERIALS AND METHODS

Experiment 1

The influence of seven field isolates, designated M, N, Q, S, U, V and W, plus one culture collection isolate, C, on gangrene development in 10 cultivars was assessed. The cultivars covered a wide range of variation

in gangrene resistance: they were Roslin Castle, Maris Piper, Pentland Ivory, Pentland Dell, Record, Catriona, Pentland Javelin, Pentland Envoy, Corrie and Pentland Falcon (Wastie, unpublished). Twenty tuber quarters of each cultivar were point inoculated with 2000 pycnospores of each isolate. After incubation for 7 weeks the surface diameter and depth of each lesion was measured.

The data from this experiment were used to estimate the relative importance of isolate and cultivar in determining gangrene lesion size. Two methods were used: the first was based on the estimation and comparison of variance components (Searle, 1971); the second was a direct comparison of the ranges in lesion size for cultivars and isolates. For both methods only five of the 10 cultivars, those currently grown in the UK, and only the seven field isolates, were used.

Experiment 2

In this experiment the influence of isolate on the relative resistances of cultivars was examined using the culture collection isolate B and the more recent field isolate N. The resistances of Maris Piper, Pentland Crown, Pentland Javelin, Pentland Envoy, Pentland Dell, Record and Roslin Castle were assessed by point inoculating 20 tuber quarters with a pycnospore suspension of each isolate. After 7.5 weeks' incubation the surface diameter and maximum depth of each lesion was measured.

3.3 RESULTS

The influence of cultivar and isolate on gangrene development.

Experiment 1

From the analyses of variance of the results for the seven field isolates (Appendix 3.1) isolate and cultivar had a significant effect on both lesion diameter and lesion depth, but there were significant cultivar x isolate interactions. The ranking order of the 10 cultivars, according to lesion diameter, was more or less similar for all seven field isolates (Table 3.1a). The coefficient of concordance of the rankings was 0.915 ($P<0.01$). Pentland Falcon had generally the widest lesions whereas the narrowest were those on Roslin Castle. For lesion depth the rankings of the 10 cultivars with the seven field isolates are shown in Table 3.1b. The coefficient of concordance was 0.837 ($P<0.01$). Corrie had usually the deepest lesions and Roslin Castle the shallowest.

The separate analyses of variance of the results for the culture collection isolate C (Appendix 3.2) show that there were differences among the cultivars in lesion depth but not in diameter. The ranking order of the 10 cultivars according to lesion depth obtained using isolate C bore little relationship to the average ranking order for the seven field isolates (Table 3.2). However, this result was most likely due to the limited discrimination

Table 3.1 The effect of field isolate of Phoma exigua var. foveata on the ranking order of 10 cultivars for gangrene lesion size

a. Lesion diameter

	Cultivar									
Isolate	PF	PJ	PE	CAT	R	COR	MP	PI	PD	RC
M	1	3	2	5	4	6	7	8	9	10
N	1	2	4	3	5	6	7	9	8	10
Q	1	2	3	4	5.5	5.5	8	7	9	10
S	1	3	2	6	5	4	7	10	9	8
U	1	2	5	3	4	6	7	10	8	9
V	1	2	5	3	4	6	8	7	9	10
W	1	3	2	6	5	4	7	9	8	10
Mean rank	1	2.4	3.3	4.3	4.6	5.4	7.3	8.6	8.6	9.6

Kendall coefficient of concordance (W) = 0.915 ($P<0.01$)

b. Lesion depth

	Cultivar									
Isolate	COR	PE	PJ	CAT	PF	PD	R	PI	MP	RC
M	1	2	3	8	7	5	6	4	10	9
N	1	2	3	4	5	6	8	7	9	10
Q	1	3	4	2	5	8	6	7	10	9
S	1	2	5	3	4	7	6	9	8	10
U	1	3	5	4	2	6	7	9	8	10
V	1	2	3	6	4	8	7	5	10	9
W	1	5	2	3	4	6	7	8	9	10
Mean rank	1	2.7	3.6	4.3	4.4	6.6	6.7	7.0	9.1	9.6

Kendall coefficient of concordance (W) = 0.837 ($P<0.01$)

Cultivar key:
PF - Pentland Falcon
PJ - Pentland Javelin
PE - Pentland Envoy
CAT - Catriona
R - Record
COR - Corrie
MP - Maris Piper
PI - Pentland Ivory
PD - Pentland Dell
RC - Roslin Castle

Table 3.2 The effect of isolate of *P. exigua* var. *foveata* on the ranking order of 10 cultivars according to lesion depth

Cultivar	Mean rank for seven field isolates	Rank for culture collection isolate
Corrie	1.0	1
P. Envoy	2.7	8
P. Javelin	3.6	2
Catriona	4.3	10
P. Falcon	4.4	3
P. Dell	6.6	6
Record	6.7	4
P. Ivory	7.0	9
M. Piper	9.1	5
R. Castle	9.6	7

among cultivars by the weakly pathogenic isolate C.

In considering the ranking order of field isolates on the different cultivars, for lesion diameter (Table 3.3a) there was a coefficient of concordance of 0.459 ($P<0.01$). However, some discrepancies in ranking are seen, notably the high rank of isolate S on Roslin Castle. With lesion depth the ranking order of isolates varied considerably on the cultivars and the coefficient of concordance was not significant (Table 3.3b).

In this experiment, taking the mean lesion radius, the range of variation among isolates was as great on the more resistant cultivars as on the very susceptible (Fig 3.1): the largest variation occurred with Pentland Ivory, a cultivar reported to be fairly resistant (Wastie, unpublished). Corrie was the most susceptible and Roslin Castle the most resistant of the 10 cultivars.

In estimating the relative influence of cultivar and isolate on lesion size it was found that cultivar had a larger effect with both of the methods of estimation used. With the variance components method, cultivar was six times more important than isolate in determining lesion diameter and eight times more so in affecting rot depth (Table 3.4). With the second method, comparing directly the ranges for cultivar and isolate, the figures were 1.6 and 2 times respectively (Fig 3.2).

Table 3.3 The effect of cultivar on the ranking order of isolates of *P. exigua* var. *foveata*

a. Lesion diameter

	Isolate						
Cultivar	W	V	U	Q	M	N	S
P. Falcon	1	5	2	6	3	4	7
P. Javelin	1	5	2	4	6	3	7
P. Envoy	1	5	6	2	3	4	7
Catriona	4	1	2	5	6	3	7
Record	1	2	3	4.5	4.5	6	7
Corrie	1	3	6	2	5	7	4
M. Piper	1	3	5	4	2	6	7
P. Ivory	3	2	5	1	4	6	7
P. Dell	1	4	3	6	2	5	7
R. Castle	1	3	4	5	6	7	2
Mean rank	1.5	3.3	3.8	4	4.2	5.1	6.2

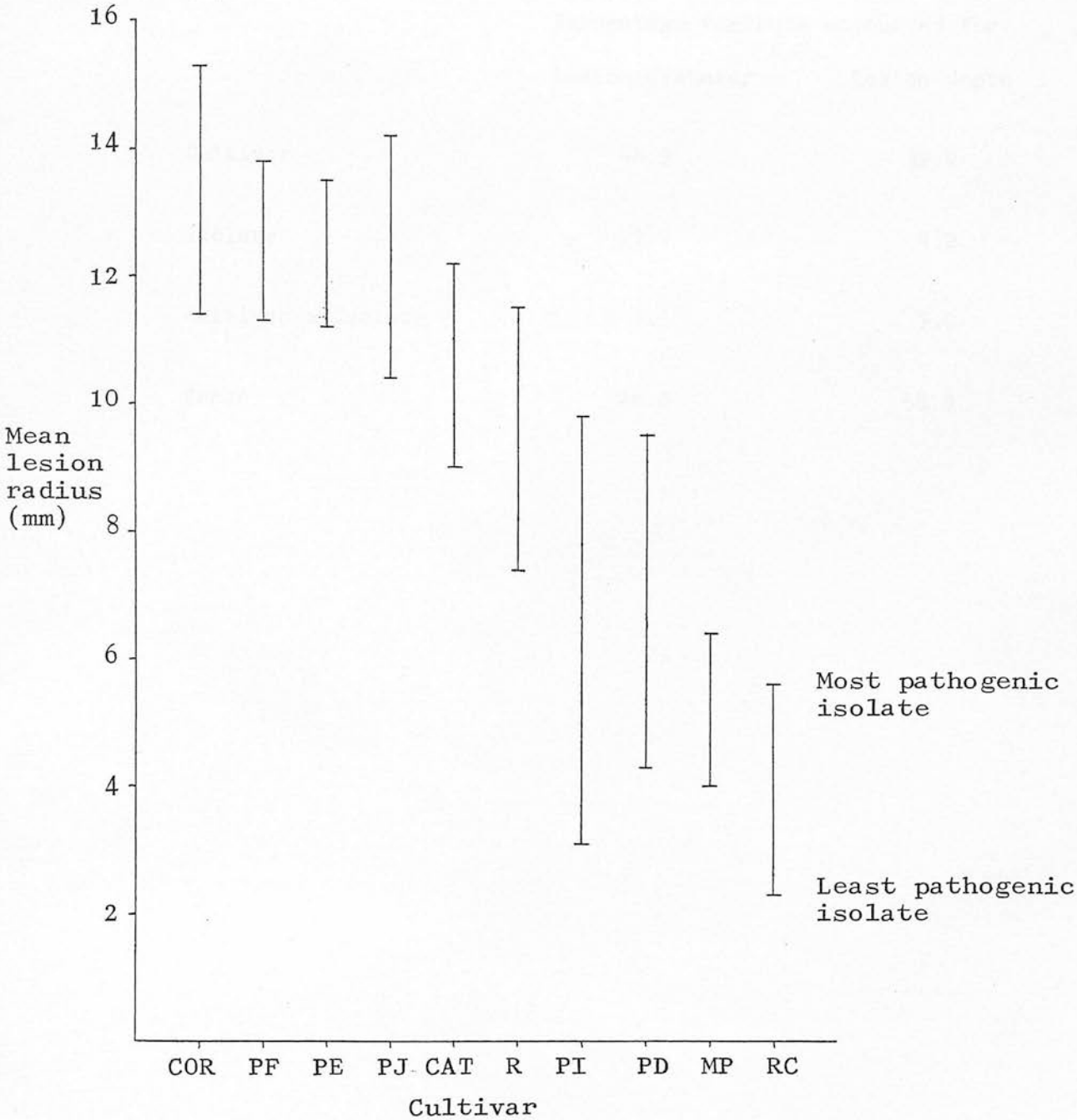
Kendall coefficient of concordance (W) = 0.459 ($P<0.01$)

b. Lesion depth

	Isolate						
Cultivar	W	V	M	U	Q	S	N
Corrie	3	1	2	6	5	7	4
P. Envoy	4	5	1	7	6	2	3
P. Javelin	1	3.5	6	5	7	3.5	2
Catriona	2	6	7	4	1	3	5
P. Falcon	1	4	7	2	6	3	5
P. Dell	1	6	3	2	5	7	4
Record	1	3	4	5	2	6	7
P. Ivory	2	1	4	6	3	7	5
R. Castle	5	2	1	4	3	6	7
M. Piper	2	4	7	1	5	3	6
Mean rank	2.2	3.6	4.2	4.2	4.3	4.7	4.8

Kendall coefficient of concordance (W) = 0.172 (NS)

Figure 3.1 The effect of cultivar on the expression of pathogenicity differences among P. exigua var. foveata field isolates

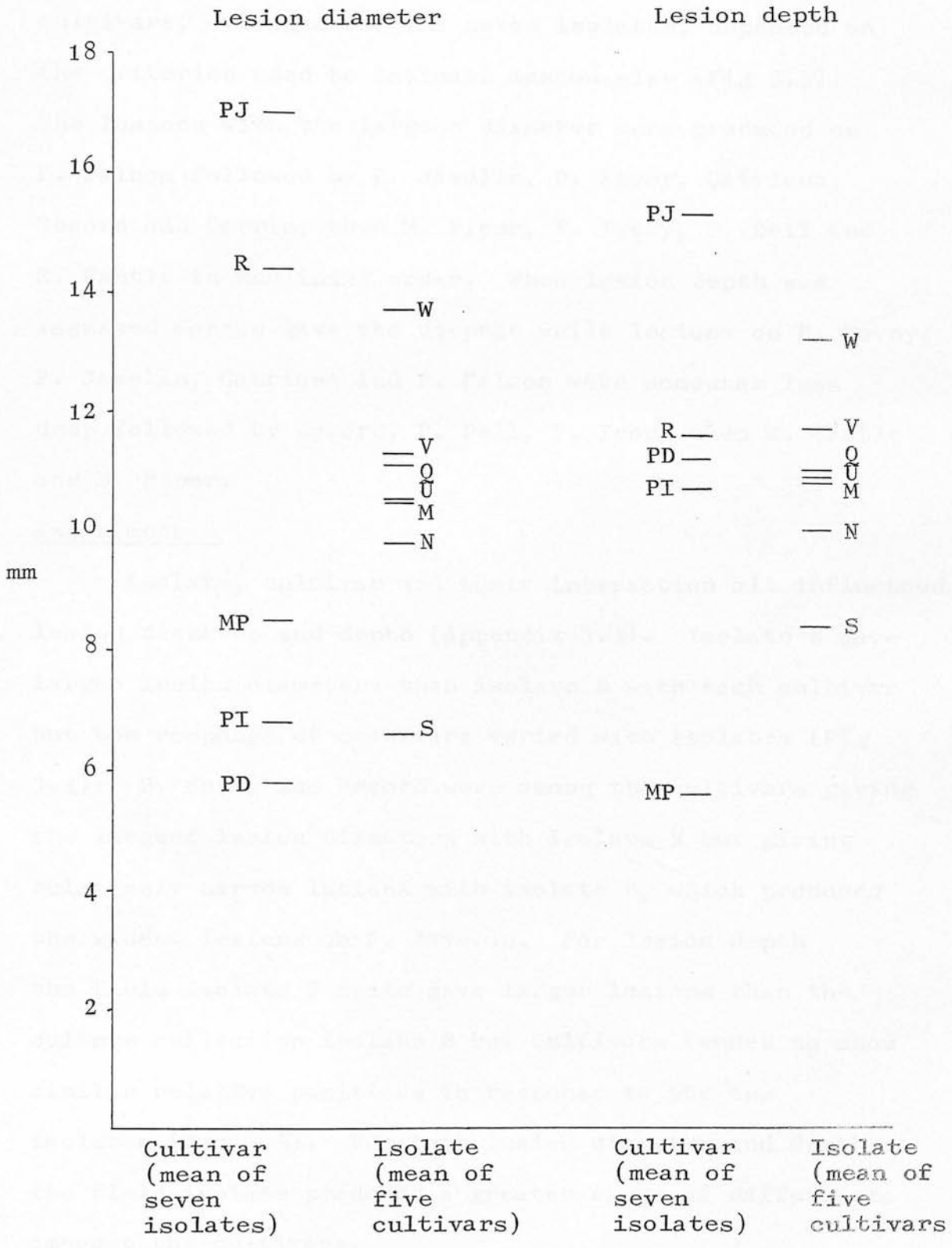


COR	Corrie	R	Record
PF	Pentland Falcon	PI	P. Ivory
PE	P. Envoy	PD	P. Dell
PJ	P. Javelin	MP	Maris Piper
CAT	Catriona	RC	Roslin Castle

Table 3.4 Estimation of the relative influence of cultivar and isolate on rot size

	Percentage variance accounted for	
	lesion diameter	lesion depth
Cultivar	44.7	32.9
Isolate	7.7	4.2
Cultivar x Isolate	1.6	7.0
Error	46.0	55.9

Figure 3.2 Variation in gangrene lesion development in relation to cultivar and isolate of P. exigua var. foveata



Cultivar key

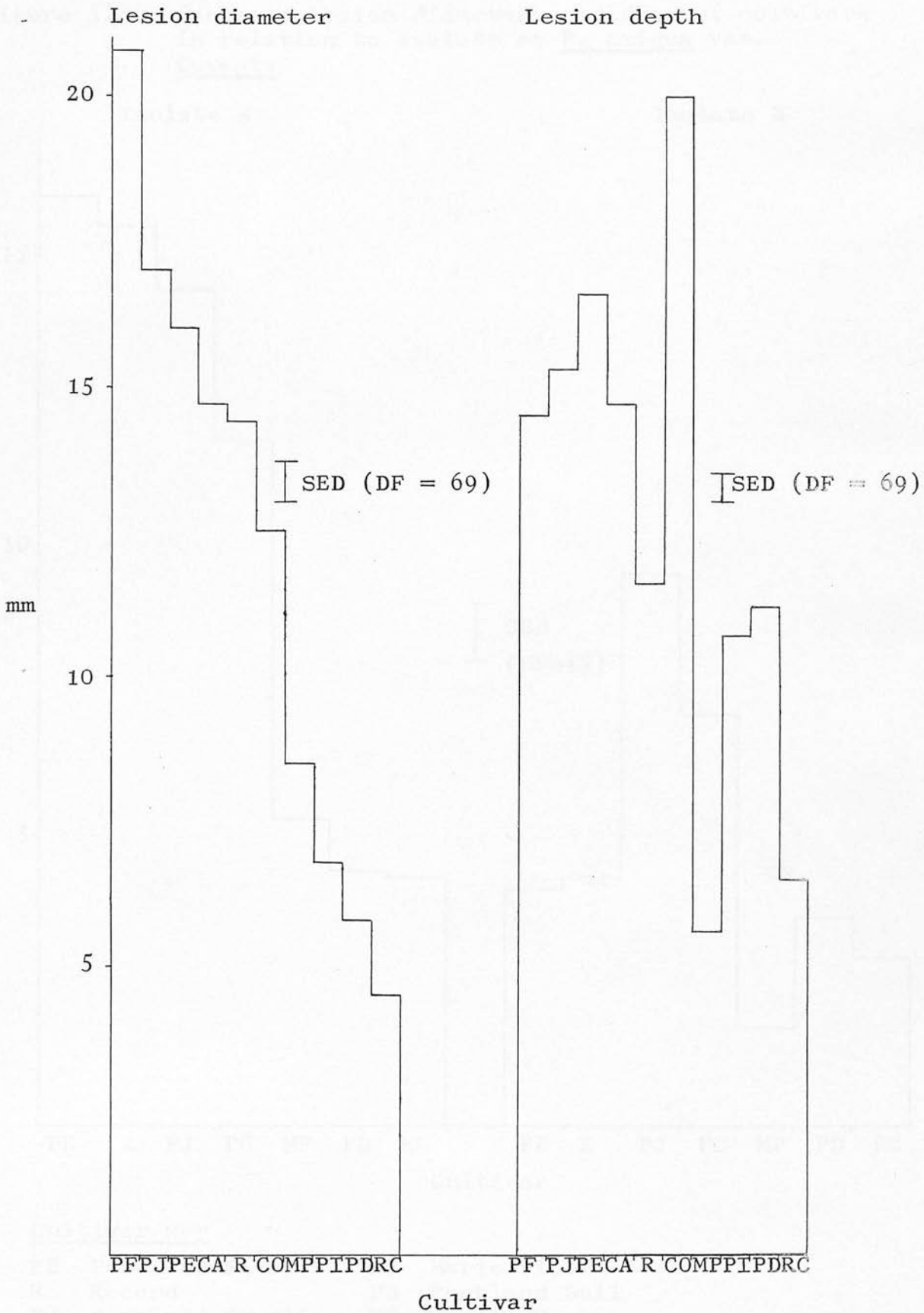
PJ Pentland Javelin
R Record
PD Pentland Dell
PI Pentland Ivory
MP Maris Piper

The relative sizes of gangrene lesions on different cultivars, averaged for the seven isolates, depended on the criterion used to estimate lesion size (Fig 3.3). The lesions with the largest diameter were produced on P. Falcon followed by P. Javelin, P. Envoy, Catriona, Record and Corrie, then M. Piper, P. Ivory, P. Dell and R. Castle in declining order. When lesion depth was assessed Corrie gave the deepest while lesions on P. Envoy, P. Javelin, Catriona and P. Falcon were somewhat less deep followed by Record, P. Dell, P. Ivory then R. Castle and M. Piper.

Experiment 2

Isolate, cultivar and their interaction all influenced lesion diameter and depth (Appendix 3.3). Isolate N gave larger lesion diameters than isolate B with each cultivar but the response of cultivars varied with isolates (Fig 3.4): P. Envoy and Record were among the cultivars giving the largest lesion diameters with isolate N but giving relatively narrow lesions with isolate B, which produced the widest lesions on P. Javelin. For lesion depth the field isolate N again gave larger lesions than the culture collection isolate B but cultivars tended to show similar relative positions in response to the two isolates (Fig 3.5). For both lesion diameter and depth the field isolate produced a greater range of differences amongst the cultivars.

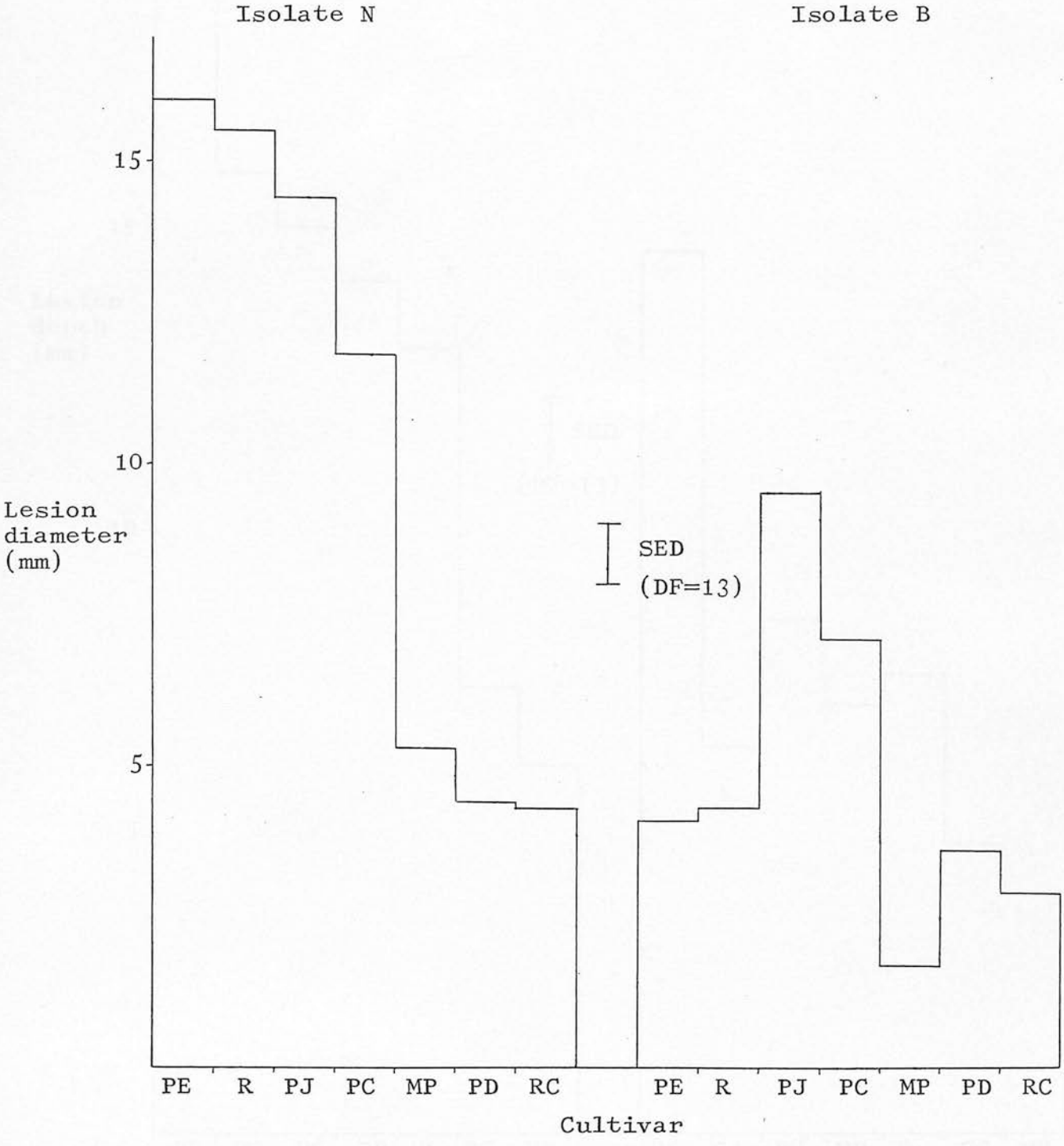
Figure 3.3 Gangrene lesion size for 10 cultivars (averaged for seven field isolates) based on diameter and depth assessments



Cultivar key

PF	Pentland Falcon	CO	Corrie
PJ	Pentland Javelin	MP	Maris Piper
PE	Pentland Envoy	PI	Pentland Ivory
CA	Catriona	PD	Pentland Dell
R	Record	RC	Roslin Castle

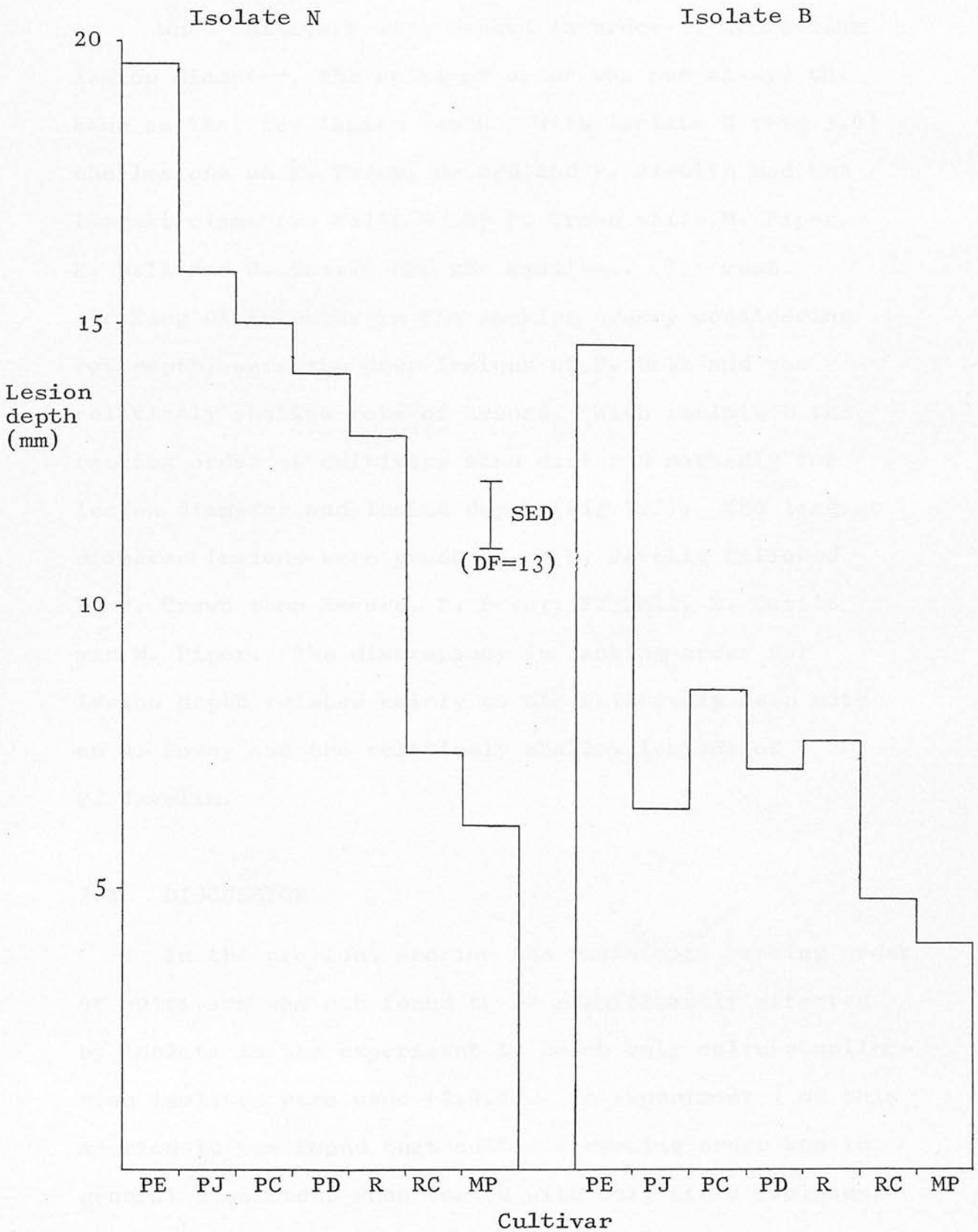
Figure 3.4 Gangrene lesion diameter on different cultivars in relation to isolate of *P. exigua* var. *foveata*



Cultivar key

PE	Pentland Envoy	MP	Maris Piper
R	Record	PD	Pentland Dell
PJ	Pentland Javelin	RC	Roslin Castle
PC	Pentland Crown		

Figure 3.5 Gangrene lesion depth in different cultivars in relation to isolate of P. exigua var. foveata



Cultivar key

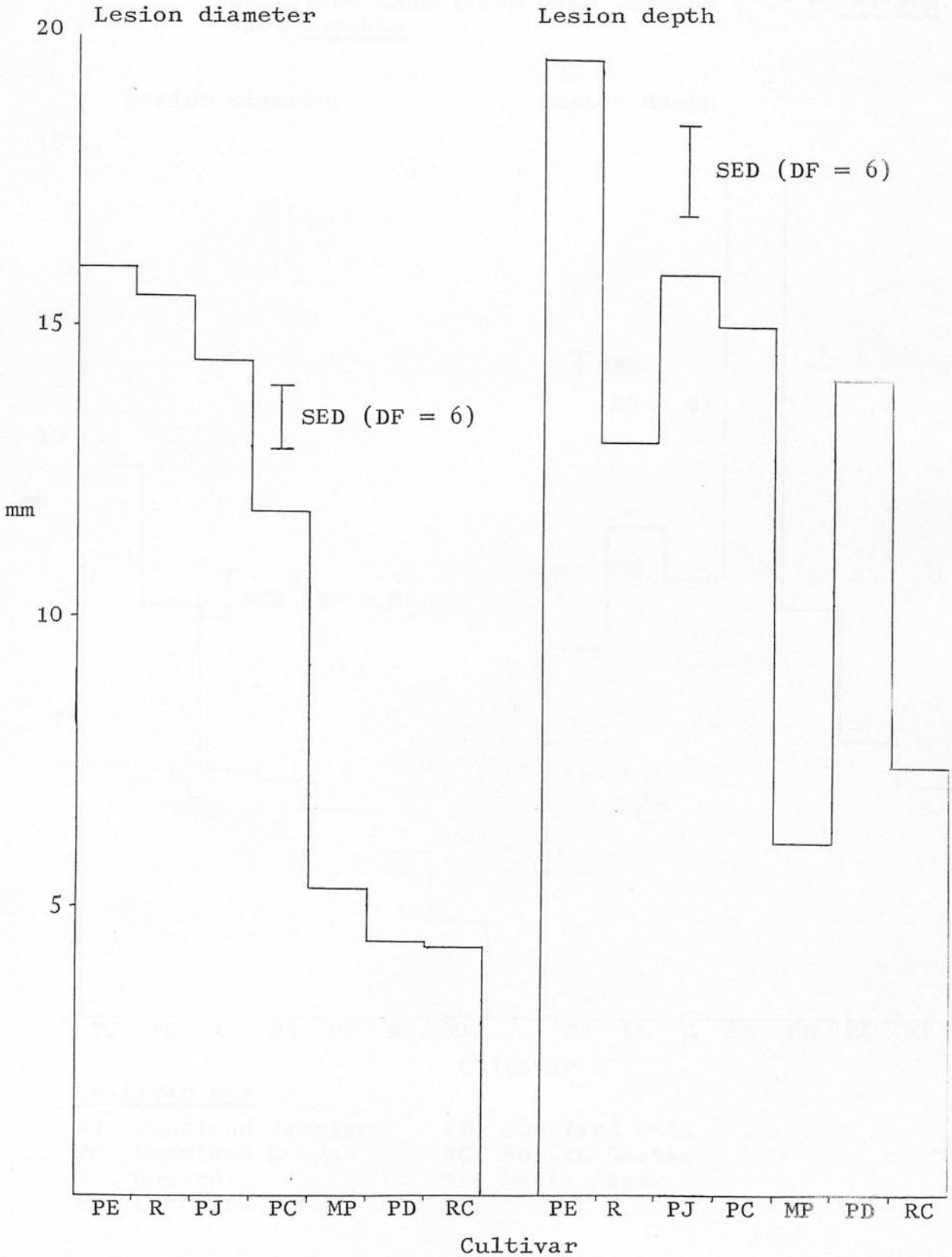
PE	Pentland Envoy	MP	Maris Piper
R	Record	PD	Pentland Dell
PJ	Pentland Javelin	RC	Roslin Castle
PC	Pentland Crown		

When cultivars were ranked in order of decreasing lesion diameter, the cultivar order was not always the same as that for lesion depth. With isolate N (Fig 3.6) the lesions on P. Envoy, Record and P. Javelin had the largest diameters followed by P. Crown while M. Piper, P. Dell and R. Castle had the smallest. The most striking differences in the ranking order, considering rot depth, were the deep lesions of P. Dell and the relatively shallow rots of Record. With isolate B the ranking order of cultivars also differed markedly for lesion diameter and lesion depth (Fig 3.7). The largest diameter lesions were produced on P. Javelin followed by P. Crown then Record, P. Envoy, P. Dell, R. Castle and M. Piper. The discrepancy in ranking order for lesion depth related mainly to the relatively deep rots on P. Envoy and the relatively shallow lesions of P. Javelin.

3.4 DISCUSSION

In the previous section the resistance ranking order of cultivars was not found to be significantly affected by isolate in the experiment in which only culture collection isolates were used (2.3.1). In experiment 1 of this section it was found that cultivar ranking order was in general consistent when tested with only field isolates. Cultivar ranking order was, however, affected by isolate

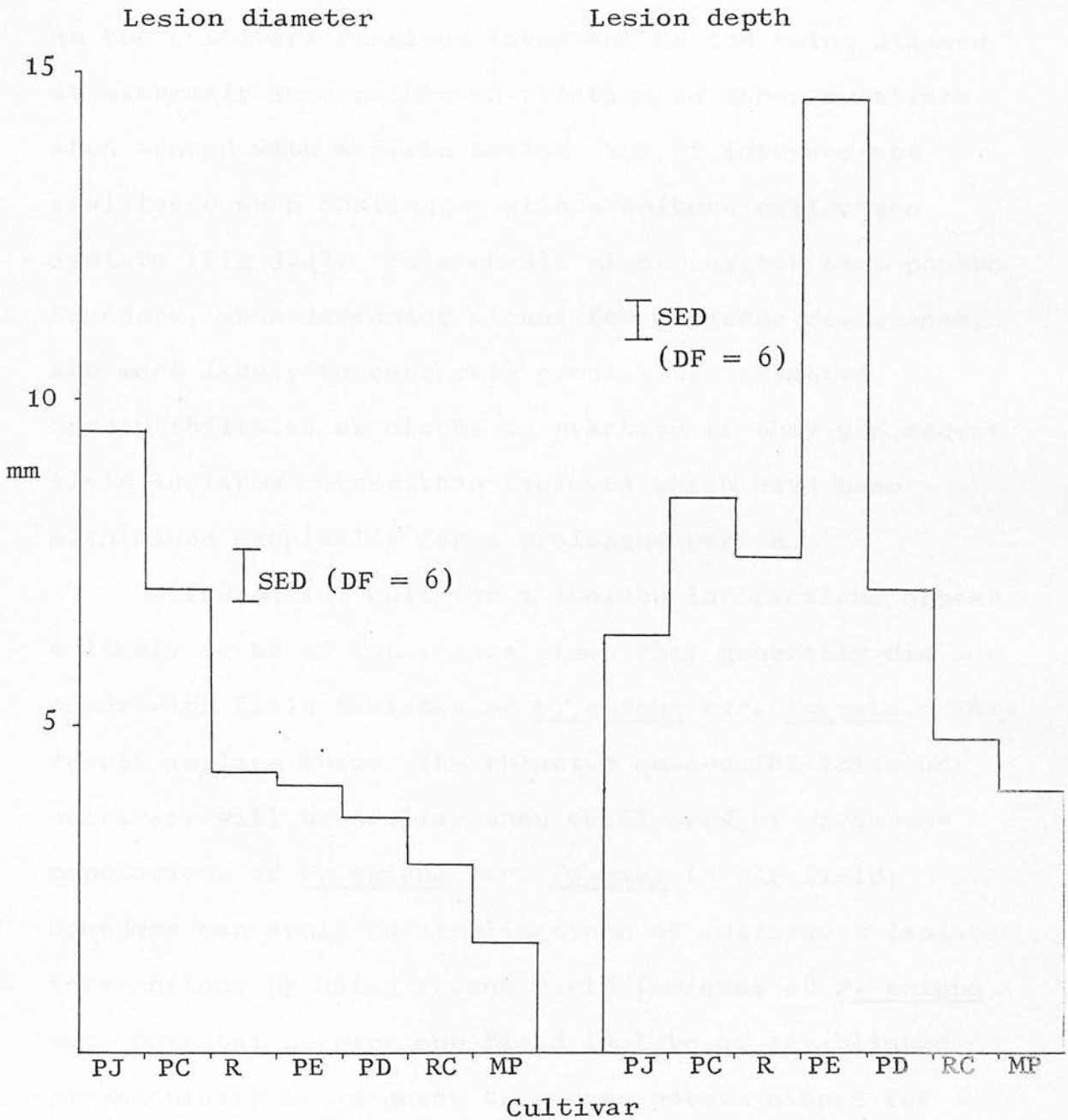
Figure 3.6 Gangrene lesion diameters and depths in different cultivars inoculated with isolate N of P. exigua var. foveata



Cultivar key

PE	Pentland Envoy	MP	Maris Piper
R	Record	PD	Pentland Dell
PJ	Pentland Javelin	RC	Roslin Castle
PC	Pentland Crown		

Figure 3.7 Gangrene lesion diameters and depths in different cultivars inoculated with isolate B of P. exigua var. foveata



Cultivar key

PJ	Pentland Javelin	PD	Pentland Dell
PC	Pentland Crown	RC	Roslin Castle
R	Record	MP	Maris Piper
PE	Pentland Envoy		

in the experiment in which both a culture collection isolate and a field isolate were used. The effect of isolate was large enough in one assessment to result in the cultivars Pentland Envoy and Record being classed as extremely susceptible in relation to other cultivars when tested with a field isolate but of intermediate resistance when challenged with a culture collection isolate (Fig 3.4). This result might suggest that potato breeders, when screening clones for gangrene resistance, are more likely to correctly predict the relative susceptibilities of clones in practice if they use recent field isolates rather than isolates which have been maintained axenically for a prolonged period.

Differential cultivar x isolate interactions appear unlikely to be of importance since they generally did not occur with field isolates of P. exigua var. foveata. This result implies that: the relative susceptibilities of cultivars will be similar when challenged by different populations of P. exigua var. foveata in the field; breeders can avoid the implications of cultivar x isolate interactions by using recent field isolates of P. exigua var. foveata; perhaps one field isolate of established pathogenicity is adequate to screen potato clones for gangrene resistance and not several as recommended previously (Jellis, 1981). Further evidence for the minor importance of differential isolate x cultivar interactions

is their inconsistency. Jellis (1981) found that the reversed relative susceptibilities of Maris Piper and Pentland Crown with two isolates were only obtained in one year out of two. The occurrence of cultivar x isolate interactions seems dependent on the tuber tissue inoculated and also how gangrene development is assessed. Jellis (1981) found that his cultivar x isolate interaction occurred only when the cortex was inoculated, not when the medullary tissue was challenged. In this study cultivar x isolate interactions consistently depended on the method of lesion measurement as Rogers & Killick (1974) found.

Differences in the range of resistance among cultivars were greater for field isolates than the less pathogenic culture collection isolates. Thus another benefit to breeders of using recent field isolates is that the screening procedure will be more sensitive, therefore facilitating the selection of genotypes. With the use of recent field isolates screening will also be faster since the rate of lesion development is greater than with less pathogenic culture collection isolates.

When assessing lesion size after point inoculation it is of value to measure both the diameter of the lesion at the tuber surface and the depth of the rot. It was repeatedly found in the experiments of this investigation that the resistance ranking order of cultivars was

markedly different for surface lesion diameter compared with lesion depth. The different cultivar ranking orders for lesion diameter and depth following cortical inoculation were probably due to the fact that the tuber tissue resistance of the medulla of a cultivar is not necessarily related to the resistance of its cortex (Pietkiewicz & Jellis, 1975).

Although cultivar ranking order was little affected by field isolate the pathogenicity ranking order of field isolates of P. exigua var. foveata was found to depend on the host cultivar on which pathogenicity was assessed. This finding has implications. For example, in studies investigating the basis of pathogenicity differences among isolates it would be necessary to screen isolate pathogenicity using several cultivars and to select for further study those isolates whose pathogenicity was stable on several cultivars. If this was not done then it is unlikely that the pathogenicity of isolates could be correctly related to other isolate characteristics.

It was found that the extent of lesion development varied more widely with cultivar than with isolate. With the comparison of variance components method, cultivar contributed approximately seven times more to the variation in lesion size than isolate, while from a

direct comparison of the ranges in lesion size, cultivar showed twice the range of isolate.

It is unlikely that the influence of field isolate on gangrene development is greater with susceptible cultivars compared with more resistant ones since it was found that the range of variation in isolate pathogenicity was as great on more resistant cultivars as the extremely susceptible. For culture collection isolates, isolate differences were much greater on the more susceptible cultivars (Fig 2.3). This result was probably due to isolate differences not being expressed on the more resistant cultivars because of the generally low level of pathogenicity of the culture collection isolates. The different result obtained for field and culture collection isolates again emphasises the desirability of using recent field isolates.

CHAPTER 4

The effect of inoculum level, and its interaction with cultivar, tuber damage method, damage incidence and isolate of *Phoma exigua* var. *foveata*, on gangrene development

4.1 INTRODUCTION

Previous work has established that, in general, the higher the level of inoculum on tubers at harvest the greater the level of gangrene will be in store if conditions suitable for gangrene development prevail (Wellving, 1976; Adams, 1980a). The aim of this section was to examine the relationship between gangrene development and inoculum level and the influence on the relationship of cultivar, method of tuber damage, tuber damage level and isolate of the pathogen.

To examine how the spore loads used corresponded to the range occurring on commercial seed stocks, the level of P. exigua var. foveata in tubersphere soil from a number of stored commercial seed crops was assessed.

In considering tuber damage methods three techniques were used. In two of them no account was taken of cultivar resistance to damage: tubers were either uniformly wounded or left undamaged. In the other treatment resistance to wounding was included: the tubers were wounded by passing them over a grader.

This section also aimed to provide more information on the influence of inoculum level in the screening of clones for gangrene resistance.

The experimental studies were as follows:

4.2.1 Incidence of P. exigua var. foveata propagules in tubersphere soil from stored seed potato crops (Experiment 1).

4.2.2 The influence of inoculum level, and its interaction with cultivar and isolate, on gangrene development (Experiments 2 & 3).

4.2.3 The influence of inoculum density and its interaction with cultivar, tuber damage treatment and damage level, on gangrene incidence (Experiment 4).

4.2- MATERIALS AND METHODS

4.2.1 Experiment 1

The levels of P. exigua var. foveata in tubersphere soil from 17 Foundation Stock crops of Desiree were determined. Nine of the crops had been grown in Aberdeenshire, the others in Fife. Crops from these two areas were chosen since it has been shown that, in general, Aberdeenshire crops have a high gangrene potential whereas Fife crops have low potentials (Carnegie et al., 1981). For each crop soil was removed from 50 tubers which had been selected at random from the stored crop. The tubers were kindly supplied by Dr S.F. Carnegie, DAFS, Edinburgh. The soil samples were air dried, sieved to remove particles greater than

2 mm and then were thoroughly mixed. A subsample of 5 g was then blended in 100 ml sterile distilled water for 1 minute at 12,000 rpm. Five ml of the solution was immediately removed, using a wide-mouthed pipette (3 mm aperture), and added to 45 ml sterile distilled water. This suspension was stirred for 2 minutes then 0.5 ml aliquots were plated out on a modified McCracken & Logan selective medium (Appendix 4.1). The suspension was further diluted using the same procedure to produce two further tenfold dilutions which were plated out as before. The number of P. exigua var. foveata colonies per plate was assessed after incubation for 10 days at 20°C. The number of colonies per plate was then used to calculate the number of P. exigua var. foveata propagules per gram of dried soil.

4.2.2 Experiment 2

Two cultivars, the susceptible Pentland Envoy and the more resistant Maris Piper, were inoculated with 625, 1250, 2500 or 5000 pycnospores per inoculation of the culture collection isolates A and C. Twenty tuber quarters of each cultivar were point inoculated with each dose of each isolate. Two replicate suspensions of each inoculum level were used. After 8 weeks' incubation the surface diameter and maximum depth of each lesion were measured.

Experiment 3

Three cultivars, Maris Piper, Pentland Crown and Pentland Dell were inoculated with eight spore loads, ie 5, 14, 41, 123, 370, 1111, 3333 and 10000 pycnospores per inoculation of the three field isolates, M, N and V. There were two replicate pycnospore suspensions for each dose. Twenty tuber quarters of each cultivar were point inoculated with each replicate suspension. After 6.5 weeks' incubation each quarter was scored for the presence of a lesion. The quarter was classified as having a lesion if, when the quarter was cut through the inoculation site, the rot at the cut surface extended more than one millimetre at any point. At the same time, for 20 quarters of each treatment, the surface diameter and maximum depth of each lesion were assessed.

4.2.3 Experiment 4

In this experiment four levels of inoculum were used. The tubers were contaminated by dipping them in soil slurries (Adams, 1980a) of field isolate U. The slurries consisted of 4 kg of air-dried, sieved (2 mm), sterilized Kettering Loam, 4 l sterilized tap water and either 10^2, 10^3, 10^4 or 10^5 pycnospores per ml of water used. Blemish-free tubers of the susceptible cultivars Pentland Crown and Pentland Javelin and the more resistant Record were immersed in the slurry, air-dried and then stored at 4°C for 1 week.

Three different damage treatments, namely grader damage, uniform wounding or no damage, were used. In the first the contaminated tubers were wounded by passing them over a reciprocating riddle-type grader (Cooch model 6AT). Prior to use this was steam-cleaned and disinfected with a Sodium hypochlorite solution (2.5% available chlorine). To investigate the effect of damage level on the relationship between inoculum density and gangrene development batches of tubers were passed either once, twice or three times over the grader. Two replicate batches of 25 contaminated tubers were used per treatment. The grader was thoroughly washed between different inoculum level treatments. The levels of tuber damage on each cultivar after one, two or three passes over the grader were assessed. Fifty non-inoculated tubers of each cultivar were damaged at each level and the number of cut or crush wounds per tuber was recorded. The second damage treatment, uniform wounding, was achieved by stabbing each contaminated tuber at four points, mid-way between the rose and heel ends, with a sterilized modified masonry nail (Dr S.F. Carnegie, personal communication). The nail inflicted a wound 3 mm in diameter and 3 mm deep. Twenty replicate tubers were used. For the third damage treatment, ie no damage, twenty tubers of each cultivar/inoculum level were merely incubated. After incubation for 8 weeks the number of lesions, greater than 4 mm in diameter, per tuber was recorded.

4.3 RESULTS

4.3.1 Experiment 1

The incidence of P. exigua var. foveata propagules in tubersphere soil from stored seed potato crops.

The level of P. exigua var. foveata in tubersphere soil varied for the different crops (Table 4.1). The levels ranged from 10^5 propagules per gram of soil to a level which was undetectable using the soil dilution plate method. The soil with the highest level of inoculum, ie A6, was reassessed since the range of inoculum levels used in Experiment 4 was determined by it. The second assessment confirmed that approximately 10^5 propagules of P. exigua var. foveata were present in this soil.

The pattern of results obtained with the soil dilution plate method was also achieved when the gangrene potentials of the stocks were assessed (by Dr S.F. Carnegie, DAFS, Edinburgh) (Table 4.1). Gangrene potential was estimated by damaging 50 tubers per stock with sterilized modified masonry nails. Each tuber was wounded at 10 points to a depth of 5 mm. After 10 weeks' incubation at 5°C the percentage of tubers with gangrene was determined. All the soils in which P. exigua var. foveata was detected by the soil dilution plate (SDP) method were from stocks which gave a very high percentage of gangrene. Some stocks gave large

Table 4.1 Level of *P. exigua* var. *foveata* in tubersphere soil from commercial seed stocks grown in Aberdeenshire (A) and Fife (F)

Seed stock	Number of propagules per gram of soil	Gangrene potential (percentage of tubers with gangrene)	Percentage of surface area of plates contaminated
A6	92,000 100,000*	100	-
A7	20,000	100	-
A3	30,800	98	-
A5	Not detectable	94	100
A9	"	90	70-80
A1	24,000	86	-
F4	Not detectable	68	75-90
F3	"	63	100
A2	"	58	-
F9	"	58	-
F1	"	34	-
A4	"	16	-
F2	"	14	40-75
F6	"	6	-
F5	"	4	-
F8	"	2	75-90
A8	"	0	-

*Repeated assessment

amounts of gangrene but P. exigua var. foveata could not be detected in the soil using the SDP method, eg soils A5, A9, F4 and F3. There was a high level of contaminating fungi in soils from these stocks (Table 4.1).

4.3.2 The influence of inoculum level, and its interaction with cultivar and isolate, on gangrene development.

Experiment 2

From the analysis of variance (Appendix 4.2) lesion radius was found to be affected by cultivar, isolate, inoculum dose and the interaction between cultivar and isolate.

Lesion size was greatest at the highest inoculum density (Table 4.2). Despite the significant cultivar x isolate interaction isolate A produced larger lesions than isolate C on both cultivars and larger rots developed on Pentland Envoy with both isolates (Table 4.3).

Experiment 3

Lesion number was affected by isolate, inoculum dose and the interactions between cultivar and dose, and isolate and dose (Appendix 4.3).

The interaction between cultivar and inoculum level is shown in Fig. 4.1. For all three cultivars infection frequency increased with increasing spore load up to 1111 pycnospores per inoculation at which level all wounds were

Table 4.2 The influence of inoculum density on gangrene lesion radius (mm)

Inoculum density (pycnospores per inoculation)				SED
625	1250	2500	5000	
4.4*	4.8	4.3	5.4	0.38 (DF = 284)

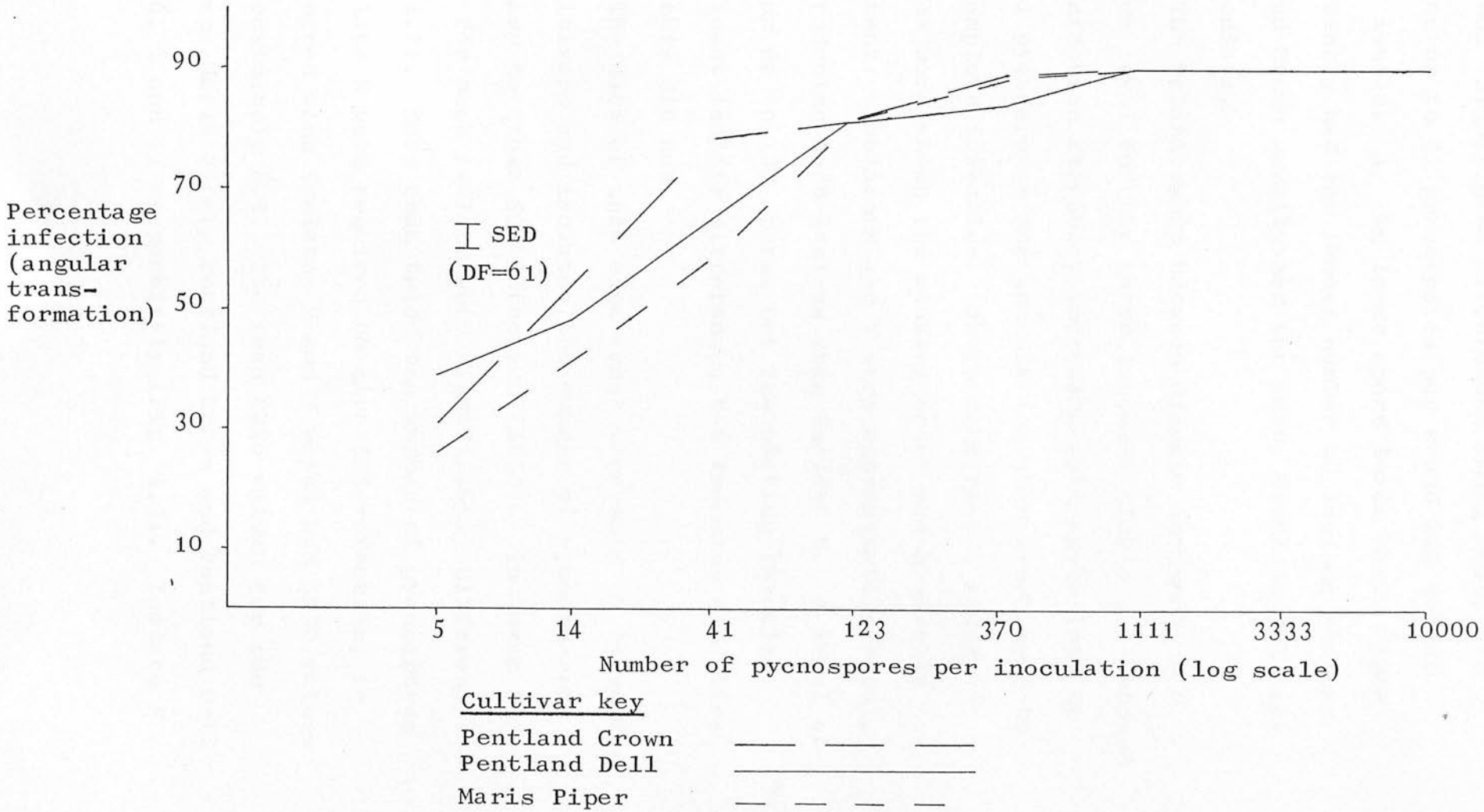
*Mean values for 2 cultivars and 2 isolates

Table 4.3 The influence of cultivar and isolate of P. exigua var. foveata on gangrene lesion radius (mm)

Cultivar	Isolate		
	A	C	Mean
Maris Piper	3.0	2.0	2.5
Pentland Envoy	9.2	4.6	6.9
Mean	6.1	3.3	4.7

Factor	Cultivar	Isolate	Cultivar x Isolate
SED	0.27	0.27	0.38
DF	284	284	284

Figure 4.1 The relationship between inoculum level and gangrene incidence in three cultivars (average of three isolates)



infected. Significant differences among cultivars occurred up to 41 pycnospores per wound but not at higher levels. At the lower spore loads Maris Piper consistently had the fewest number of lesions whereas Pentland Crown usually had the most; Pentland Dell was intermediate.

The relationship between disease incidence and inoculum level for the three isolates (Table 4.4) showed that infection frequency increased with spore load up to 1111 pycnospores per inoculation when every isolate gave complete infection. Where differences among isolates were shown the ranking order was generally consistent. Isolates M and V were approximately equal, both producing more lesions than isolate N. A level of inoculum up to 123 spores per inoculation revealed significant isolate differences but inoculum densities above this did not.

The data of this experiment were used to calculate for cultivars and isolates the number of pycnospores necessary to cause 50% infection (ED50). The mean ED50 values for each isolate were significantly different (Fig. 4.2). More than twice the number of pycnospores of isolate N were required to give 50% infection, ie 20, compared with isolates M and V which had ED50 values of approximately 8.5. The mean ED50 values for the cultivars Maris Piper, Pentland Crown and Pentland Dell were 16, 8 and 11 respectively (Fig. 4.3). Isolate N

Table 4.4 The effect of isolate of P. exigua var. foveata and inoculum density on infection frequency (angular transformation of percentage infection)

Pycnospores per inoculation	Isolate (average on three cultivars) M	V	N	Mean
5	36.8	36.7	21.6	31.7
14	53.1	51.0	39.0	47.7
41	73.5	70.8	57.1	67.1
123	83.4	84.8	74.4	80.9
370	87.9	87.9	84.1	86.6
1111	90.0	90.0	90.0	90.0
3333	90.0	90.0	90.0	90.0
10000	90.0	90.0	90.0	90.0
Mean	75.6	75.1	68.3	73.0

Factor	Isolate	Inoculum density	Interaction
SED	2.48	1.90	3.95*
DF	8	61	-

*SED = 3.28 for values with same level of isolate

Figure 4.2 ED50 values and their fiducial limits for three P. exigua var. foveata isolates (mean values for three cultivars)

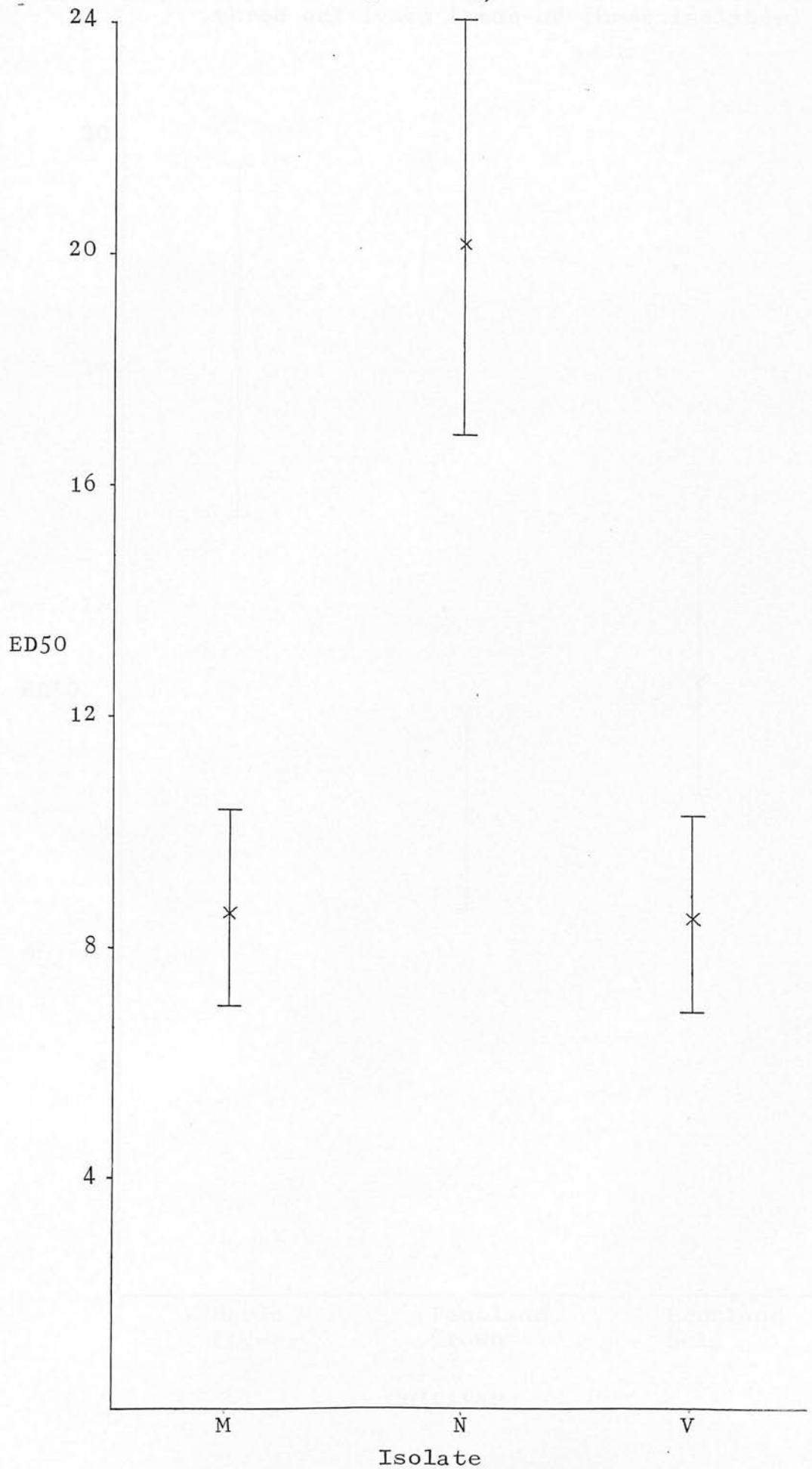
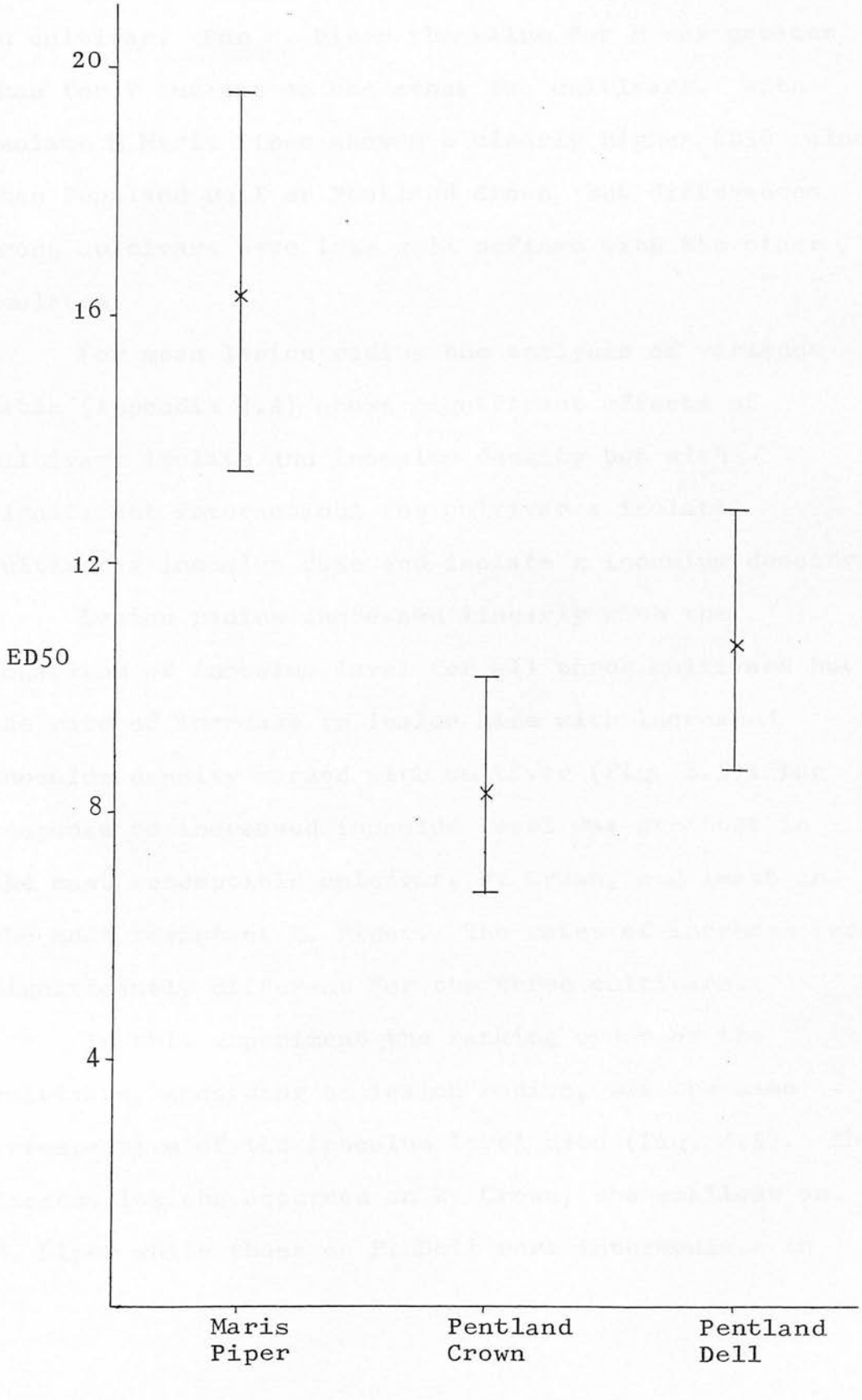


Figure 4.3 ED50 values and their fiducial limits for three cultivars (mean of three isolates)



had the largest ED50 value on all three cultivars (Fig. 4.4). The relative ED50 levels for M and V depended on cultivar. For M. Piper the value for M was greater than for V but not on the other two cultivars. With isolate M Maris Piper showed a clearly higher ED50 value than Pentland Dell or Pentland Crown, but differences among cultivars were less well defined with the other isolates.

For mean lesion radius the analysis of variance table (Appendix 4.4) shows significant effects of cultivar, isolate and inoculum density but with significant interactions for cultivar x isolate, cultivar x inoculum dose and isolate x inoculum density.

Lesion radius increased linearly with the logarithm of inoculum level for all three cultivars but the rate of increase in lesion size with increased inoculum density varied with cultivar (Fig. 4.5): the response to increased inoculum level was greatest in the most susceptible cultivar, P. Crown, and least in the most resistant M. Piper. The rates of increase were significantly different for the three cultivars.

In this experiment the ranking order of the cultivars, according to lesion radius, was the same irrespective of the inoculum level used (Fig. 4.5). The largest lesions occurred on P. Crown, the smallest on M. Piper while those on P. Dell were intermediate in

Figure 4.4 The influence of cultivar on the ED50 values of three P. exigua var. foveata isolates

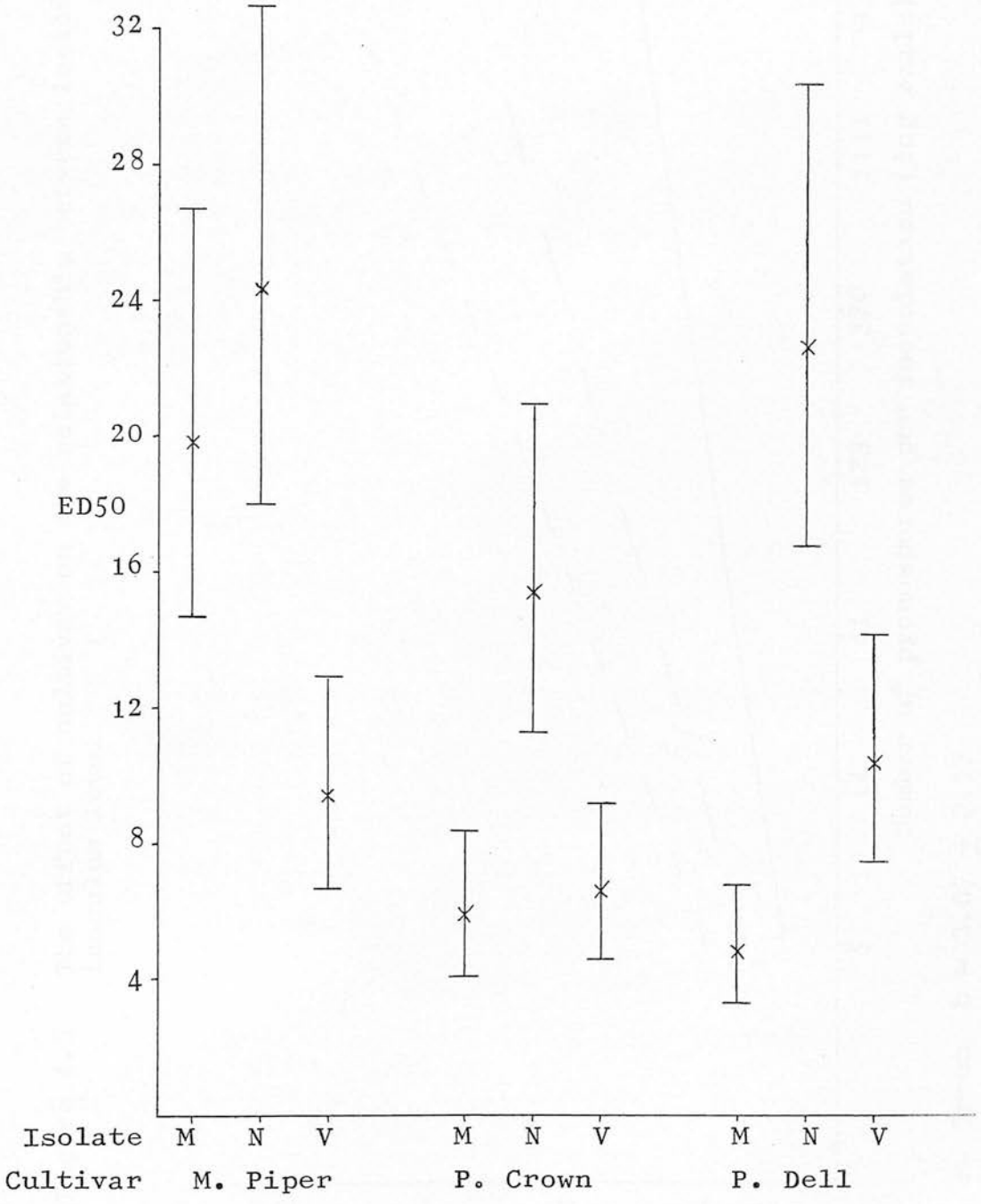
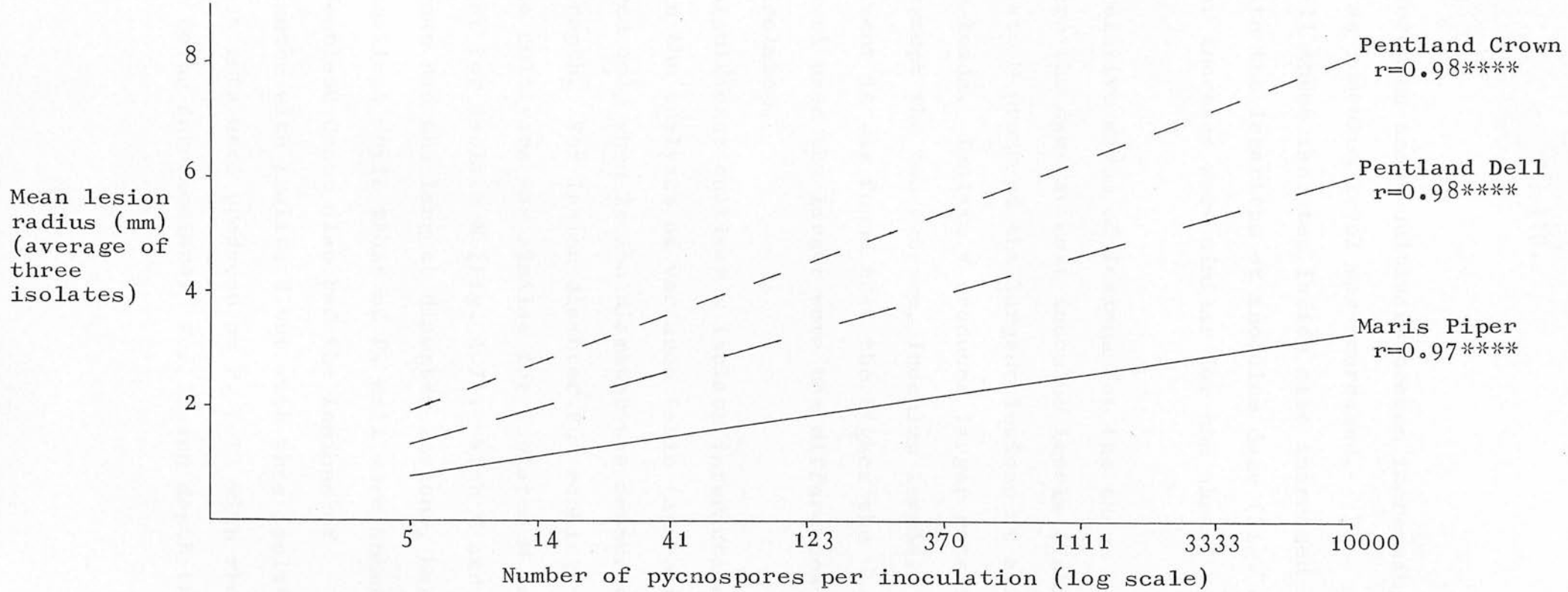


Figure 4.5 The effect of cultivar on the relationship between lesion size and inoculum level



P. Crown b = 2.07 ± 0.17
P. Dell b = 1.59 ± 0.14
M. Piper b = 0.77 ± 0.07

**** (P<0.002)

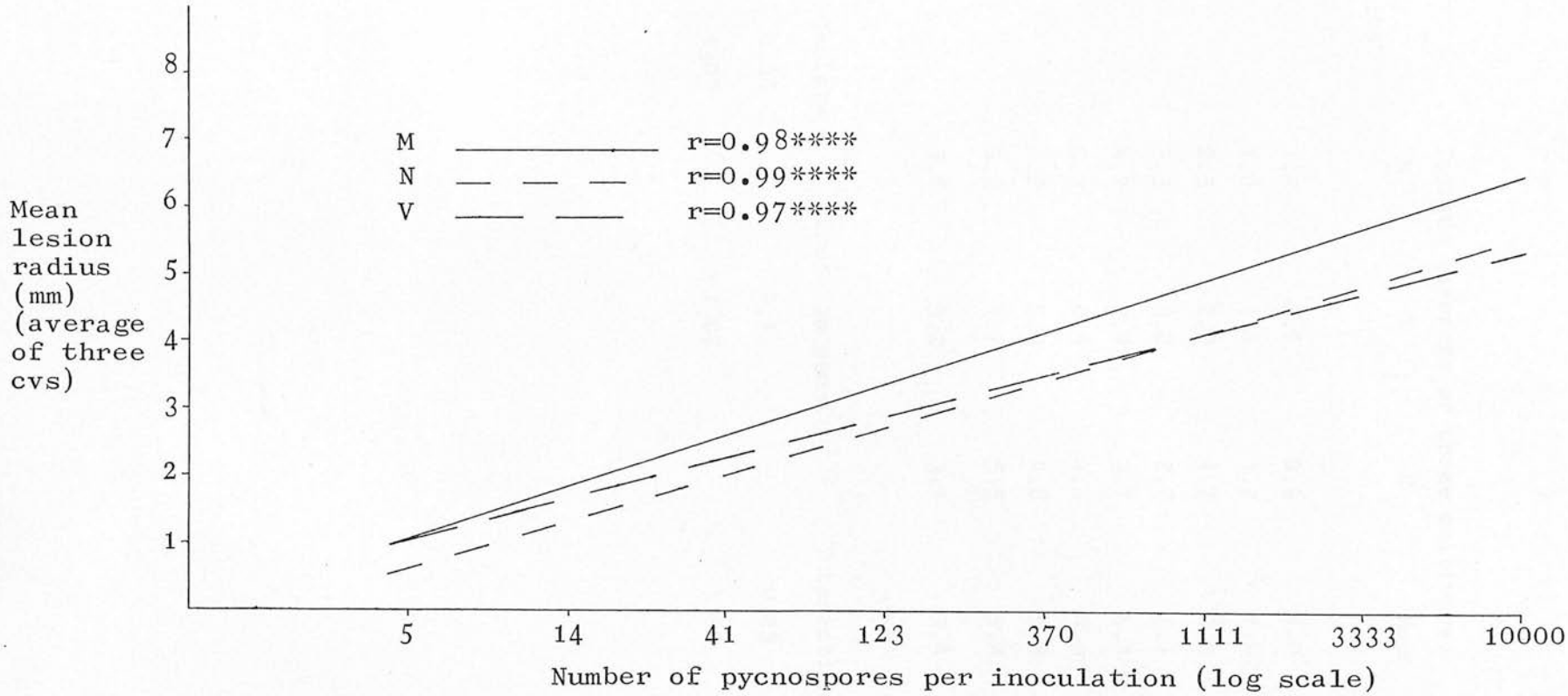
size. Differences among cultivars became increasingly pronounced as inoculum level was increased.

For all three isolates lesion size increased linearly with the logarithm of inoculum dose (Fig. 4.6). The rates of increase were similar for the three isolates.

The relative sizes of lesions for the three isolates were the same at most inoculum levels (Table 4.5). Isolate M produced the largest lesions at all eight spore loads. Isolate V produced larger rots than N at all, except the two extreme, inoculum levels. In this experiment it was found that the higher the inoculum level used the larger were the differences among the isolates.

The significant cultivar x isolate interaction indicated in the analysis of variance table (Appendix 4.4) occurred only when lesion diameter was measured not lesion depth. For lesion diameter the ranking order of the three cultivars was similar for isolates M and V but different for isolate N (Fig. 4.7). With M and V Pentland Crown had the largest diameter lesions, Maris Piper the smallest while those of P. Dell were intermediate. Pentland Crown also had the lesions of largest diameter with isolate N but with this isolate the narrowest rots were produced on P. Dell with those of M. Piper being intermediate. For lesion depth the

Figure 4.6 The influence of isolate on the relationship between lesion size and inoculum level



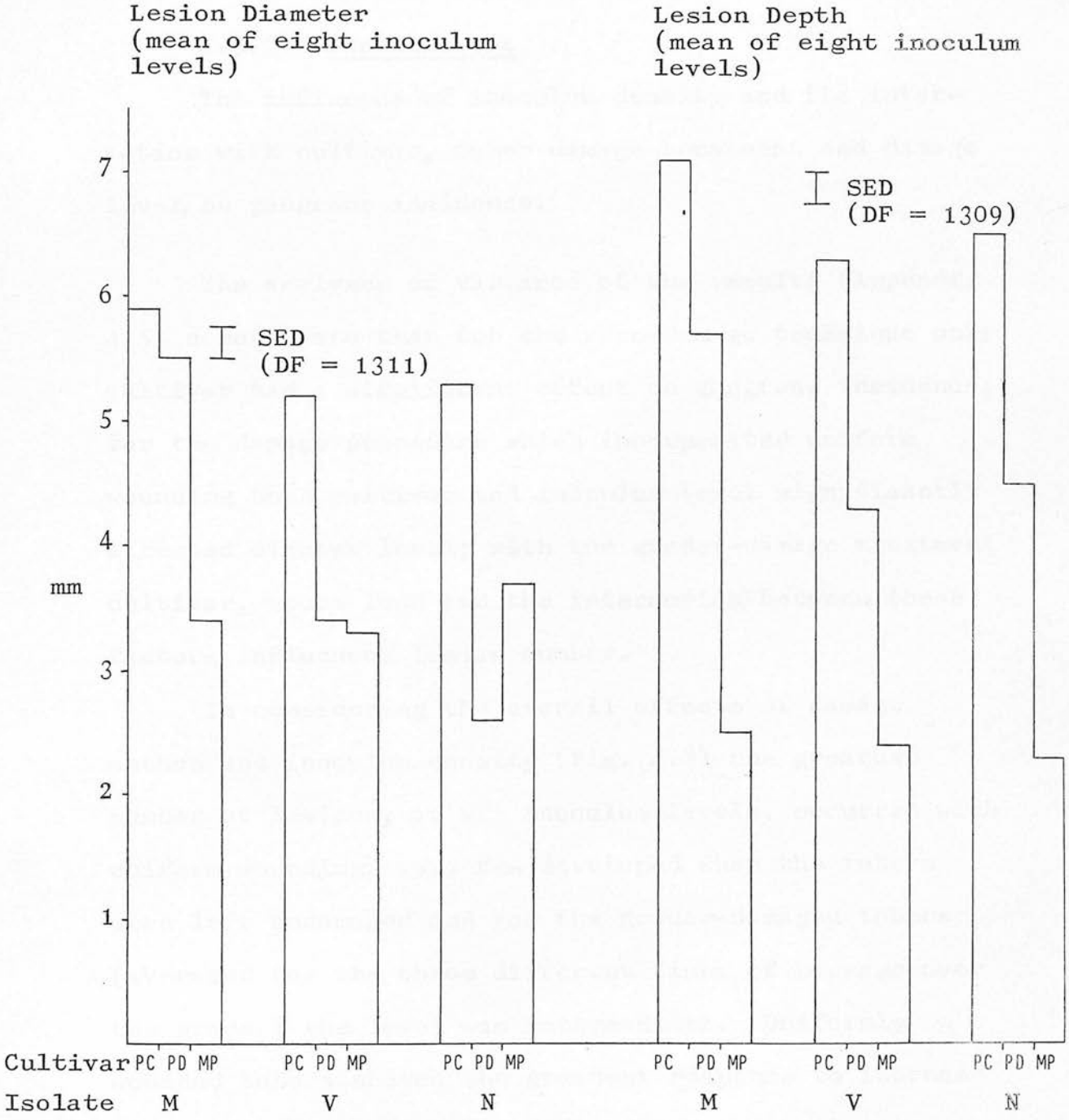
M b = 1.63 ± 0.13
N b = 1.48 ± 0.07
V b = 1.32 ± 0.14 **** (P<0.002)

Table 4.5 The effect of inoculum level and isolate of P. exigua var. foveata on gangrene lesion radius (mm)

Pycnospores per inoculation	Isolate (average of three cultivars) M	V	N	Mean
5	1.2	0.9	0.9	1.0
14	1.6	1.4	1.2	1.4
41	2.3	2.0	1.7	2.0
123	3.3	3.2	2.7	3.1
370	4.7	3.9	3.7	4.1
1111	5.0	4.5	4.4	4.6
3333	6.2	5.0	4.8	5.3
10000	5.9	4.7	5.5	5.4
Mean	3.8	3.2	3.1	3.4

Factor	Isolate	Inoculum density	Interaction
SED	0.08	0.13	0.23
DF	1309	1309	1309

Figure 4.7 The effect of field isolate of P. exigua var. foveata on the ranking order of cultivars for gangrene lesion size



Cultivar key

PC Pentland Crown
PD Pentland Dell
MP Maris Piper

same ranking order resulted with all three isolates. Pentland Crown had the deepest lesions, then P. Dell followed by M. Piper.

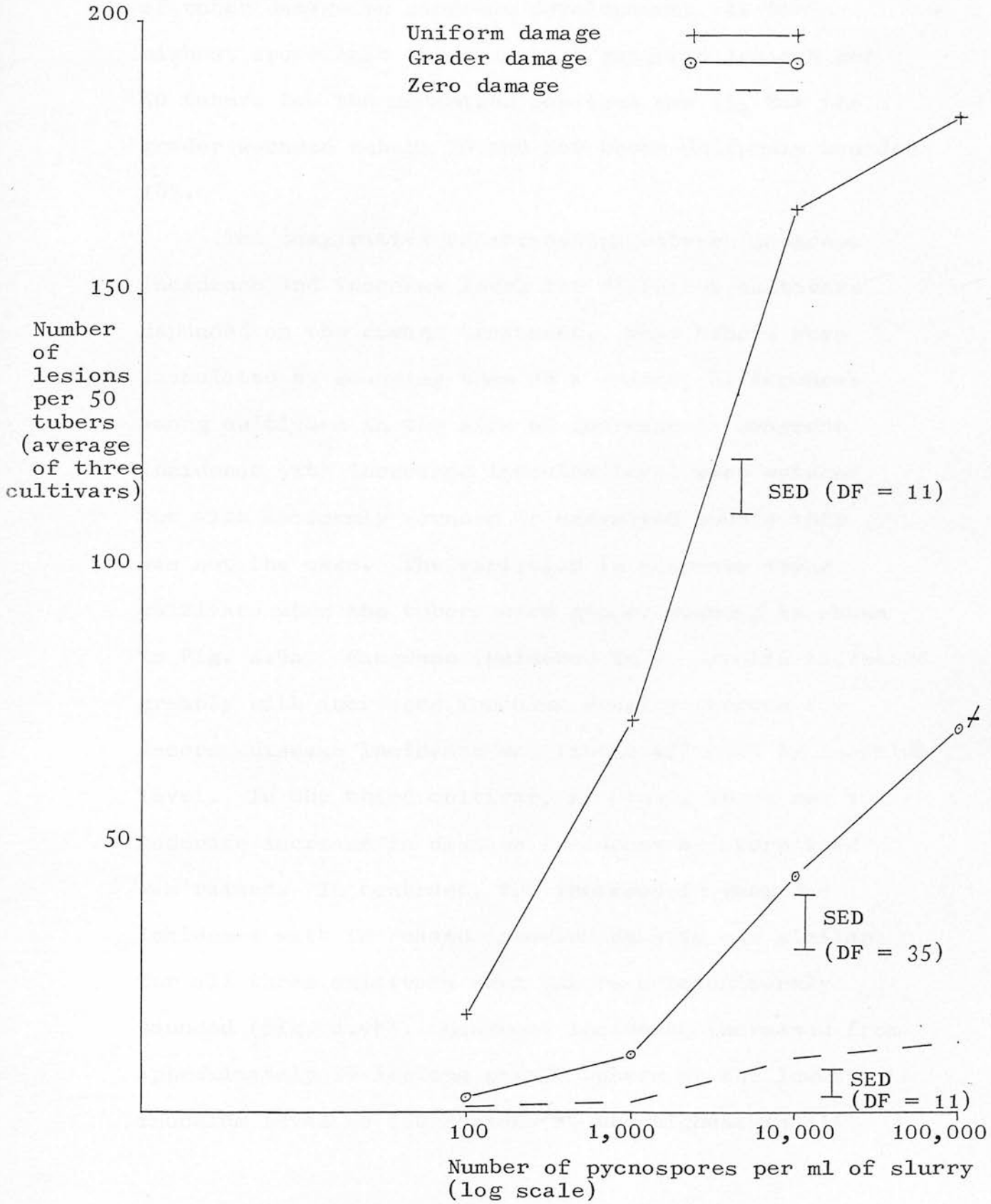
4.3.3 Experiment 4

The influence of inoculum density, and its interaction with cultivar, tuber damage treatment and damage level, on gangrene incidence.

The analyses of variance of the results (Appendix 4.5) demonstrate that for the zero-damage technique only cultivar had a significant effect on gangrene incidence; for the damage procedure which incorporated uniform wounding both cultivar and inoculum level significantly affected disease level; with the grader-damage treatment cultivar, spore load and the interaction between these factors influenced lesion number.

In considering the overall effects of damage method and inoculum density (Fig. 4.8) the greatest number of lesions, at all inoculum levels, occurred with uniform wounding, very few developed when the tubers were left undamaged and for the grader-damaged tubers (averaged for the three different times of passage over the grader) the level was intermediate. Uniformly wounded tubers showed the greatest response to increase in inoculum density and undamaged tubers the least.

Figure 4.8 The effect of damage method on the relationship between gangrene incidence and inoculum level

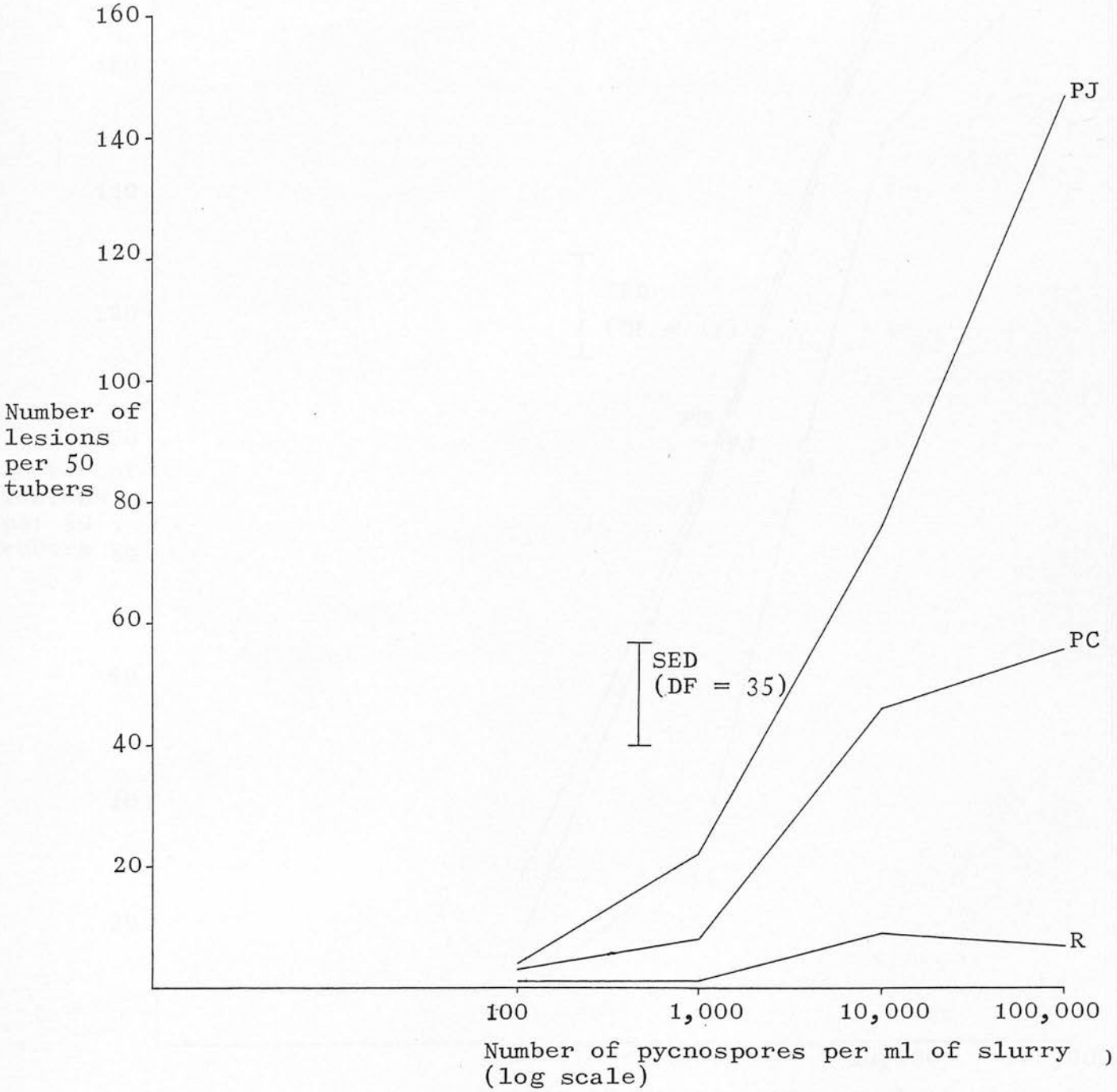


/ mean values for 1, 2 and 3 passes of tubers over grader.

The results in Fig. 4.8 confirmed the importance of tuber damage in gangrene development. At the highest spore load the number of gangrene lesions per 50 tubers for the undamaged potatoes was 13, for the grader wounded tubers 70 and for those uniformly wounded 183.

The comparative relationships between gangrene incidence and inoculum level for different cultivars depended on the damage treatment. When tubers were inoculated by wounding them on a grader, differences among cultivars in the size of increase in gangrene incidence with increased inoculum level were evident but with uniformly wounded or undamaged tubers this was not the case. The variation in response among cultivars when the tubers were grader wounded is shown in Fig. 4.9a. Gangrene incidence in P. Javelin increased greatly with increased inoculum density whereas for Record disease incidence was little affected by inoculum level. In the third cultivar, P. Crown, there was a moderate increase in disease incidence as spore load was raised. In contrast, the increase in gangrene incidence with increased inoculum density was similar for all three cultivars when tubers were uniformly wounded (Fig. 4.9b). Gangrene incidence increased from approximately 20 lesions per 50 tubers at the lowest inoculum level to 180 lesions at the highest in all

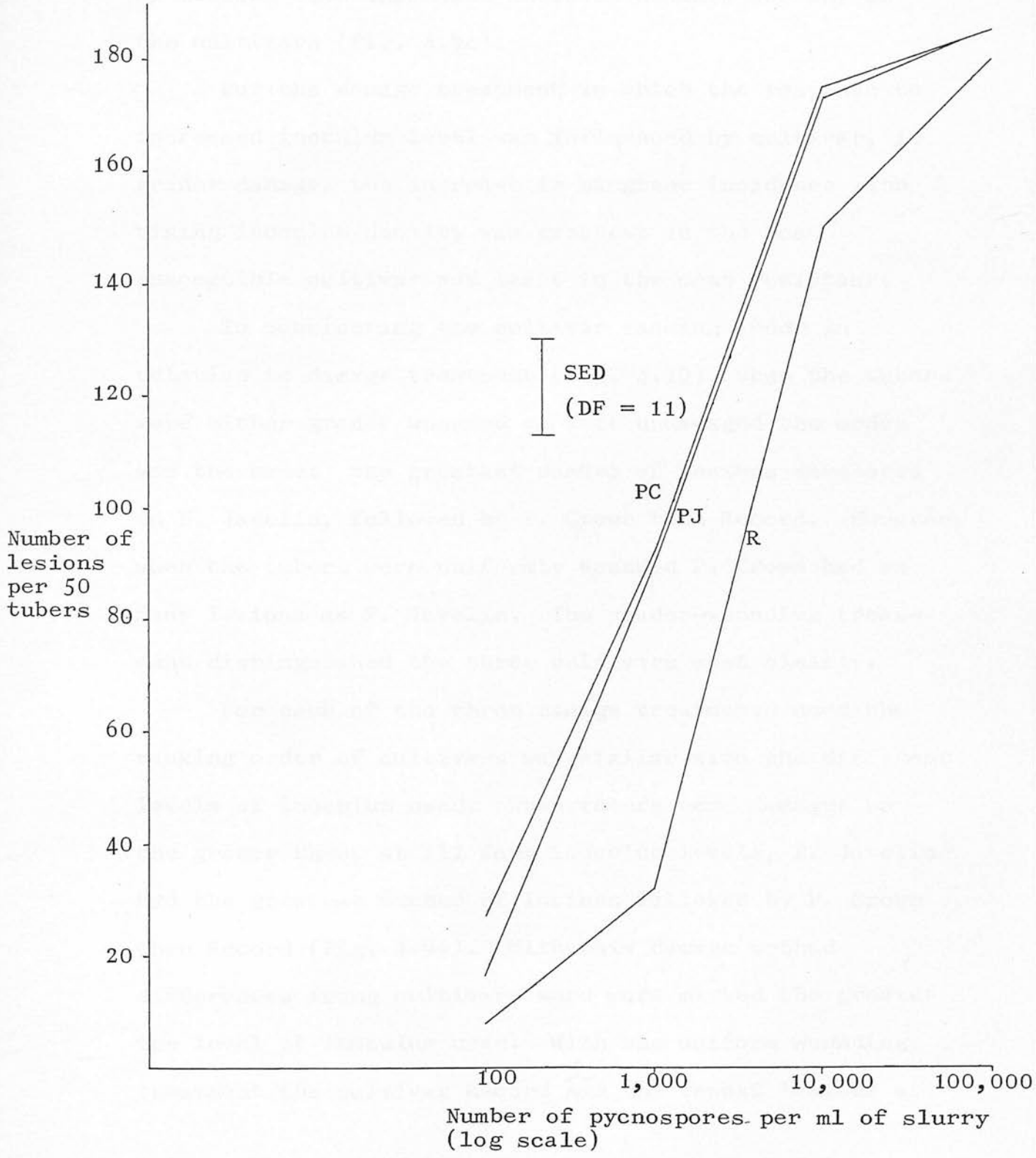
Figure 4.9a The relationship between gangrene incidence and inoculum level for different cultivars: grader damage



Cultivar key

PJ Pentland Javelin
PC Pentland Crown
R Record

Figure 4.9b The relationship between gangrene incidence and inoculum level for different cultivars: uniform damage



three cultivars. When tubers were inoculated without wounding there was no substantial increase in the number of lesions with increased inoculum density for any of the cultivars (Fig. 4.9c).

For the damage treatment in which the response to increased inoculum level was influenced by cultivar, ie grader damage, the increase in gangrene incidence with rising inoculum density was greatest in the most susceptible cultivar and least in the most resistant.

In considering the cultivar ranking order in relation to damage treatment (Fig. 4.10), when the tubers were either grader wounded or left undamaged the order was the same: the greatest number of lesions developed on P. Javelin, followed by P. Crown then Record. However, when the tubers were uniformly wounded P. Crown had as many lesions as P. Javelin. The grader-wounding treatment distinguished the three cultivars most clearly.

For each of the three damage treatments used the ranking order of cultivars was similar with the different levels of inoculum used. When tubers were damaged on the grader then, at all four inoculum levels, P. Javelin had the greatest number of lesions followed by P. Crown then Record (Fig. 4.9a). With this damage method differences among cultivars were more marked the greater the level of inoculum used. With the uniform wounding treatment the cultivar Record had the fewest lesions at

Figure 4.9c The relationship between gangrene incidence and inoculum level for different cultivars: zero damage

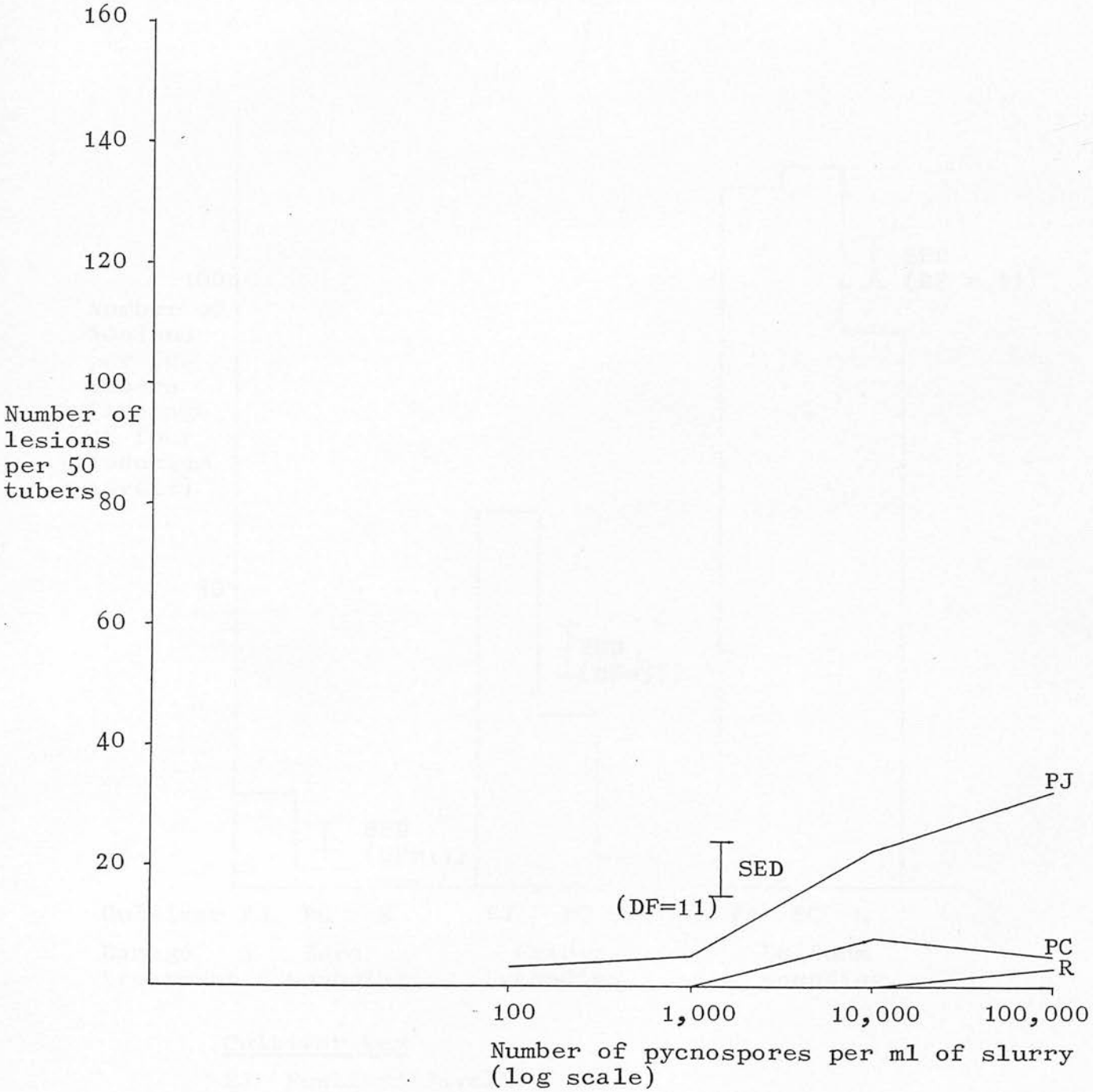
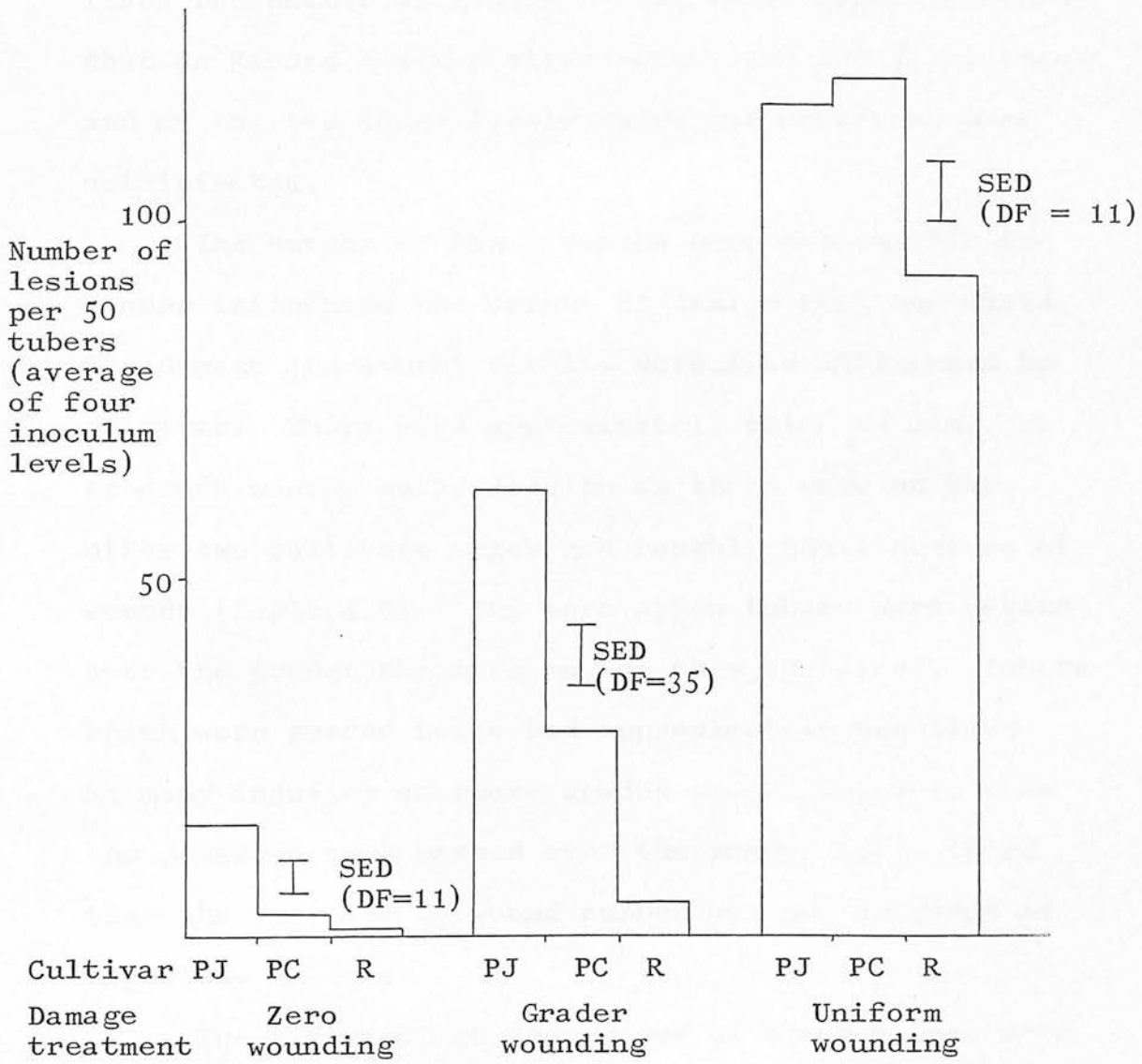


Figure 4.10 The effect of damage treatment on the ranking order of cultivars



Cultivar key

PJ Pentland Javelin
PC Pentland Crown
R Record

all four spore loads but the difference was only significant at the 1000 pycnospore level and no differences were found between Pentland Crown and Pentland Javelin (Fig. 4.9b). For undamaged tubers at all four inoculum levels P. Javelin had the largest number of lesions (Fig. 4.9c). At the two higher spore loads the number of gangrene rots on P. Crown exceeded that on Record but the differences were not significant and at the two lower levels these two cultivars were not infected.

The number of times tubers were passed over the grader influenced the extent of damage they sustained. The damage assessment results were also influenced by cultivar. There were approximately twice as many cut or crush wounds on P. Javelin as there were on the other two cultivars which had roughly equal numbers of wounds (Table 4.6). The more often tubers were passed over the grader the more wounds they sustained. Tubers which were graded twice had approximately two times as many injuries as those graded once. However, when the potatoes were passed over the grader for a third time the increase in wound number was not as great as expected.

The influence of the number of times tubers were passed over the grader on the relationship between gangrene incidence and inoculum level depended on

Table 4.6 The effect of cultivar, and the number of passes of tuber samples over the grader, on the number of cut or crush wounds per 50 tubers

Cultivar			
P. Javelin	P. Crown	Record	SED
34.5	18.3	18.7	5.2 (DF = 8)

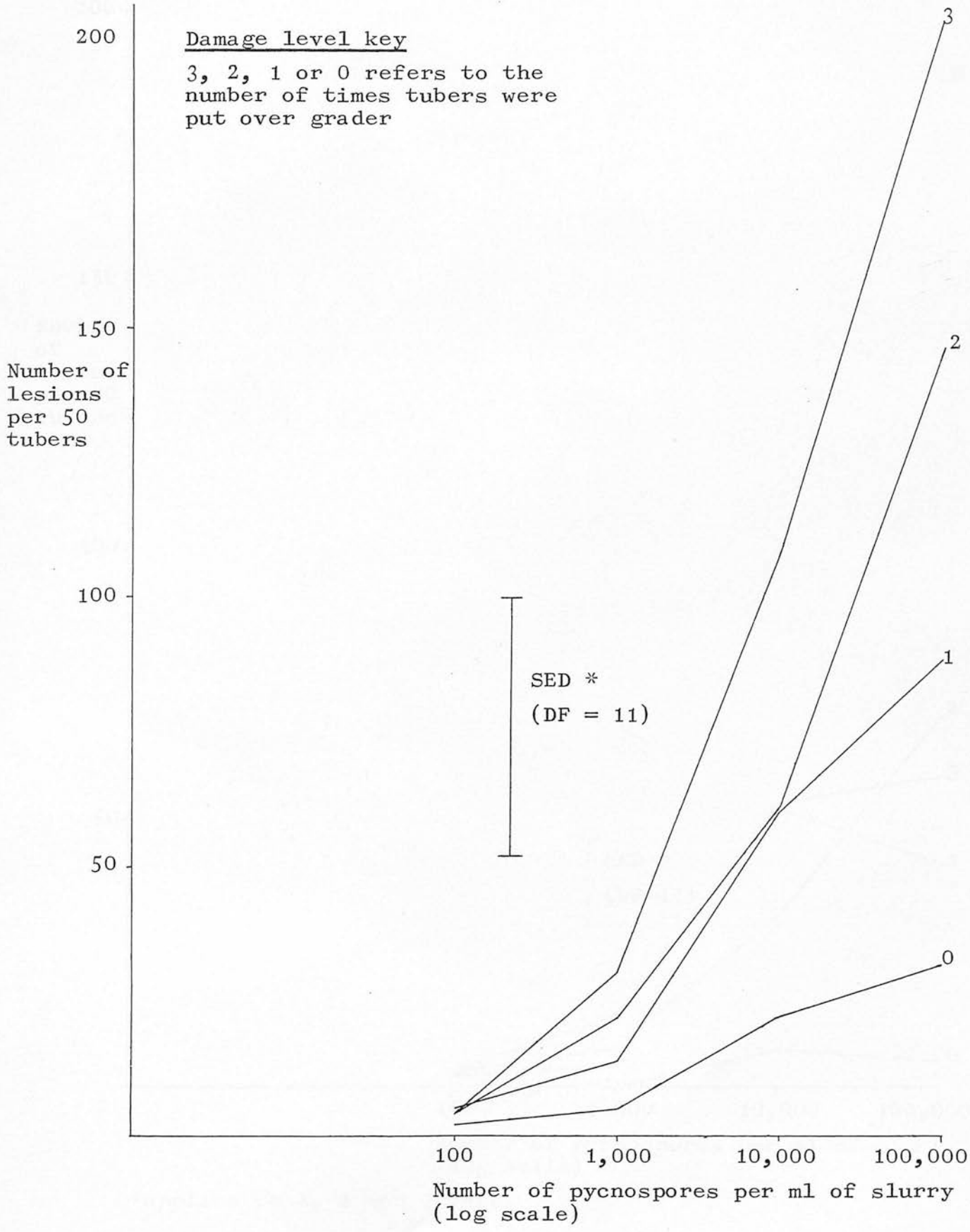
Number of passes over grader			
1	2	3	SED
14.3	25.7	31.5	5.2 (DF = 8)

cultivar. With P. Javelin the more often tubers were graded the larger was the increase in lesion number with increasing spore load (Fig. 4.11a): however, the differences between one and three passes were not significant. A similar result was obtained with P. Crown but the effect of grading frequency on the response to increased inoculum density was less marked (Fig. 4.11b). With Record the increase in disease incidence with increased spore load was not substantially greater for tubers graded three times than with ungraded tubers (Fig. 4.11c).

4.4 DISCUSSION

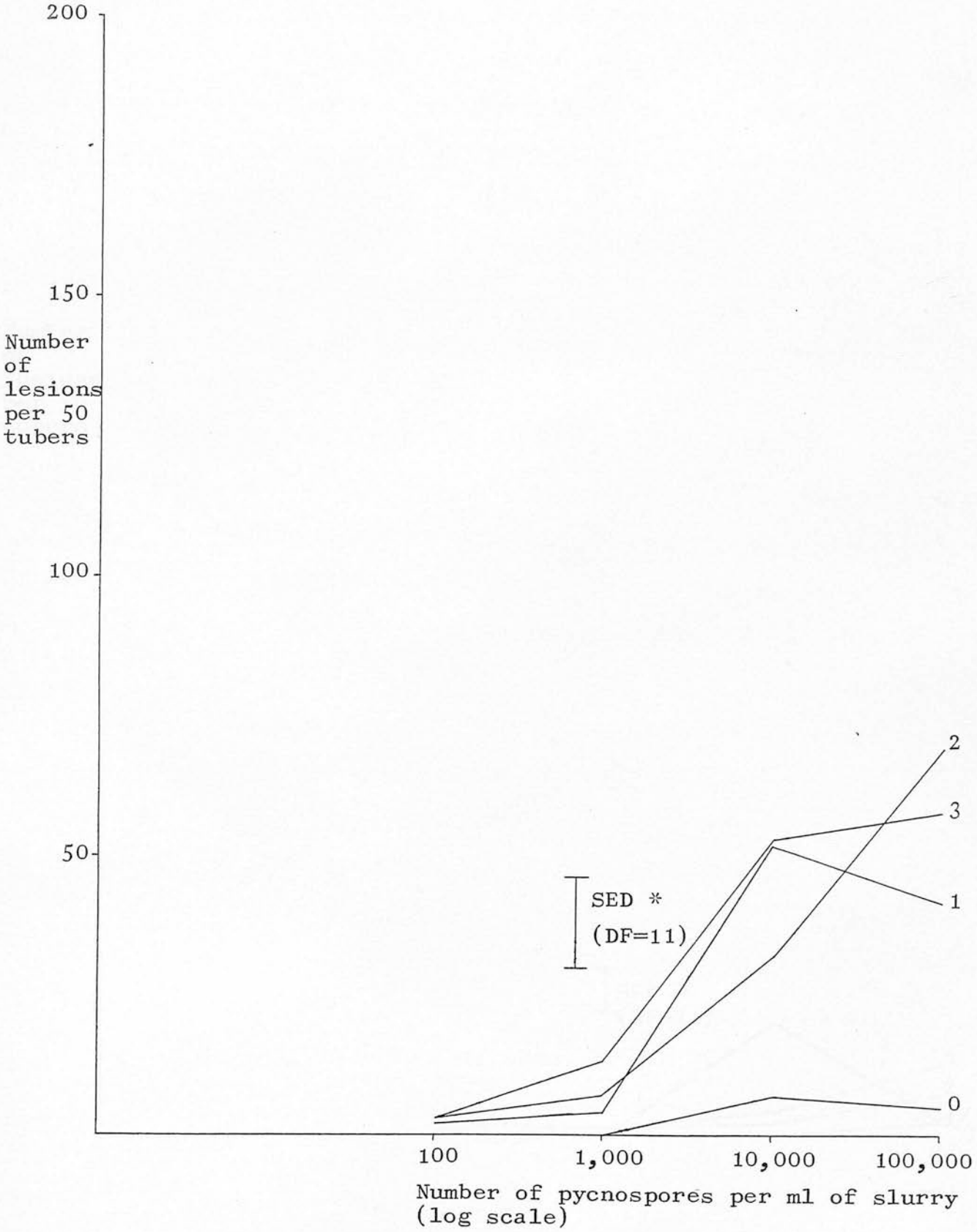
The results of this section confirm that, in general, the higher the level of inoculum on tubers the greater the development of gangrene in storage given suitable conditions for its development. However, the response to increased inoculum density was shown to be markedly affected by cultivar, the damage treatment and also by tuber damage level. The influence of cultivar on the relationship between disease incidence and inoculum level depended on the damage treatment and was considerably greater with the grader damage treatment than with the other damage procedures. This was probably because the grader treatment took into account tuber resistance to damage as well as to infection but the

Figure 4.11a The relationship between gangrene incidence and inoculum level for Pentland Javelin with different damage levels



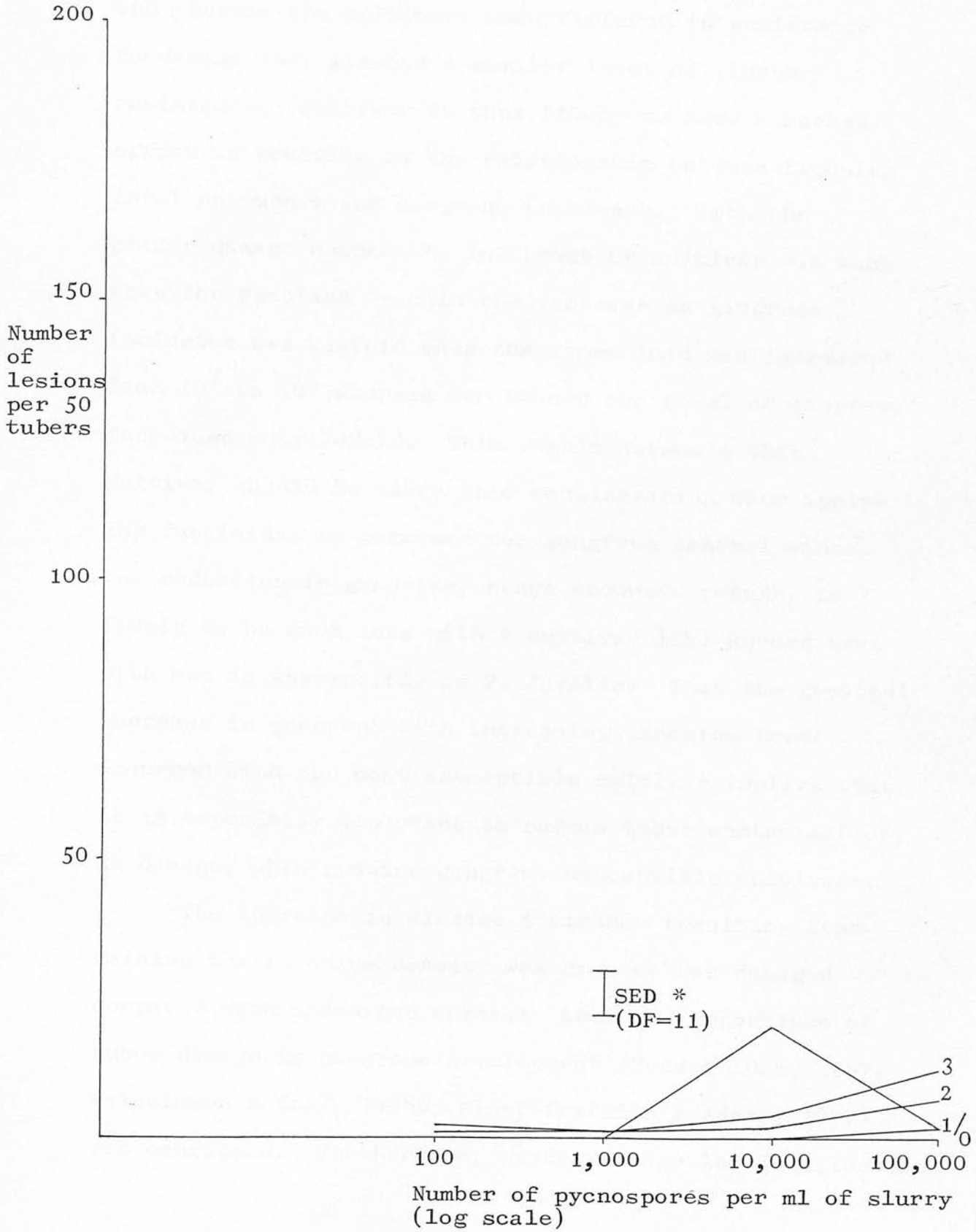
*applies to 1, 2 and 3 only.

Figure 4.11b The relationship between gangrene incidence and inoculum level for Pentland Crown with different damage levels



*applies to 1, 2 and 3 only.

Figure 4.11c The relationship between gangrene incidence and inoculum level for Record with different damage levels



*applies to 1, 2 and 3 only.

other methods only took tissue resistance into account and whereas the cultivars used differed in resistance to damage they all had a similar level of tissue resistance. Cultivar is thus likely to have a marked effect in practice on the relationship between inoculum level on tubers and gangrene incidence. With the grader damage method the influence of cultivar was such that for Pentland Javelin the increase in gangrene incidence was 25-fold when the spore load was increased from 10^2 to 10^5 whereas for Record the level of gangrene increased only 7-fold. This result suggests that cultivar should be taken into consideration when applying fungicides to potatoes for gangrene control since the reduction in gangrene, hence economic return, is likely to be much less with a cultivar like Record than with one as susceptible as P. Javelin. That the greatest increase in gangrene with increasing inoculum level occurred with the most susceptible cultivar implies that it is especially important to reduce tuber contamination, or damage, when growing gangrene susceptible cultivars.

The increase in disease incidence resulting from raising the inoculum density was greater for damaged compared with undamaged tubers: thus the importance of tuber damage in gangrene development (Todd & Adam, 1967; Malcolmson & Gray, 1968b; Hide, Griffith & Adams, 1977) was confirmed. Furthermore, grader damage level influenced

the response to increased spore load. The impact of damage level on gangrene incidence was greater the higher the tuber inoculum density. Also, the higher the incidence of wounding the greater the influence of inoculum level on gangrene development. These effects were most pronounced on the most susceptible cultivar, Pentland Javelin and least for the most resistant, Record. Adams (1980a) suggested that priority should be given to controlling gangrene by reducing the incidence of tuber damage, through the breeding of damage-resistant cultivars and better husbandry, rather than by reducing the level of daughter tuber contamination. However, it is unlikely that gangrene could be effectively controlled by minimising tuber damage alone since damage is unlikely to be completely eliminated either through breeding of resistant cultivars or by better production techniques. The results of this study suggest that gangrene would be most effectively controlled by lowering both tuber contamination level and damage incidence.

The number of pycnospores of P. exigua var. foveata required to cause 50% infection (ED50) depended on cultivar: the values ranged from 8 for the susceptible Pentland Crown to 16 for the more resistant Maris Piper. The ED50 values also varied among field isolates of the pathogen. The variation in ED50 values for the three isolates is likely to be an underestimate of the variation

in the P. exigua var. foveata population since the three isolates used turned out to have similar pathogenicity levels.

Wellving's finding that the method used to inoculate tubers with P. exigua var. foveata affected the cultivar resistance ranking order (Wellving, 1976) was supported by the results of this section. The best inoculation method is the one which accurately predicts the relative resistances of cultivars in practice. In this study the most appropriate inoculation method in screening for gangrene resistance was that which consisted of damaging artificially-contaminated tubers on a commercial grader since it incorporated an assessment of cultivar resistance to tuber damage in addition to assessing the resistance of tuber tissue to infection. The resistance of tubers to damage is a very important component of overall resistance to gangrene since P. exigua var. foveata is primarily a wound pathogen (Todd & Adam, 1967; Walker & Wade, 1976). Greater differences in resistance were evident among cultivars when their damage resistance was assessed in the inoculation method. Wellving (1976) and Jellis (1982) also found that when the inoculation procedure assessed damage resistance cultivars were more easily discriminated. One drawback with the grader damage inoculation method is that a large number of tubers per cultivar is required. Therefore, this inoculation method

could only be used late on in the breeding programme when the number of clones to be screened has been reduced and more tubers of each clone are available. This means that, for the early screening of cultivars, methods which only assess tissue resistance to infection will have to be used (Langton, 1971b; Wiersema, 1977). The results of this study have confirmed that point inoculation methods can give misleading results. For example, when assessed using a point inoculation method the cultivar Record was frequently classed as a susceptible cultivar whereas due to this cultivar's good damage resistance it is likely to be fairly resistant to gangrene in practice.

The results of this section suggest that when cultivars are being screened for resistance to gangrene the cultivar ranking order is likely to be the same irrespective of the level of inoculum with which the tubers are inoculated. The extent to which cultivars can be discriminated will however be greatly influenced by the inoculum density. Which inoculum level distinguishes cultivars best in turn depends on the inoculation method used. When resistance is to be assessed in terms of lesion number then with inoculation techniques in which the number of possible gangrene lesions is not pre-determined, for example the grader-damage inoculation method, the higher the level of inoculum used the greater

the separation among cultivars. For point inoculation methods, in which possible lesion number is limited there will be an optimum inoculum level for discriminating the cultivars. When cultivar resistance is based on lesion size then with point inoculation methods the greater the inoculum level the greater the differences among cultivars.

CHAPTER 5

The influence of cultivar tuber tissue resistance on gangrene development

5.1 INTRODUCTION

When screening potato clones for resistance to gangrene it is desirable to understand which components of resistance are being assessed as this will have implications for disease control in practice. Point inoculation techniques favour those cultivars with cortical, or medullary, tissue which when exposed by mechanical injury is more resistant to infection or colonisation by P. exigua var. foveata. Reference has already been made to the three phases of tissue resistance suggested by Walker & Wade (1976). The phase which operates can be influenced by tuber incubation temperature. Walker & Wade demonstrated that at 10°C lesion development was arrested whereas at 6°C rot progress was merely retarded. These workers did not, however, indicate the extent to which the level of cultivar tissue resistance related to whether lesion retardation or arrest occurred. The purpose of this section was to investigate the relationship between level of tissue resistance in cultivars and lesion development characteristics. In one experiment the influence of inoculum level and incubation temperature on the expression of tissue resistance in different cultivars was considered since the two factors have been shown to greatly affect disease development. In a second experiment lesion development over time was compared in a susceptible and a more resistant cultivar.

5.2 MATERIALS AND METHODS

Experiment 1

Resistance expression was investigated in the extremely susceptible cultivar Pentland Falcon and the more resistant Stormont Enterprise, Maris Piper and Roslin Castle. Two temperatures and two spore loads were used. Each tuber was point inoculated with either 200 or 2000 pycnospores of field isolate M. Forty replicate tubers were incubated at 4°C and 40 at 10°C. In order to determine whether lesion arrest had occurred within a normal period of incubation for gangrene-inoculated tubers, lesion size at the end of this period was compared with lesion size after an incubation period twice as long. Twenty replicate tubers which had been incubated at 10°C were scored after 5 weeks and another 20 after 10 weeks. Half of the tubers incubated at 4°C were scored after 8.5 weeks and the others after 17 weeks. Scoring consisted of measuring the surface diameter and maximum depth of each lesion.

Experiment 2

The extremely susceptible cultivar Corrie and the moderately resistant Pentland Dell were used. Tubers were point inoculated with 2000 pycnospores of field isolate M. After 5 weeks at 4°C 20 tubers of each cultivar were scored for surface diameter and maximum

depth of lesion. Rot development was similarly assessed after 6, 7, 8, 9 and 10 weeks.

5.3 RESULTS

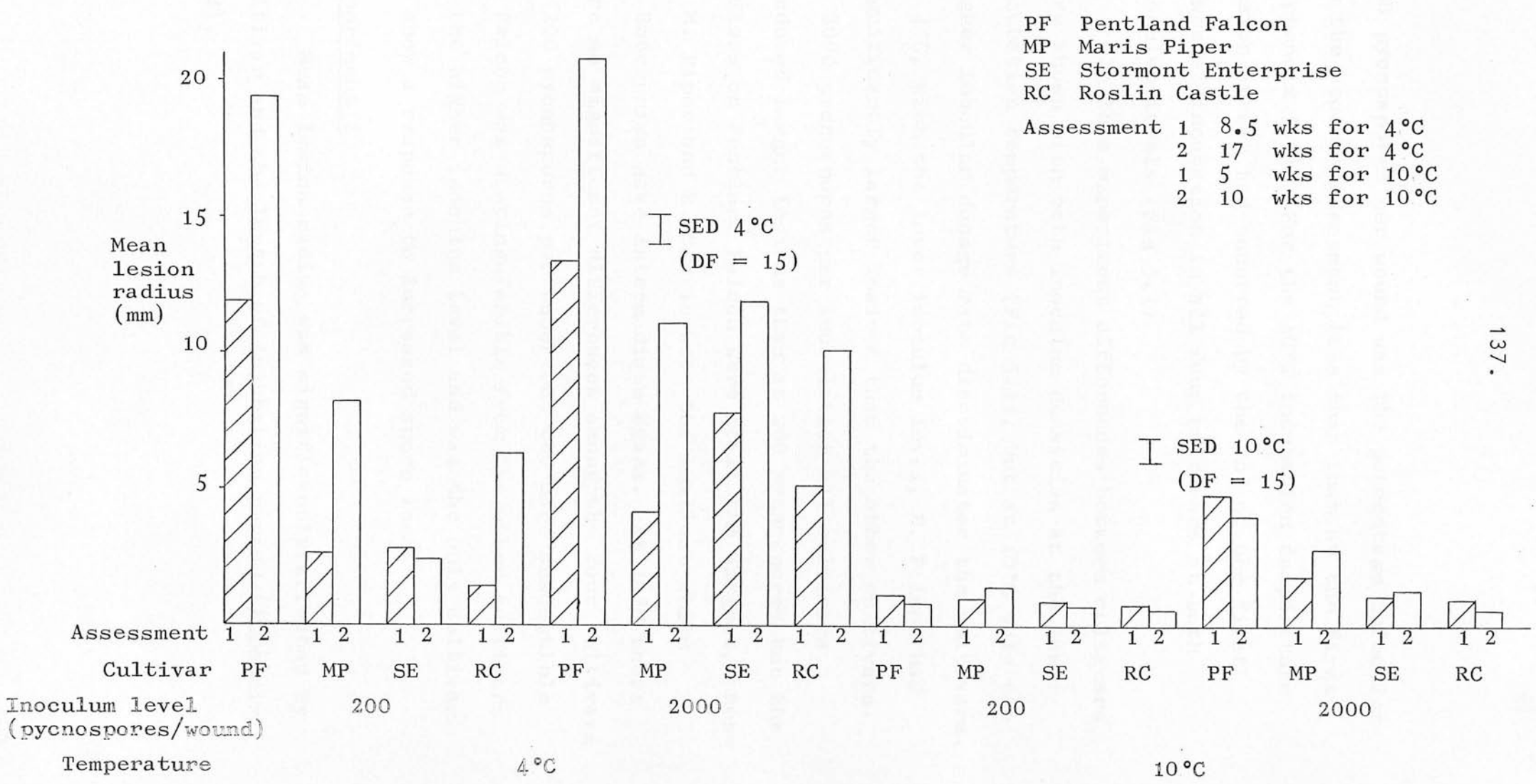
The influence of cultivar tuber tissue resistance on gangrene development.

Experiment 1

At the 4°C incubation temperature, lesion radius was significantly affected by the time of assessment, cultivar, inoculum level and the assessment time x cultivar and cultivar x inoculum density interactions (Appendix 5.1). At 10°C only cultivar and inoculum level and the interaction between these two factors had a significant effect on lesion radius (Appendix 5.2).

At 4°C, resistance expression was generally reflected in lesion retardation whereas at 10°C resistance expression involved lesion arrest (Fig 5.1). For the 4°C incubation temperature, with the high inoculum dose, no lesion arrest occurred in any of the four cultivars by the end of the normal incubation period. The same was true for Maris Piper, Roslin Castle and Pentland Falcon at the lower dose. Lesion arrest appears to have occurred in Stormont Enterprise at the lower inoculum level but there is evidence that the result was due to a low level of infection of the second assessment tubers: only for Stormont Enterprise inoculated with

Figure 5.1 Gangrene lesion development in resistant and susceptible cultivars at 4°C and 10°C in relation to inoculum density and assessment time



200 pycnospores per wound was the percentage infection at the second assessment less than that at the first (Appendix 5.3). For the 10°C incubation temperature lesion arrest had occurred by the end of the first 5 weeks' incubation in all four cultivars at both inoculum levels (Fig 5.1).

In this experiment differences between cultivars were shown with both inoculum densities at the lower incubation temperature (Fig 5.1), but at 10°C only the higher inoculum dosage rate discriminated the cultivars. At 4°C, with the lower inoculum level, P. Falcon had significantly larger lesions than the other cultivars. At 2000 pycnospores per inoculation all cultivars produced larger lesions than at 200 pycnospores but the lesions on Pentland Falcon were still the largest, those of M. Piper and R. Castle were the smallest while S. Enterprise gave intermediate sizes. At 10°C there were no significant differences among the four cultivars at 200 pycnospores per tuber but the more susceptible P. Falcon was distinguishable from the other cultivars at the higher inoculum level and was the only cultivar to show a response to increased spore load.

Experiment 2

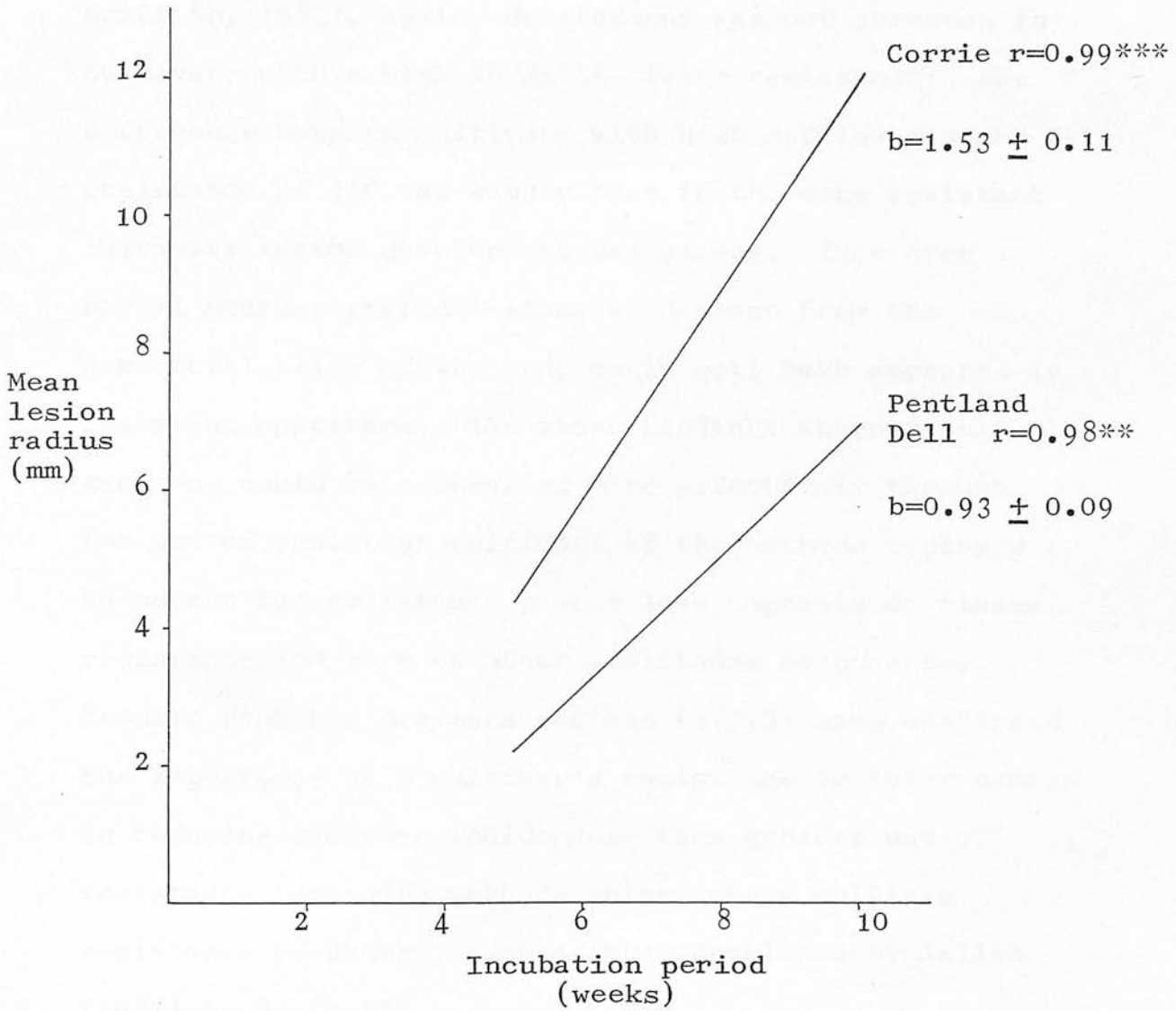
Mean lesion radius was significantly affected by cultivar and the length of incubation period (Appendix 5.4).

There was no lesion arrest in either cultivar within the 10 week incubation period. The increase in lesion radius with duration of incubation was linear for both cultivars (Fig 5.2), but the rate of lesion development was significantly greater in Corrie than in P. Dell.

5.4 DISCUSSION

Results obtained in this and previous sections indicate that selection for tuber tissue resistance may not necessarily be reflected in a large reduction in gangrene incidence in practice. It was found in an earlier experiment (Fig 4.1) that, over a wide range of inoculum densities, the level of tissue resistance in different cultivars had only a moderate effect on the incidence of gangrene at 4°C yet two of the cultivars used in the experiment, namely Pentland Crown and Maris Piper, are generally accepted to differ greatly in resistance: Pentland Crown is reported to be susceptible and M. Piper recognised to be much more resistant (Logan & Woodward, 1971; Anon., 1980; Anon., 1981). Furthermore, it was found in the present section that differences in tissue resistance to infection among cultivars may be transient: there were differences in infection frequency among the extremely susceptible Pentland Falcon and the highly resistant cultivars

Figure 5.2 Gangrene lesion development in relation to time in the susceptible cultivar Corrie and the more resistant Pentland Dell at 4°C



*** (P<0.01)

** (P<0.02)

Maris Piper (Jellis, 1982) and Roslin Castle (Gans, 1978) after 8.5 weeks at 4°C but such differences were much reduced by 17 weeks (Appendix 5.3).

At 4°C, the temperature at which gangrene development is widely accepted to be greatest (Malcolmson, 1958; Malcolmson & Gray, 1968b; Langton, 1972; Adams & Griffith, 1983), lesion development was not arrested in cultivars with a high level of tissue resistance: the difference between cultivars with high and low tissue resistance at 4°C was simply that in the more resistant cultivars lesion development was slower. Thus over a normal storage period lesions to detract from the commercial value of the crop could well have appeared in resistant cultivars. The above findings suggest that gangrene could be controlled more effectively through the use of resistant cultivars if the methods employed to screen for resistance placed less emphasis on tissue resistance and more on other resistance components. Results from the previous section (4.3.3) have confirmed the importance of a cultivar's resistance to tuber damage in reducing gangrene incidence: thus greater use of resistance screening methods which assess cultivar resistance to damage, such as that developed by Jellis (1982) is desirable.

Wellving (1976) reported a considerable variation among cultivars in tissue resistance to infection. He

found that, following point inoculation and incubation at 3°C, the level of infection in the most susceptible cultivar used was many times greater than that in the most resistant and exceeded the range found in the present study (Fig 4.1, Appendix 5.3). An explanation for the divergence in findings in the respective studies may be that, whereas in the relevant experiments in this thesis infection frequency was assessed independently of lesion size, Wellving's lesion number assessment may have been confounded with rot size. Wellving defined infection frequency as the number of actively growing lesions as a percentage of the total possible number of rots. It is likely that the decision as to whether or not a rot was active would be influenced by its size, and differences between cultivars might be exaggerated where tubers with slow growing lesions in a more resistant cultivar might not be included in the infection frequency score. It is therefore likely that the cultivar differences which Wellving attributed to differences in tissue resistance to infection were in fact at least partly due to differences in lesion development rate.

It could be argued that although cultivar tissue resistance level appears to have little practical influence on gangrene incidence at 4°C it may do at higher incubation temperatures. It was found however that at 10°C lesion arrest occurred in all cultivars

irrespective of their tissue resistance level. It is surprising therefore that an incubation temperature of 10°C has been used by breeders when screening clones for gangrene resistance (Jellis, 1975) and that cultivar differences are reported to be greater at 10°C than 5°C (Langton, 1971a). The results obtained by these authors probably arose because the pathogen was favoured by the inoculation techniques used: tubers were inoculated with actively growing mycelium whereas in this study pycnospore suspensions were used. Although Walker & Wade (1976) have shown that lesion development is initially greater at 10°C than 6°C or 2°C, nevertheless lesions incubated at 10°C would, from the present study's findings, never attain a large size.

CHAPTER 6

The effect of gamma irradiation treatment of tubers on gangrene development after wounding

6.1 INTRODUCTION

The treatment of potato tubers with gamma irradiation increases the susceptibility of their tissue to some tuber pathogens and is recognised to interfere with certain physiological processes in the tuber which have been proposed as possible tissue resistance mechanisms (1.2.4). The objective of this section was to investigate which processes may be involved in tissue resistance to gangrene by looking at the effect of gamma irradiation on susceptibility to infection by P. exigua var. foveata and considering this in relation to the known effects of irradiation on specific physiological processes.

Gamma irradiation can affect several of the possible resistance mechanisms of the tuber simultaneously. However, the response of these processes to increasing doses of irradiation differs. In experiments 1 and 2 the influence of irradiation dose on the susceptibility of tubers to P. exigua var. foveata was examined to allow an identification of those mechanisms with a similar dose-response relationship to susceptibility. Such processes are more likely to be involved in resistance than those with a different dose-response pattern.

Gamma irradiation has a temporary effect on some tuber physiological processes but a permanent effect on others. By determining whether the effect of irradiation on susceptibility is long or short term it should be possible to state whether or not particular physiological

processes are likely to be involved in tissue resistance. Three experiments to examine the longevity of the effect of irradiation on susceptibility were carried out. In experiment 3 the influence of irradiation on the development of resistance in ageing wounds was investigated. In another experiment the impact of delay between irradiation and wounding/inoculation of tubers on lesion development was examined (experiment 4). The rate of lesion development in irradiated tubers was assessed to determine if it was constant during incubation (experiment 5).

Experiment 6 was designed to confirm that the increased susceptibility of irradiated tubers was not simply due to irradiation-induced tuber cell death but to an impairment of host tissue resistance.

6.2 MATERIALS AND METHODS

Experiment 1

In this experiment the effect of gamma irradiation on the resistance of two gangrene susceptible cultivars, Pentland Javelin and Pentland Envoy, and two resistant cultivars, Maris Piper and Roslin Castle, was investigated. Twenty tubers of each cultivar were exposed to 0, 10, 25, 50, 100 or 200 krads of gamma irradiation. Each tuber was then point inoculated with 2000 pycnospores of culture collection isolate B. After incubation for 8 weeks the surface diameter and maximum depth of each lesion was measured.

Experiment 2

In this experiment two cultivars were used, Pentland Envoy and Maris Piper. Eighteen tubers of each cultivar were treated with 0, 10, 25, 50, 100 or 200 krads of irradiation. All tubers were then point inoculated with 2000 pycnospores of field isolate N. After incubation for 7 weeks the surface diameter and maximum depth of each lesion was measured.

Experiment 3

Catriona tubers were treated with either 0 or 100 krads of gamma irradiation. The tubers were then wounded by removing a 7 mm wide and 3 mm long core of tissue from midway between the rose and heel ends. Half of the irradiated tubers and half of the untreated tubers were allocated for storage at 4°C and 100% humidity. Twenty of these tubers from each irradiation treatment were inoculated with 2000 pycnospores of culture collection isolate B either at the time of wounding or 7, 14, 21, 28, 40 or 60 days later. The remaining tubers were assigned for storage at 15°C and 100% relative humidity and twenty from each irradiation treatment were inoculated as before either at wounding or 2, 5, 7, 9, 12 or 16 days later. After inoculation, tubers were incubated at 4°C and 100% relative humidity. Ten weeks after inoculation each tuber was scored for the presence or absence of a lesion as described earlier (4.2.2, Experiment 3).

Experiment 4

Pentland Javelin tubers were exposed to 0 or 50 krads of gamma irradiation. Twenty tubers of each treatment were wounded, and inoculated with 2000 pycnospores of field isolate M per wound, either 1, 5 or 12 days after the irradiation treatment. After 7 weeks' incubation the surface diameter and maximum depth of each tuber lesion was measured.

Experiment 5

Pentland Dell tubers, treated with either 0 or 100 krads of gamma irradiation, were point inoculated with 2000 pycnospores of field isolate M. Lesion development was assessed, using 20 tubers from each irradiation treatment, after 3.5, 4, 4.5, 5, 5.5 and 6 weeks' incubation by measuring the surface diameter and maximum depth of each lesion.

Experiment 6

Batches of 20 Roslin Castle tubers were exposed to 0, 10, 25, 50, 100, 200, 500, 1000 or 2000 krads of gamma irradiation. Sixteen tubers from each treatment were point inoculated with 2000 pycnospores of field isolate N per tuber in order to determine the effect of irradiation treatment on gangrene susceptibility. After 5 weeks' incubation the surface diameter and maximum depth of each lesion was measured. Two non-inoculated

tubers from each irradiation treatment were used to determine the relationship between level of irradiation and the amount of tuber cell death. From each tuber a core, 17 mm in diameter, was removed. From 3 mm below the core periderm two discs, 6 mm in diameter and 0.5 mm thick were taken. The discs were stained in a 0.1% solution of Evans Blue for five minutes then rinsed with tap water (Gahan, 1981).
The discs were examined microscopically and scored for the percentage of their area which contained dead cells.

6.3 RESULTS

The effect of gamma irradiation treatment of tubers on gangrene development after wounding.

Experiment 1

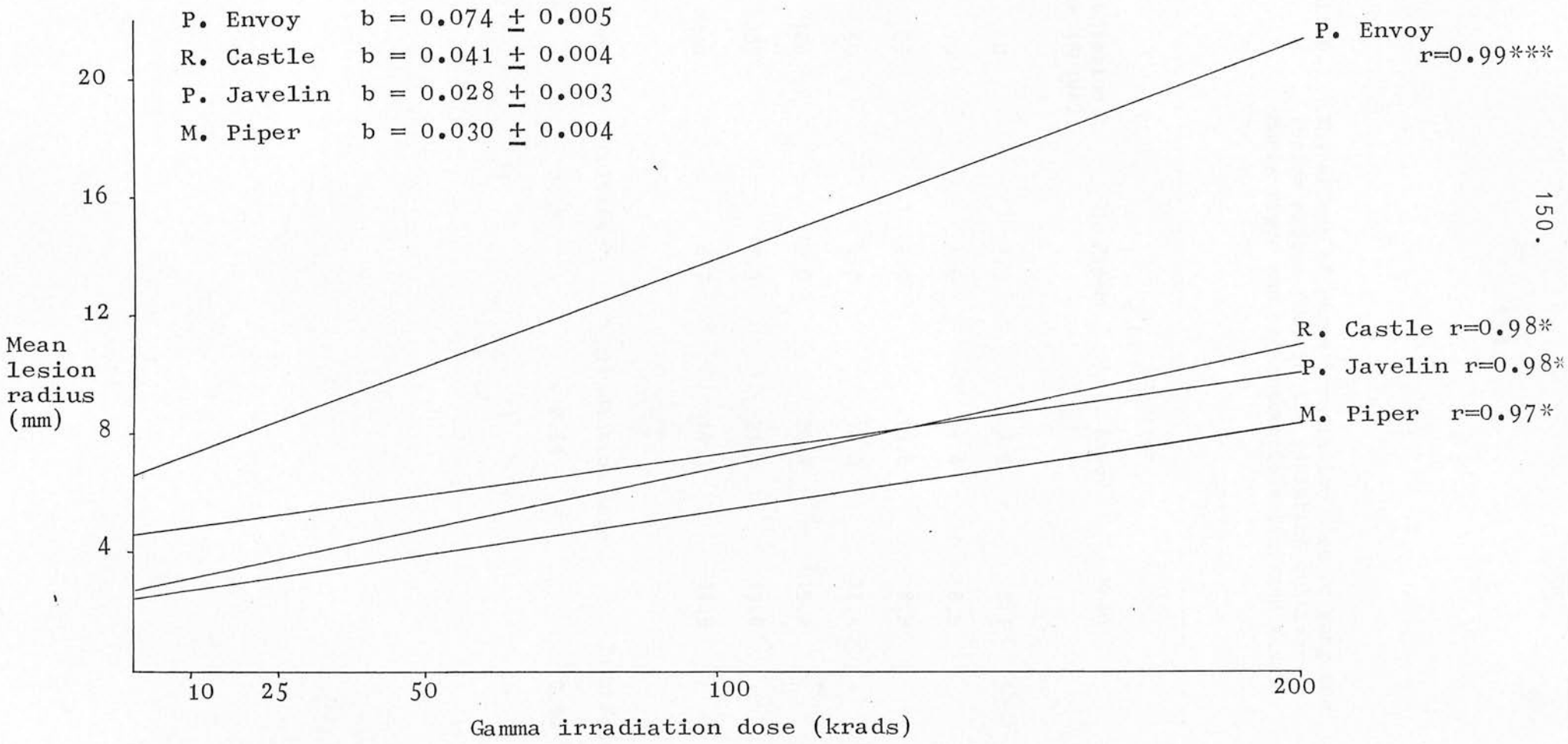
For all four cultivars lesion radius increased linearly with irradiation dose (Fig 6.1). The rate of increase was greatest in Pentland Envoy, lowest in Maris Piper and Pentland Javelin and intermediate for Roslin Castle.

Experiment 2

Cultivar, irradiation dose and the interaction between these two factors all significantly influenced mean lesion radius (Appendix 6.1).

For both cultivars lesion radius increased with increasing irradiation dose (Table 6.1). For Maris Piper

Figure 6.1 The effect of gamma irradiation dose on gangrene lesion size for four cultivars



* P<0.05 *** P<0.01

Table 6.1 The effect of gamma irradiation dose on gangrene lesion radius (mm) in the resistant cultivar Maris Piper and the susceptible Pentland Envoy

Irradiation dose (krads)	Cultivar M. Piper	P. Envoy	Mean
0	3.3	12.8	8.1
10	1.6	11.4	6.5
25	2.9	13.6	8.2
50	4.7	18.0	11.4
100	10.0	20.4	15.2
200	17.9	21.7	19.8
Mean	6.7	16.3	11.5

Factor	Cultivar	Irradiation dose	Interaction
SED	0.38	0.65	0.92
DF	11	11	11

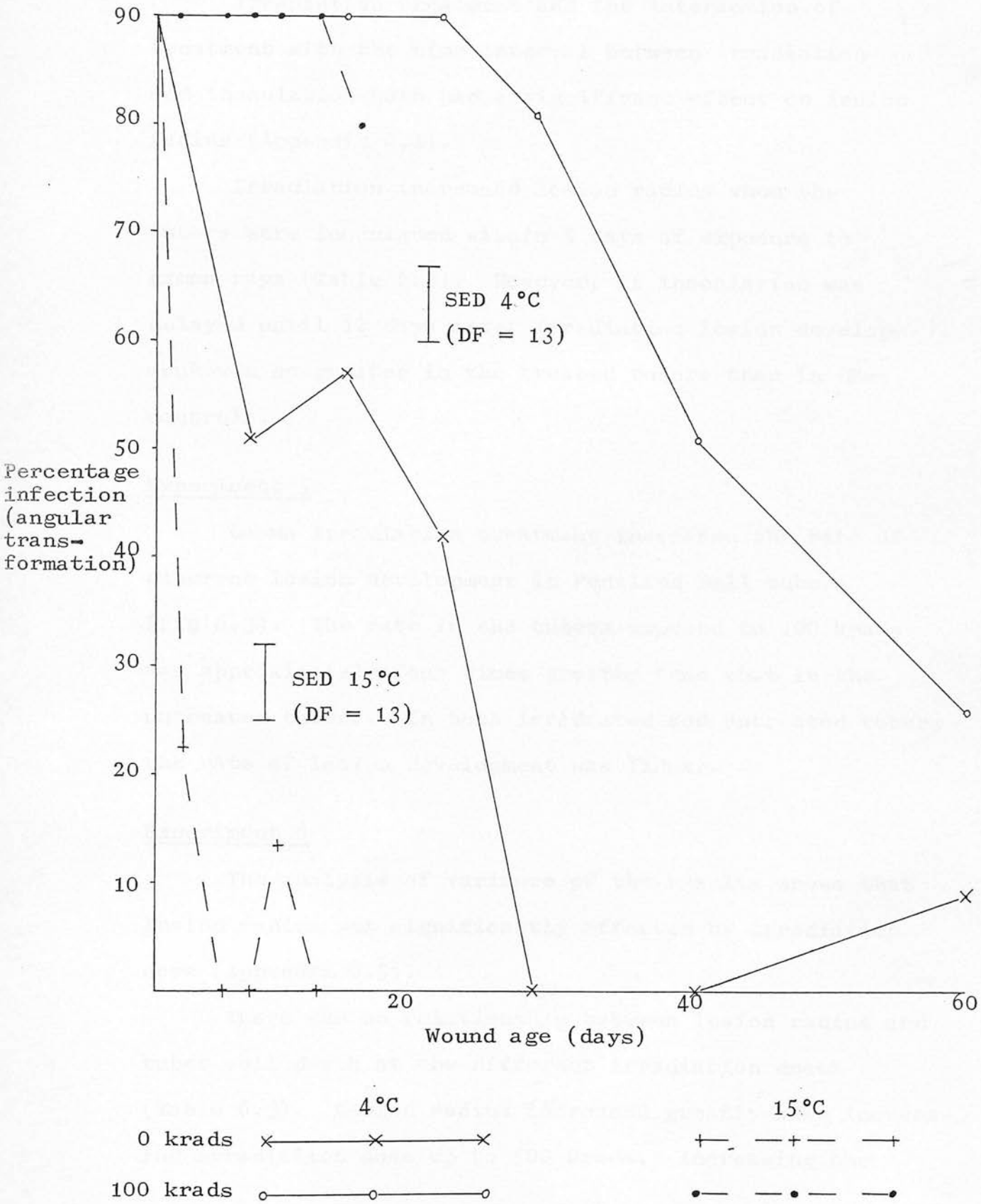
this relationship was linear but for Pentland Envoy the increase tended towards a plateau at the higher dose levels. At comparable doses P. Envoy always gave larger lesions than M. Piper but the comparative resistance of M. Piper was lost with increasing irradiation dose.

Experiment 3

From the analyses of variance of the results at 4°C (Appendix 6.2) and 15°C (Appendix 6.3) infection level was significantly influenced by irradiation and the time interval between the irradiation/wounding treatment of tubers and the inoculation of the wounds. There was also a significant irradiation x time interval interaction at both temperatures.

For wounds, in tubers which had not been exposed to gamma irradiation, cured at either 4°C or 15°C, the level of gangrene infection declined as the age of the wounds increased (Fig 6.2). Zero infection occurred sooner when wounds were incubated at 15°C than at 4°C: there was little or no infection after only 5 days at 15°C whereas 28 days were required at 4°C. At both incubation temperatures the reduction in gangrene with increase in interval between wounding and inoculation was delayed in the irradiated tubers compared with the untreated ones.

Figure 6.2 The susceptibility to infection by P. exigua var. foveata of wounds in gamma-irradiated and non-irradiated tubers healed at 4°C or 15°C



Experiment 4

Irradiation treatment and the interaction of treatment with the time interval between irradiation and inoculation both had a significant effect on lesion radius (Appendix 6.4).

Irradiation increased lesion radius when the tubers were inoculated within 5 days of exposure to gamma rays (Table 6.2). However, if inoculation was delayed until 12 days after irradiation lesion development was no greater in the treated tubers than in the controls.

Experiment 5

Gamma irradiation treatment increased the rate of gangrene lesion development in Pentland Dell tubers (Fig 6.3). The rate in the tubers exposed to 100 krads was approximately four times greater than that in the untreated tubers. In both irradiated and untreated tubers the rate of lesion development was linear.

Experiment 6

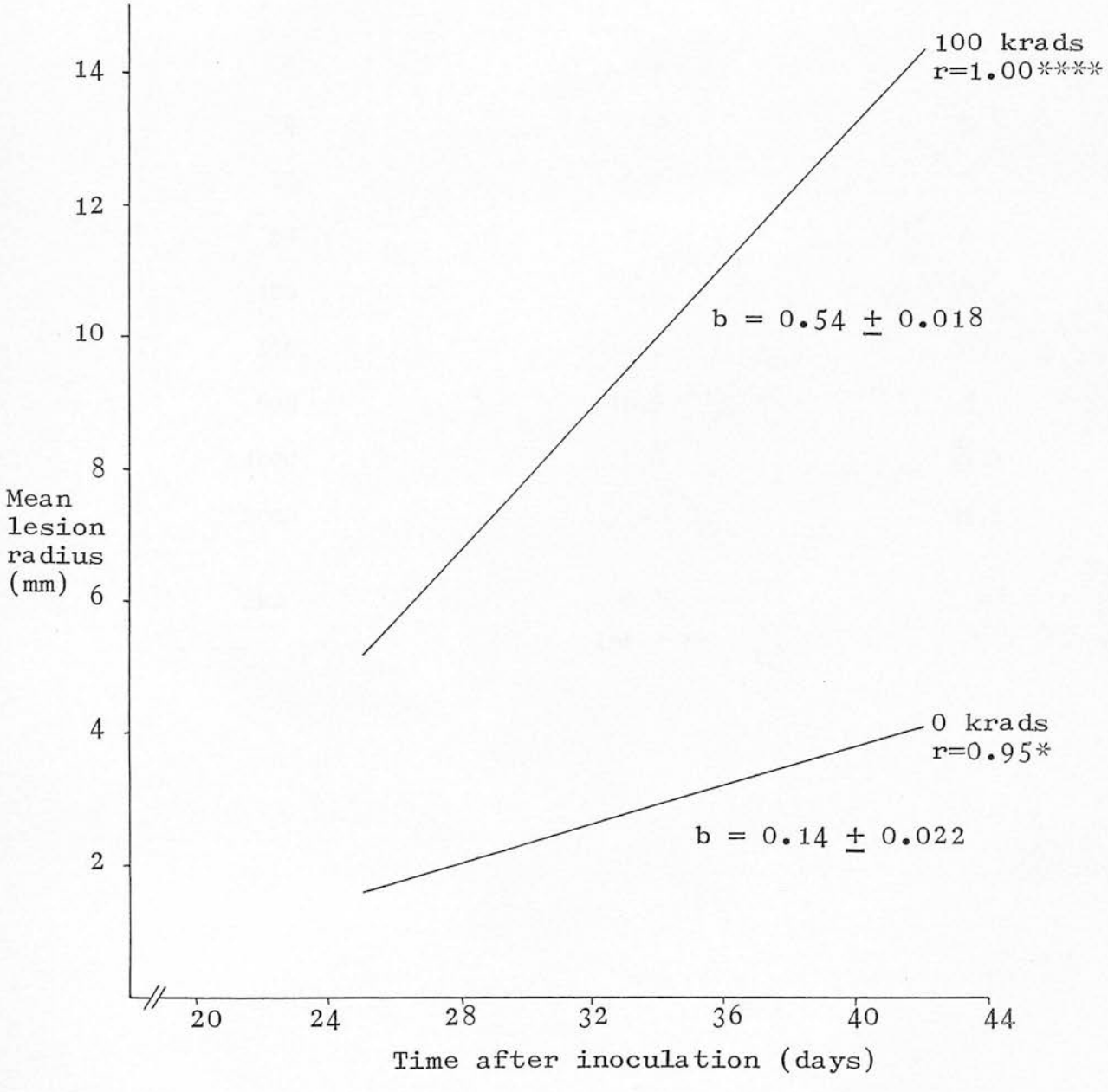
The analysis of variance of the results shows that lesion radius was significantly affected by irradiation dose (Appendix 6.5).

There was no relationship between lesion radius and tuber cell death at the different irradiation doses (Table 6.3). Lesion radius increased greatly with increasing irradiation dose up to 500 krads. Increasing the

Table 6.2 The effect of time interval between irradiation and wounding/inoculation on the difference in lesion development between irradiated tubers and controls (mean lesion radius, mm)

Period between irradiation and wounding/inoculation (days)	Gamma irradiation dose (krads)	
	0	50
1	9.2	11.7
5	10.2	14.5
12	11.2	10.1
	SED	0.95
	(DF = 5)	

Figure 6.3 The rate of gangrene lesion development in gamma-irradiated, compared with non-irradiated, tubers



* P<0.05 **** P<0.002

Table 6.3 The relationship between gangrene lesion radius and cell death in tubers exposed to different levels of gamma irradiation

Irradiation dose (krads)	Lesion radius (mm)	Percentage cell death
0	2.4	0
10	1.3	0
25	5.2	0
50	6.6	0
100	8.5	0
200	11.7	0
500	16.6	2
1000	17.6	32.3
2000	-	64.5
SED	0.77 (DF = 7)	-

dose from 500 to 1000 krads did not significantly increase rot size. In contrast tuber cell death was not detectable until the 500 krad dose was reached. Increasing the dose to 1000 or 2000 krads increased the level of cell death very markedly.

6.4 DISCUSSION

The results of the first two experiments suggest that wound periderm formation is not a primary factor in tissue resistance to P. exigua var. foveata. It was consistently found for various cultivars in these two experiments that susceptibility to lesion development increased with increasing irradiation dose, whereas it is recognised that at low doses of irradiation, ie 10 krads, wound periderm formation is completely inhibited in potato tubers (Brownell et al., 1957; Isleib, 1957). That gangrene susceptibility continues to increase with increasing irradiation dose suggests that some other tuber physiological process, or processes, less sensitive to irradiation, are involved in tissue resistance. Further evidence for the minor role of wound periderm formation is provided by Walker & Wade (1978) who found that gangrene lesion arrest can precede wound periderm formation.

In considering the longevity of the effect of gamma irradiation on tuber susceptibility, wound healing was

found to be only temporarily inhibited by irradiation. Furthermore, lesion development was greater in irradiated tubers, compared with controls only if the tubers were inoculated within several days of irradiation. In one experiment, however, the rate of lesion development in irradiated tubers was constant over time and was higher than the rate in non-irradiated tubers. This finding might suggest that gamma irradiation has both a temporary and long term effect on susceptibility and that irradiation influences at least two distinct resistance mechanisms. It is possible that the mechanism temporarily inhibited is suberisation since it is well documented that suberisation is only temporarily affected by irradiation (Brownell et al., 1957; Isleib, 1957; Skou & Henriksen, 1964; Thomas & Delincee, 1979). Hooker & Duncan (1959) also found, when assessing the effect of gamma irradiation of potato tubers on their susceptibility to ring rot (Corynebacterium sepedonicum), dry rot (Fusarium sambucinum) and soft rot (Erwinia carotovora), that two different processes were affected, one temporarily, the other for an indefinite period. However, it might be that the apparent long term effect demonstrated in this study (Fig 6.3) is simply a consequence of the earlier, more rapid lesion development in irradiated tubers compared with non-irradiated ones which show a longer lag phase.

Although no conclusions can be reached regarding the mechanism by which gamma irradiation increases the susceptibility of tubers to gangrene the results of the last experiment indicate that the increased susceptibility is not due to irradiation-induced tuber cell death.

CHAPTER 7

Aspects of inoculum production by *P. exigua* var. *foveata*

7.1 INTRODUCTION

Current methods of assessing isolate pathogenicity consist of determining the amount of gangrene produced after inoculating tubers with a fixed level of inoculum of each isolate. Such methods measure only the tuber rotting abilities of isolates and take no account of the possibility that isolates may differ in the extent to which they transmit inoculum from seed to daughter tubers during the growing season. If isolates do differ in their transmission rates then such differences could influence the potential disease risks from different isolates in the field.

One of the major factors determining the incidence of gangrene in the stored crop is the level of tuber inoculum (Adams, 1980a). The level of daughter tuber contamination at harvest largely depends on the extent of transmission of P. exigua var. foveata from the seed to the progeny tubers. This transmission may be direct when the mother tuber rots and releases inoculum into the soil or indirect, by means of pycnospores released from pycnidia on the senescing stems of the infected plant. Adams (1980b) has demonstrated that the transmission of P. exigua var. foveata from seed to daughter tubers can be at least as extensive when seed is merely contaminated as it is when seed has gangrene lesions. This suggests that the use of potato cultivars with

greater tuber resistance is not necessarily going to reduce the spread of the pathogen from seed to the daughter tubers.

One aim of the experiment in this section was to investigate the relative abilities of two isolates to cause gangrene when the assessment method used took into account transmission of inoculum to progeny tubers as well as tuber rotting ability. A further objective was to examine the relative transmission rates of P. exigua var. foveata for different cultivars using inoculated or contaminated seed tubers.

7.2 MATERIALS AND METHODS

Inoculum transmission from seed to progeny tubers was investigated using two field isolates, J and M, and three cultivars, Maris Piper, Pentland Crown and Pentland Dell. The choice of the two isolates was based on the assumption that pycnospore production would be an important factor in inoculum transmission: isolate M was observed to produce abundant pycnospores in vitro and isolate J produced relatively few. In order to investigate how inoculum transmission was influenced by the nature of the association between the pathogen and the mother tuber, seed was either inoculated or contaminated.

Seed of the three cultivars, all from the same source, was surface sterilized prior to treatment. Seed was inoculated by inserting a mycelial disc of the appropriate isolate into each tuber. In order to estimate the relative pathogenicity of the two isolates, and the tuber resistances of the cultivars, the surface diameter of each lesion was measured after 4 weeks incubation at 4°C. The inoculated seed was planted 2 days after this assessment. Seed was contaminated by dipping in a soil slurry (1 kg sterilized loam:1 l sterile water) containing macerated mycelium of the appropriate isolate. The macerated mycelium was obtained by blending 10 mycelial mats, from 6 day-old malt extract agar membrane filter cultures, in 500 ml sterile water for 1 minute. Contaminated tubers were air-dried for 1 week prior to planting. Controls in this experiment were wounded tubers for the inoculation treatment and tubers dipped in sterilized soil slurry for the contamination treatment.

The seed was planted in late April by hand, 40 cm apart in rows 70 cm wide. Plots were randomised within two replicate blocks. Each plot consisted of six treatment plants surrounded by 14 guard plants of the same cultivar. In order to prevent spread of inoculum between treatments, treatment plots were separated from each other by four rows of guards or 2.26 m within rows.

Cross contamination was further minimised by using disposable gloves to handle seed at planting, by omitting mechanical cultivation of plots and by applying aphid and blight sprays from guard rows.

Four weeks after haulm desiccation, using diquat dibromide (Reglone), the relative numbers of stem pycnidia were estimated using two harvested plants per plot. Plants were scored using a 1 (few pycnidia) to 3 (abundant pycnidia) scale. The procedure described by Adams (1980b) was used to assess whether the stem pycnidia were P. exigua var. foveata. A few drops of sterile water were placed over pycnidia on a stem, left for approximately 2 minutes, and then were streaked out on the selective medium of McCracken & Logan (Appendix 4.1). The plates were scored for the presence of P. exigua var. foveata colonies after 10 days' incubation at 20°C. Stems were also scored for their degree of senescence at harvest on a 1 to 3 scale (1 partially green, 3 completely desiccated). The tubers were harvested, the same day as stem samples were taken, using forks which were disinfected between plots by dipping in a Chloros solution. The level of inoculum on the harvested tubers was assessed by removing soil from them, mixing it thoroughly following air-drying and inserting it into wounds, 7 mm wide and 3 mm deep, in Record tubers. Forty such wounds, two in each of

20 tubers, were filled with soil from each treatment. After 10 weeks incubation the surface diameter and maximum depth of each lesion was assessed. In order to confirm that the lesions were caused by the isolates used to treat the seed, isolations were made from two lesions per treatment on to the McCracken & Logan selective medium. The levels of pycnospore and anthraquinone production by these isolates were compared with isolates J and M.

The production of pycnospores *in vitro* by isolates J and M was assessed by growing each isolate on three malt extract agar plates at 20°C. Pycnospore production was determined after 4 weeks by washing the cultures with a known volume of water and determining pycnospore concentration, using a haemocytometer.

7.3 RESULTS

Aspects of inoculum production by *P. exigua* var. *foveata*.

The size of lesion produced by tubersphere soil was affected by isolate, method of seed treatment and the interactions between isolate x cultivar and isolate x seed treatment (Appendix 7.1).

In Table 7.1 the amount of gangrene produced from daughter tubersphere soil for the two isolates, their pathogenicity and *in vitro* pycnospore production are

Table 7.1 Tubersphere soil gangrene potential, pathogenicity, *in vitro* pycnospore production and pycnidia production on stems for two isolates of *P. exigua* var. *foveata*

	Isolate J	Isolate M	Control	SED
Tubersphere soil gangrene potential (mean lesion radius on Record test tubers, average of three cultivars, mm)	5.0	3.5	1.5	0.39 (DF=17)
Pathogenicity (diameter of gangrene lesions on seed tubers, mean of three cultivars, mm)	17.2	12.1	-	0.60 (DF=115)
In vitro pycnospore production (pycnospores per agar plate)	8.3×10^4	2.7×10^7	-	1×10^7 (DF=2)
Pycnidia on stems (1 = few; 3 = abundant, average of three cultivars)	1.7	1.7	2.0	0.20 (DF=17)

given, along with the extent of pycnidia production on stems. The gangrene potential of tubersphere soil was significantly greater for isolate J than isolate M. The soil gangrene potentials of the two isolates were related to isolate pathogenicity not to pycnospore production *in vitro*. Isolate J, which had the greater tubersphere soil gangrene potential, was the more pathogenic isolate but its pycnospore production *in vitro* was less than that of M. The results obtained for pycnospore production *in vivo* on the stems of plants were inconclusive because large numbers of pycnidia not produced by *P. exigua* var. *foveata* were present in the control plots. As a result it could not be assumed that the pycnidia on the treatment plants were only *P. exigua* var. *foveata* despite the production of anthraquinone pigments by the samples taken from their stems (Table 7.2).

A low level of gangrene developed in test tubers inoculated with tubersphere soil from control tubers. There is, however, little evidence to suggest that the results of this experiment were much affected by cross contamination between plots or the presence of contaminant *P. exigua* var. *foveata*. The lesions produced by tubersphere soil from the various treatments yielded the isolate with which the seed had been treated except for one replicate of one treatment (Appendix 7.2). Furthermore only for one replicate of one control (Pentland

Table 7.2 The variety of *P. exigua* responsible for stem pycnidia on Pentland Dell plants

Seed treatment	Presence of anthraquinone pigments in cultures derived from stem pycnospores
Isolate J/Inoculated	+
Isolate M/Inoculated	+
Isolate J/Contaminated	+
Isolate M/Contaminated	+
Contamination control	-
Inoculation control	-

Crown, contaminated) was gangrene produced from tubersphere soil.

With contaminated mother tubers there was a greater carry over of inoculum than from seed showing lesions. The mean lesion radii produced from tubersphere soil being 3.9 and 2.7 mm respectively (SED = 0.32, DF = 17).

The relative soil gangrene potentials of the two isolates depended on the cultivar tested (Table 7.3): that of isolate J was greater than that of M for Maris Piper and Pentland Dell but there were no significant differences between the isolates for Pentland Crown. When isolate J was used Maris Piper gave a greater transmission of P. exigua var. foveata than Pentland Crown but with isolate M there was no discernable variation among cultivars.

Overall there were no significant differences among the three cultivars in transmission of inoculum to progeny tubers although there were differences in lesion development on mother tubers and differences in the extent of haulm desiccation at lifting (Table 7.4). Pentland Crown seed had significantly larger lesions at planting than the other two cultivars. Maris Piper haulm was significantly more desiccated than either Pentland Crown or Pentland Dell.

Table 7.3 The effect of cultivar and isolate of *P. exigua* var. *foveata* on the gangrene potential of daughter tubersphere soil (mean lesion radius produced after inoculation of tubersphere soil into Record test tubers, mean of two seed treatments, mm)

Cultivar	Isolate		Control	SED
	J	M		
Maris Piper	6.1	3.1	1.3	
Pentland Crown	3.8	3.9	1.8	
Pentland Dell	5.0	3.6	1.3	0.68 (DF=17)

Table 7.4 Lesion size on seed tubers, lesion size produced from tubersphere soil of daughter tubers and the extent of haulm desiccation in relation to cultivar

Cultivar	Lesion size on seed tuber (diameter, mm)	Lesion size produced from tubersphere soil (radius, mm)	Haulm desiccation (1 = partially green 3 = completely desiccated)
Maris Piper	13.7	3.5	2.4
Pentland Crown	17.0	3.2	1.2
Pentland Dell	13.2	3.3	1.5
SED	0.73	0.39	0.18
DF	115	17	17

7.4 DISCUSSION

The results of this section show that when the disease potential of P. exigua var. foveata isolates was assessed using a method which took into account the isolates' abilities to transmit inoculum from the seed to the daughter tubers, in addition to their tuber rotting abilities, there was variation between the isolates. However, the relative disease potentials of the isolates obtained with this method were the same as those which resulted when only the tuber rotting abilities of the isolates were assessed. There are several possible explanations for this result. Firstly, the isolates may not in fact have differed in inoculum transmission in vivo even although they differed considerably in pycnospore production in vitro. Secondly, isolate M may have been transmitted to a greater extent to progeny tubers than isolate J but the difference between the isolates was not large enough to mask the difference in tuber rotting ability. Another possibility is that in vivo pycnospore production may have depended on the relative capabilities of the isolates to colonise the host plant. As a result of the greater pathogenicity of isolate J this isolate may have colonised the host to a greater extent and therefore produced as many pycnospores as the faster sporing isolate M. Finally, it is possible that mycelium may be an important form of

inoculum: therefore, differences in pycnospore production among isolates would have little influence on inoculum transmission. It is obvious that further experimental work, using a greater number of isolates and assessing the transmission of P. exigua var. foveata per se, is necessary to confirm whether the pathogenicity ranking order of isolates is affected when their ability to transmit inoculum is taken into consideration.

Some evidence is provided that potato cultivars may differ in the extent to which they transmit P. exigua var. foveata from seed to progeny tubers in the field although this occurred with only one of the two isolates used in comparing Maris Piper and Pentland Crown. Adams (1980b) found that the degree of transmission of P. exigua var. foveata from seed to progeny tubers depended on cultivar. In his experiments, to examine the role of seed-tuber and stem inoculum in gangrene development, the two cultivars Pentland Crown and Ulster Sceptre were used. It was found that the contamination of U. Sceptre progeny tubers was greater than that of Pentland Crown. Ulster Sceptre is a first early whereas Pentland Crown is a maincrop cultivar, therefore the difference in progeny tuber contamination levels for these two cultivars may have been due to the earlier senescence of, and hence greater build-up of

P. exigua var. foveata on, Ulster Sceptre. The results of the present study show that cultivars of a similar maturity class also differ in the degree of inoculum transmission to progeny tubers, but that this may be again related to the rate of haulm senescence: Maris Piper showing a higher degree of haulm desiccation at lifting than Pentland Crown. Haulm desiccation appeared to be more important than seed infection in relation to transmission (Table 7.4). However, more studies of this aspect of gangrene resistance are necessary in order to determine whether the variation between cultivars is large, and consistent, enough to affect the resistance ranking order of cultivars based on tuber resistance, ie resistance to damage and infection alone.

It may be that variation in tuber resistance among cultivars is of little significance as far as the transmission of inoculum from the seed to progeny tubers is concerned. It was found in this study that the cultivar with the largest gangrene lesions at planting was not the cultivar which had the most highly contaminated daughter tubers. Furthermore, the level of progeny tuber contamination was generally greater when the seed had only been contaminated with P. exigua var. foveata rather than having had gangrene lesions at the time of planting. This confirmed Adams' result, already referred to (Adams, 1980b). That the transmission of P. exigua var. foveata

from seed to progeny tubers can be at least as great with contaminated seed, compared with seed with gangrene lesions, implies that in order to reduce seed-borne transmission, and so reduce daughter tuber inoculum levels, it is necessary for the potato grower to not only ensure that the seed which is planted is free from gangrene lesions but that it is not heavily contaminated with P. exigua var. foveata.

CHAPTER 8

Concluding Discussion

A recurring theme in this thesis has been the appraisal of screening tests for cultivar resistance to potato gangrene. Several factors influence to varying degrees the occurrence of the disease. These factors include the pathogenicity of the isolate, the tuber inoculum level, tuber damage, the temperature of incubation and the resistance of tubers as determined by their damage resistance and tissue resistance characteristics. The balance of these factors in resistance screening tests will determine the value of the results as an indicator of the performance of cultivars when grown commercially.

In testing the pathogenicity of different isolates variation was associated with isolate source and history. In considering isolates taken from different potato fields, crop to crop variation was found but the range of variation was generally greater within crops. There was however little evidence of much variation within cultures derived from a single lesion. In general recent field isolates gave higher disease indices than those maintained for several years in culture collections, but repeated axenic subculturing over a short period did not normally result in diminished pathogenicity. The importance of using highly pathogenic isolates for a sensitive discrimination between cultivars is emphasised and it is recommended that in any screening programme

recent field, or tuber, isolates are used. Although isolates varied slightly in their comparative pathogenicity on different cultivars the ranking order of cultivars tended to be constant with different isolates and one highly pathogenic isolate would seem to be adequate for testing cultivars: there was no evidence of cultivar x isolate interactions of an order to suggest that physiological specialisation would be of practical importance in this pathogen.

Inoculum density was shown to be an important factor in revealing differences among cultivars. The optimum inoculum level in screening tests depended on the assessment technique employed. Results obtained indicated that with point inoculation methods low spore loads, ie 10-100 pycnospores per inoculation, are likely to give the clearest results if infection frequency is the resistance criterion whereas high inoculum densities (2000-10000 pycnospores per inoculation) discriminated cultivars better when lesion size was measured. For screening tests in which account was taken of cultivar resistance to damage, such as the grader damage procedure, high spore loads separated cultivars more clearly.

The inoculum potential of tubersphere soil was found to be influenced by cultivar and isolate although the underlying mechanisms involved appear complex: in the case of isolate variation a more pathogenic isolate gave tubersphere soil with a greater disease potential

than one which was less pathogenic but produced more pycnospores *in vitro*; in the case of cultivars, the one showing the greatest degree of haulm senescence at harvest was associated with tubersphere soil with the greatest gangrene potential. However, much more work is needed on the implications of the *P. exigua* var. *foveata* transmission characteristics associated with different cultivars in order to assess their significance in the field.

Tuber damage is well recognised as an important factor in the epidemiology of gangrene and should be taken into account when choosing a method to screen clones for gangrene resistance. It is considered that a technique which simulated a commercial grading procedure gave the most reliable estimate of a cultivar's gangrene resistance in practice since this technique included an assessment of cultivar damage resistance. Using undamaged tubers is unrealistic, whereas using a standard wound inoculation procedure circumvents damage resistance. A further disadvantage of uniform wound inoculation is that results are generally assessed in terms of lesion size whereas with the grader damage method lesion frequency is recorded. Gangrene causes economic loss primarily through infected tubers having to be discarded resulting in a reduced marketable yield: it is the presence of lesions rather than their size which is critical (Kerr, DAFS, Dundee, personal communication).

There are two broadly recognised components of tuber resistance to gangrene, damage resistance and tissue resistance, which appear to occur independently: the former influences primarily lesion frequency while the latter influences mainly lesion size, the criterion of less practical importance despite its continued use in cultivar resistance tests. Whether tissue resistance brings about lesion retardation or lesion arrest depends upon incubation temperature. At 10°C lesion arrest occurred in all cultivars, although slightly earlier in more resistant cultivars. At 4°C, the recommended storage temperature for potatoes, lesion retardation was related to the level of cultivar tissue resistance, but lesions increased in size with time in all cultivars. Thus over longer storage periods the advantage associated with tissue resistance diminishes. Damage resistance which restricts the establishment of infection will thus contribute more significantly to the control of gangrene in practice and should thus be given the greater emphasis in screening tests.

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APPENDICES

Appendix 1.1 Inoculation dates of experiments

Section	Experiment	Date
2	1	10/80
	2 Whole tuber inoculation method	1/82
	2 Point inoculation method	2/83
	3	3/83
	4	10/81
	5	7/83
	6	1/83
	7	7/83
3	1	11/82
	2	10/82
4	2	10/81
	3	9/82
	4	2/83
5	1	12/82
	2	10/82
6	1	1/82
	2	10/82
	3	12/82
	4	12/82
	5	10/82
	6	11/82
7	1	10/83

Appendix 2.1 Experiment 1. Point inoculation method

Source of variation	DF	Lesion diameter		Lesion depth		Mean lesion radius	
		Mean square	F ratio	Mean square	F ratio	Mean square	F ratio
Reps stratum	2	187.7	5.0	25.0	1.8	18.4	2.2
Reps Plots stratum							
Isolate	7	6735.6	148.45***	1967.0	103.98***	1819.2	256.98***
Cultivar	4	1051.2	23.17***	216.2	11.43***	218.0	30.80***
Isolate Cultivar	28	207.4	4.57***	42.2	2.23**	38.4	5.43***
Residual	78	45.4	1.22	18.9	1.35	7.1	0.85
Total	117	518.8	13.93	147.8	10.53	130.2	15.57
Reps Plots Subplots stratum	360	37.3		14.0		8.4	
Grand Total	479						

*** P<0.001
** P<0.01
* P<0.05

Appendix 2.2 Experiment 1. Whole tuber inoculation method

Source of variation	DF(MV)	Surface lesion index Mean square	F ratio
Reps stratum	3	39.4	6.63
Reps Plots stratum			
Isolate	8	427.0	40.39***
Cultivar	4	263.0	24.88***
Isolate Cultivar	32	59.3	5.60***
Residual	132	10.6	1.78
Total	176	44.1	7.41
Reps Plots Subplots stratum	539(1)	6.0	
Grand Total	718		

Appendix 2.3 Experiment 2. Whole tuber inoculation method

Source of variation	DF	Surface lesion index Mean square	F ratio
Reps stratum	3	8.7	1.31
Reps Plots stratum			
Isolate	16	295.8	22.78***
Residual	48	13.0	1.96
Total	64	83.7	12.66
Reps Plots Subplots stratum	272	6.6	
Grand Total	339		

Appendix 2.4 Experiment 2. Point inoculation method

Source of variation	DF	Lesion diameter		Lesion depth	
		Mean square	F ratio	Mean square	F ratio
Reps stratum	1	6.5	0.52	29.5	2.29
Reps Plots stratum					
Isolate	14	465.9	51.54***	273.7	22.55***
Residual	14	9.0	0.72	12.1	0.94
Total	28	237.5	19.00	142.9	11.11
Reps Plots Subplots stratum	270	12.5		12.9	
Grand Total	299				

Appendix 2.5 Experiment 3. Isolate variation

Mean lesion radius

Source of variation	DF(MV)	Mean square	F ratio
Reps stratum	1	63.0	21.05
Reps Plots stratum			
Isolate	55	15.1	4.36***
Residual	55	3.5	1.16
Total	110	9.3	3.10
Reps Plots Subplots stratum	997(11)	3.0	
Grand Total	1108		

Appendix 2.6 Experiment 3. Crop variation

Source of variation	DF(MV)	Mean lesion radius Mean square	F ratio
Reps stratum	13	18.4	6.16
Reps Plots stratum			
Crop	7	23.8	3.21**
Residual	91	7.4	2.49
Total	98	8.6	2.88
Reps Plots Subplots stratum	997(11)	3.0	
Grand Total	1108		

Appendix 2.7 Experiment 4

Mean lesion radius

Source of variation	DF	Mean square	F ratio
Reps stratum	3	1.9	0.69
Reps Plots stratum	16	1.7	0.62
Reps Plots Units stratum			
Isolate	19	153.7	55.70***
Residual	361	2.8	
Total	380	10.3	
Grand Total	399		

Appendix 2.8 Experiment 5

Source of variation	DF(MV)	Lesion diameter Mean square	F ratio
Reps stratum	1	138.5	10.92
Reps Plots stratum			
Isolate	24	321.2	5.23***
Residual	24	61.4	4.84
Total	48	191.3	15.09
Reps Plots Subplots stratum	445(5)	12.7	
Grand Total	494		

Appendix 2.9 Experiment 6

Mean lesion radius

Source of variation	DF	Mean square	F ratio
Reps stratum	1	95.0	21.29
Reps Plots stratum			
Isolate	11	93.5	11.18***
Residual	11	8.4	1.88
Total	22	50.9	11.42
Reps Plots Subplots stratum	216	4.5	
Grand Total	239		

Appendix 2.10 Experiment 7

Mean lesion radius

Source of variation	DF(MV)	Mean square	F ratio
Reps stratum	1	0.9	0.72
Reps Plots stratum			
Isolate	3	4.9	2.05
Assessment	1	5.2	2.15
Isolate Assessment	3	0.2	0.08
Residual	7	2.4	1.94
Total	14	2.7	2.16
Reps Plots Subplots stratum	142(2)	1.2	
Grand Total	157		

Appendix 3.1 Experiment 1. Seven field isolates

Source of variation	DF(MV)	Lesion diameter		Lesion depth	
		Mean square	F ratio	Mean square	F ratio
Reps stratum	1	0.3	0.02	62.0	3.75
Reps Plots stratum					
Cultivar	9	4124.1	131.96***	2798.6	142.08***
Isolate	6	817.8	26.17***	162.6	8.26***
Cultivar Isolate	54	59.9	1.92**	71.2	3.61***
Residual	69	31.3	2.04	19.7	1.19
Total	138	343.6	22.47	227.3	13.74
Reps Plots Subplots stratum	1249(11)	15.3		16.5	
Grand Total	1388				

Appendix 3.2 Experiment 1. Culture collection isolate

Source of variation	DF(MV)	Lesion diameter		Lesion depth	
		Mean square	F ratio	Mean square	F ratio
Reps stratum	1	1.2	4.91	2.1	1.97
Reps Plots stratum					
Cultivar	9	0.2	0.30	17.3	42.43***
Residual	9	0.7	3.07	0.4	0.38
Total	18	0.5	2.00	8.8	8.33
Reps Plots Subplots stratum	179(1)	0.2		1.1	
Grand Total	198				

Appendix 3.3 Experiment 2

Source of variation	DF(MV)	Lesion diameter		Lesion depth	
		Mean square	F ratio	Mean square	F ratio
Reps stratum	1	13.7	1.12	2.1	0.22
Reps Plots stratum					
Isolate	1	2128.5	210.00***	2084.6	156.16***
Cultivar	6	524.9	51.79***	628.9	47.11***
Isolate Cultivar	6	197.2	19.45***	66.6	5.00**
Residual	13	10.1	0.83	13.3	1.43
Total	26	253.6	20.66	247.4	26.43
Reps Plots Subplots stratum	252	12.3		9.4	
Grand Total	279				

Appendix 4.1 Selective medium for the isolation of P. exigua var. foveata from soil (McCracken & Logan, 1977)

	ppm	%
Thiophanate-methyl	1.5	-
Pentachloronitrobenzene	10.0	-
Methoxyethylmercury chloride	7.5	-
Propionic acid	100.0	-
Chloramphenicol	48.0	-
Streptomycin	133.0	-
Aureomycin	50.0	-
Malt extract	-	3
Agar (Oxoid No. 3)	-	1.2
Filter paper (Whatman No. 1)	1 circle	
pH	5.8	

Appendix 4.2 Experiment 2

Source of variation	DF(MV)	Mean lesion radius Mean square	F ratio
Reps stratum	19	6.2	1.07
Reps Units stratum			
Cultivar (CV)	1	1547.2	267.42***
Isolate (I)	1	630.4	108.96***
Inoculum level (IL)	3	19.8	3.42*
Cv. I	1	254.5	43.98***
Cv. IL	3	7.1	1.22
I. IL	3	8.9	1.53
Cv. I. IL	3	12.8	2.22
Residual	284(1)	5.8	
Total	299	14.1	
Grand Total	318		

Appendix 4.3 Experiment 3

Angular transformation of percentage infection

Source of variation	DF(MV)	Mean square	F ratio
Reps stratum	1	178.8	5.53
Reps Plots stratum			
Cultivar	2	267.0	1.81
Isolate	2	803.1	5.43*
Cultivar Isolate	4	171.9	1.16
Residual	8	147.8	4.57
Total	16	250.7	7.75
Reps Plots Subplots stratum			
Inoculum level (IL)	7	8985.3	277.77***
Cultivar IL	14	123.1	3.81***
Isolate IL	14	96.9	3.00**
Cultivar Isolate IL	28	36.3	1.12
Residual	61(2)	32.4	
Total	124	556.2	
Grand Total	141		

Appendix 4.4 Experiment 3

Mean lesion radius

Source of variation	DF(MV)	Mean square	F ratio
Reps stratum	19	1.6	0.98
Reps Units stratum			
Cultivar	2	825.7	521.68***
Isolate	2	61.3	38.73***
Inoculum level (IL)	7	543.4	343.29***
Cultivar Isolate	4	22.6	14.29***
Cultivar IL	14	40.4	25.54***
Isolate IL	14	4.8	3.06***
Cultivar Isolate IL	26(2)	2.4	1.49
Residual	1309(40)	1.6	
Total	1378	6.1	
Grand Total	1397		

Appendix 4.5 Experiment 4

Zero damage treatment

Source of variation	DF	Number of gangrene lesions per 50 tubers: Mean square	F ratio
Reps stratum	1	84.4	1.12
Reps Plots stratum			
Cultivar	2	516.7	6.86*
Inoculum level (IL)	3	228.8	3.04
Cultivar IL	6	106.9	1.42
Residual	11	75.3	
Total	22	145.0	
Grand Total	23		

Uniform damage treatment

Source of variation	DF(MV)	Number of gangrene lesions per 50 tubers: Mean square	F ratio
Reps stratum	1	24334	21.36
Reps Plots stratum			
Cultivar	2	17887	6.03*
Inoculum level (IL)	3	370343	124.90***
Cultivar IL	6	3832	1.29
Residual	11	2965	2.60
Total	22	54655	47.98
Reps Plots Subplots stratum	211(5)	1139	
Grand Total	234		

Appendix 4.5 (Contd)

Grader damage treatment

Source of variation	DF(MV)	Number of gangrene lesions per 50 tubers Mean square	F ratio
Reps stratum	1	139385	64.00
Reps Plots stratum			
Inoculum level (IL)	3	439238	19.57***
Damage level (DL)	2	45465	2.03
Cultivar	2	512380	22.83***
IL DL	6	22004	0.98
IL Cultivar	6	145563	6.49***
DL Cultivar	4	31125	1.39
IL DL Cultivar	12	10766	0.48
Residual	35	22441	10.30
Total	70	63971	29.37
Reps Plots Subplots stratum	1719(9)	2178	
Grand Total	1790		

Appendix 5.1 Experiment 1. 4°C

Source of variation	DF(MV)	Mean lesion radius Mean square	F ratio
Reps stratum	1	32.9	3.43
Reps Plots stratum			
Assessment time (AT)	1	2105.4	177.16***
Cultivar	3	2105.4	177.16***
Inoculum level (IL)	1	1069.1	89.97***
AT Cultivar	3	115.9	9.75**
AT IL	1	46.7	3.93
Cultivar IL	3	128.6	10.82**
AT Cultivar IL	3	21.8	1.84
Residual	15	11.9	1.24
Total	30	350.5	36.52
Reps Plots Subplots stratum	285(3)	9.6	
Grand Total	316		

Appendix 5.2 Experiment 1. 10°C

Source of variation	DF(MV)	Mean lesion radius Mean square	F ratio
Reps stratum	1	38.4	4.13
Reps Plots stratum			
Assessment time (AT)	1	0.04	0.01
Cultivar	3	58.4	7.66*
Inoculum level (IL)	1	131.6	17.26***
AT Cultivar	3	6.0	0.79
AT IL	1	0.05	0.01
Cultivar IL	3	45.0	5.90*
AT Cultivar IL	3	1.3	0.16
Residual	15	7.6	0.82
Total	30	19.3	2.07
Reps Plots Subplots stratum	287(1)	9.3	
Grand Total	318		

Appendix 5.3 Experiment 1. 4°C

Source of variation	DF	Angular transformation of percentage infection: Mean square	F ratio
Reps stratum	1	840.4	12.46
Reps Plots stratum			
Assessment time (AT)	1	278.9	4.13
Cultivar	3	1628.2	24.13***
Inoculum level (IL)	1	5218.8	77.35***
AT Cultivar	3	699.8	10.37***
AT IL	1	53.4	0.79
Cultivar IL	3	833.7	12.36***
AT Cultivar IL	3	351.0	5.20*
Residual	15	67.5	
Total	30	570.1	
Grand Total	31		

Table of means: Angular transformation of percentage infection

Cultivar	Assessment time (weeks)	Inoculum level (Pycnospores per inoculation) 200	2000
P. Falcon	8.5	90	90
	17	90	90
M. Piper	8.5	57	90
	17	72	90
S. Enterprise	8.5	45	81
	17	17	81
R. Castle	8.5	33	77
	17	81	90

SED 8.2 (DF = 15)

Appendix 5.4 Experiment 2

Mean lesion radius

Source of variation	DF(MV)	Mean square	F ratio
Reps stratum	1	1.0	0.20
Reps Plots stratum			
Time	5	214.1	32.00***
Cultivar	1	828.8	123.92***
Time Cultivar	5	16.7	2.49
Residual	11	6.7	1.36
Total	22	93.5	19.04
Reps Plots Subplots stratum	215(1)	4.9	
Grand Total	238		

Appendix 6.1 Experiment 2

Mean lesion radius

Source of variation	DF	Mean square	F ratio
Reps stratum	1	2.6	0.42
Reps Plots stratum			
Cultivar	1	4978.6	650.94***
Irradiation level (IrL)	5	937.0	122.51***
Cultivar IrL	5	88.0	11.50***
Residual	11	7.6	1.25
Total	22	463.1	75.71
Reps Plots Subplots stratum	192	6.1	
Grand Total	215		

Appendix 6.2 Experiment 3. 4°C

Angular transformation of percentage infection

Source of variation	DF	Mean square	F ratio
Reps stratum	1	58.7	1.22
Reps Plots stratum			
Time	6	2947.5	61.19***
Irradiation level (IrL)	1	10273.6	213.29***
Time IrL	6	670.4	13.92***
Residual	13	48.2	
Total	26	1254.1	
Grand Total	27		

Appendix 6.3 Experiment 3. 15°C

Angular transformation of percentage infection

Source of variation	DF	Mean square	F ratio
Reps stratum	1	72.3	1.41
Reps Plots stratum			
Time	6	1191.8	23.25***
Irradiation level (IrL)	1	34431.0	671.64***
Time IrL	6	1032.7	20.15***
Residual	13	51.3	
Total	26	1863.3	
Grand Total	27		

Appendix 6.4 Experiment 4

Mean lesion radius

Source of variation	DF(MV)	Mean square	F ratio
Reps stratum	1	10.0	2.46
Reps Plots stratum			
Time	2	43.6	4.80
Irradiation level (IrL)	1	108.9	12.00*
Time IrL	2	76.9	8.48*
Residual	5	9.1	2.22
Total	10	39.5	9.69
Reps Plots Subplots stratum	107(1)	4.1	
Grand Total	118		

Appendix 6.5 Experiment 6

Mean lesion radius

Source of variation	DF(MV)	Mean square	F ratio
Reps stratum	1	9.1	2.72
Reps Plots stratum			
Irradiation level	7	595.6	126.60***
Residual	7	4.7	1.40
Total	14	300.2	89.45
Reps Plots Subplots stratum	111(1)	3.4	
Grand Total	126		

Appendix 7.1 Experiment 1

Source of variation	DF(MV)	Mean lesion radius Mean square	F ratio
Reps stratum	1	6.3	
Reps Plots stratum			
Cultivar (CV)	2	741.9	0.30
Isolate (I)	2	281.8	40.54***
Seed treatment (ST)	1	63.1	15.40**
CV I	4	41.5	3.45*
CV ST	2	99.5	2.27
I ST	2	22.9	5.44*
CV I ST	4	18.3	1.25
Residual	17	79.8	
Total	34		
Reps Plots Tubers stratum	321(3)	8.1	
Reps Plots Tubers Rots stratum	354(6)	6.8	
Grand Total	710		

Appendix 7.2 Experiment 1. Culture characteristics of P. exigua var. foveata isolated from Record test tubers inoculated with tubersphere soil from the various treatments

Treatment				Culture characteristics	
Cultivar	Isolate	Seed Treatment	Rep	Pycnospore production	Anthraquinone production
M. Piper	M	Inoculated	1	++	+
"	M	"	2	++	+
"	M	Contaminated	1	++	+
"	M	"	2	++	+
"	J	Inoculated	1	+	++
"	J	"	2	+	++
"	J	Contaminated	1	+	++
"	J	"	2	+	++
P. Crown	M	Inoculated	1	++	+
"	M	"	2	++	+
"	M	Contaminated	1	++	+
"	M	"	2	No P. exigua var. foveata	
"	J	Inoculated	1	+	++
"	J	"	2	+	++
"	J	Contaminated	1	+	++
"	J	"	2	+	++
"	Control	"	1 tuber 1	++	+
"	"	"	1 tuber 2	++	0
"	"	"	1 tuber 3	++	+
P. Dell	M	Inoculated	1	++	+
"	M	"	2	++	+
"	M	Contaminated	1	++	+
"	M	"	2	+	++
"	J	Inoculated	1	+	++
"	J	"	2	+	++
"	J	Contaminated	1	+	0
"	J	"	2	+	++