DEVELOPMENT AND INFECTION OF LENTICELS ON POTATO TUBERS DURING GROWTH AND STORAGE

by

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ABSTRACT

Lenticels arose from stomata as the internodes on which they were situated aged during tuber growth. Anatomical studies showed that the youngest two internodes had stomata, progressively raised and eventually ruptured by cell division to form lenticels, the filling cells of which suberized in dry conditions by about the fifth internode from the apex. Sometimes a cork barrier formed below the lenticel. Proliferation of lenticels in wet conditions was studied anatomically. In the field and laboratory, lenticels proliferated less readily as tubers matured, and always less on Pentland Crown than Majestic or King Edward. Stored tubers allowed to sprout, proliferated more readily than if eyes were removed.

Laboratory, glasshouse and field experiments did not detect which lenticels were susceptible to Streptomyces scabies as accurately as chance observations from 1967, when only young lenticels on the third and fourth internodes from the apex seemed susceptible. Cultivars differing in scab susceptibility did not differ in the way or speed lenticels developed. On glass slides, S. scabies spores germinated and grew similarly in wet and dry autoclaved soils, but better in dry than wet non-sterile soil, suggesting antagonism in wet soil. Bacillus subtilis inhibited germination of S. scabies spores, probably by antibiosis.

In laboratory tests on mature tubers, small wounds were more susceptible than lenticels to Erwinia carotovora but proliferation increased lenticel susceptibility. Keeping tubers wet or dry for 3 weeks prior to lifting at different stages of

growth did not affect susceptibility to E. carotovora, Phytophthora infestans or penetration by a dye solution, but fewer lenticels were infected or penetrated as tubers aged. Dye penetration was not directly related to the presence or absence of cork barriers below lenticels, or to tuber turgidity. Tuber lenticels may be infected by bacteria during growth, but at and after harvest, infection is most likely through wounds.

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CONTENTS

Chapter 1: GENERAL INTRODUCTION

Many plant pathogens do not penetrate intact surfaces, but enter the host by means of wounds inflicted during agricultural operations or through natural openings. This is particularly so on secondary structures covered with cork, as this is very resistant to biological attack (Wood, 1967). The mature potato tuber is covered with a periderm, and most pathogens either enter through wounds at or after harvest, penetrate the less 'protected' tissue around eyes, or infect the lenticels which are frequent on the tuber surface. Table 1, which summarizes the infection sites of the more important potato tuber pathogens in the United Kingdom, indicates that only mechanical damage may be of greater importance than the lenticel as an infection site.

Lenticels of potatoes, as well as other plants, are not uniformly susceptible to pathogens, and our present knowledge suggests that their susceptibility is at least partly determined by anatomy which is in turn influenced by the age of the tissue and by previous environmental conditions. This is so for the three pathogens used in this study, in which an attempt has been made to relate infection, environmental conditions and lenticel structure.

Streptomyces scabies (Thaxt.) Waksman & Henrici is a soil-borne pathogen causing common scab, which infects lenticels only during a brief period early in their development and provided soil is dry (Lapwood & Hering, 1970). By contrast, Phythophthora infestans (Mont.) de Bary (the fungus causing late blight), and the Erwinia carotovora (Jones) Bergey et al. group of bacteria (causing blackleg in the field and soft-rotting in store) are characteristically spread under wet

conditions, when lenticels may change structurally and thereby become more susceptible.

Table 1. Infection sites used by the major potato tuber pathogens of the U.K.*

Disease (Pathogen)	Wounds at or after harvest	Lenticel	Eye/ Epidermis	Stolon from mother tuber	Periderm
Blackleg/soft-rot (*Erwinia carotovora*)	+	+	-	+	-
Common scab (*Streptomyces scabies*)	-	+	-	-	-
Powdery scab (*Spongospora subterranea*)	-	+	+	-	-
Wart (*Synchytrium endobioticum*)	-	-	+	-	-
Watery wound rot (*Pythium ultimum*)	+	-	-	-	-
Pink rot (*Phytophthora erythroseptica*)	+	-	+	+	-
Late blight (*Phytophthora infestans*)	+	+	+	-	-
Gangrene (*Phoma exigua*)	+	-	-	-	-
Skin spot (*Oospora pustulans*)	+	+	+	+	-
Dry rot (*Fusarium* spp.)	+	-	-	-	-
Silver scurf (*Helminthosporium solani*)	-	+	-	-	+
Black scurf (*Rhizoctonia solani*)	-	-	?+	+	?+

* Data mainly from Lapwood & Hide (1971)

The detailed development of lenticels throughout the growing season and their response to environmental conditions, which is relevant to each of the diseases studied, is described in chapter 2. Investigations on common scab are described in chapter 3 and lenticel infection by the other pathogens in chapter 4. In the investigations described in chapter 5, changes in lenticel structure and susceptibility to infection by the soft-rotting bacteria and blight fungus were followed throughout the growing season in differing soil conditions and into the storage period. In each chapter the relevant literature is surveyed at the beginning and the results discussed at the end, while the major conclusions and more general implications are drawn together in chapter 6.

Chapter 2: LENTICEL DEVELOPMENT AND PHYSIOLOGY

A. INTRODUCTION

Lenticels are defined by Esau (1965) as 'structurally differentiated portions of the periderm characterized by a relatively loose arrangement of cells'. They have been studied physiologically and anatomically in considerable detail, mostly using woody stems, but they also occur on roots, especially those showing secondary growth, and on other organs including apple fruit and potato tubers.

Whilst periderm tissue is impermeable to gases, gas exchange can occur through lenticels. Thomas et al., (1956) demonstrated that air could be drawn in via lenticels of a woody stem and through intercellular spaces to areas of the stem submerged in water, and the physiological studies of Hook et al., (1971) indicated that the oxygen for the roots of flooded seedlings of Nyssa sylvatica var. biflora entered through stem lenticels and then diffused through cortex and phloem. Anatomical studies also indicate that there is often continuity between the intercellular spaces of the lenticel and those of the internal tissue. Lenticels of woody stems are usually situated at the ends of vascular rays (Wetmore, 1926 a and b), while on roots, lenticels are often paired and associated with root junctions as shown by Templeton (1926) on cotton and Kerr (1972) on seedlings of peach.

The mature lenticel is a fissure containing filling (or complementary) cells produced by the activity of a periclinally dividing meristem (the lenticel phellogen), which is continuous with the cork phellogen. This meristem may produce phelloderm to the

inside, and filling cells to the outside, which differ in structure and form the basis for classifying different types of lenticels. In one of the more recent studies of lenticels on woody stems, Wutz (1955) distinguished four types. In the first (Gymnosperm) type, filling cells were suberized and only slightly differentiated from the phellem (cork), while in the second (Salix) type, the suberized filling cells had air spaces between them and were clearly different from the phellem. The third (Sambucus) type had unsuberized filling cells and at the end of the season, a compact layer of suberized cells (closing layer) was formed. The fourth (Prunus) type also had unsuberized filling cells, but alternating each season with several suberized closing layers, of which the outer ones ruptured as the lenticel meristem continued its activity. Wetmore (1926 a & b) showed that lenticels of woody stems could be classified as 'longitudinal' or 'transverse' depending on the orientation of the fissure relative to the stem axis and related this distinction to wood structure, arguing that this was a more 'natural' classification than systems based on the presence and structure of closing layers. While such classifications may assist our understanding of lenticel development and organization, they should probably be applied with caution to root lenticels. Thus Hook et al., (1970) noted that the circular openings of the root lenticels of Nyssa sylvatica could not be categorized as either 'transverse' or 'longitudinal'. Moreover, changes in soil moisture conditions influenced the structure of the lenticel, which also happens to lenticels on other underground structures, including potato tubers. Environmental conditions also influenced apple fruit lenticels, which were normally open in high humidity but became suberized and resisted penetration by dye solutions when stored in dry conditions (Clements, 1935).

The origin of lenticels has been frequently noted but rarely studied in detail. On apple fruits, a lenticel may arise from a broken trichome, split epidermis or a stoma (Clements, 1935) and the anatomical changes have been carefully studied by Krapf (1961). However, these fruits have only a cuticularized epidermis, so the lenticels may not be strictly comparable to those on structures with a true periderm. Where the periderm is initiated in the subepidermal layer, lenticels arise below stomata, but where the periderm is more deep-seated, lenticels form irrespective of the position of previous stomata (Esau, 1965). Secondary lenticels unrelated in position to stomata may also be formed in other stem tissues.

Whilst potato lenticels are known to be important in several diseases, there have been few critical studies of their development and struture under different environmental conditions. Artschwager (1924) and Fellows (1926) described the formation of lenticels from stomata on the young tuber, which involved division of the cells below the stomatal opening and the subsequent rupture of the epidermis or young periderm. This took place on the young tissue nearest the growing apex of the tuber and occurred continuously as fresh tissue formed behind the apical bud. It is not known whether lenticels arise secondarily on older tissue. Thus lenticels on a growing tuber are at different stages of development. Only rarely has the possible significance of this in disease development been appreciated. Fellows (1926) realised that infection of tubers by Streptomyces scabies (causing common scab) was only through some of the younger lenticels, but many subsequent pathologists failed to realise the significance of these observations. Now that the period of susceptibility has been more closely defined (see Lapwood & Hering, 1970;

chapter 3 of this thesis) a more detailed study of the formation of lenticels in relation to the growth of the potato tuber is appropriate, and this has been attempted in the study described here.

The influence of soil moisture on lenticels of potato tubers and other underground structures has been noted by several authors. Under wet conditions, callus-like cells formed by division of the lenticel meristem erupt from the fissure, a process known as proliferation (or hypertrophy). Whilst the role of water is undisputed, the importance of aeration in proliferation is uncertain. Thus, with cotton roots proliferation occurred more readily in unaerated water (Templeton, 1926) with potato tubers in aerated (Devaux, 1891) but with roots of Nyssa sylvatica aeration made no difference (Hook et al., 1970). After describing proliferation on potato tubers, Devaux (1891) suggested its function was to prevent the injection of tuber tissue with water by virtue of the air held in the intercellular spaces of the proliferated tissue, whereas Hook et al.,(1971) considered it important for facilitating gas exchange.

Since proliferation may be a factor in the susceptibility of potato lenticels to disease, it is important to understand the factors influencing the process. Devaux (1891) using tubers of unspecified age and Fox et al., (1971) with stored tubers, obtained proliferation of tubers at 100% relative humidity, but Zeck (1957) could only obtain proliferation of lenticels of immature tubers at 100% r.h., and found that a continuously wet surface was needed on mature tubers.

In the studies described here, an attempt was made to follow the progress of proliferation by making a numerical 'score' (based on the proportion of lenticels proliferated and the degree to which

individual lenticels had proliferated) on several occasions. In this way, different factors influencing proliferation could be investigated. The possibility of differences between tubers of different ages and perhaps between cultivars (although Zeck (1957) found that most of 27 European cultivars behaved similarly) seemed of particular pathological interest, and received special attention.

B. MATERIALS AND METHODS

Growth of tubers

Many of the tubers used were grown out-of-doors in boxes 3 x 4 x 1 ft (0.91 x 1.22 x 0.30 m). Each box was half-filled with soil-less nutrient compost ('Eff' products, Bracknell, Berks.) which was covered by a single layer of nylon net (fine mesh curtain material) stapled to the box sides. Above the net was a layer of about 4 in (150 mm) of sedge peat, in which the sprouted seed tuber was planted. Roots penetrated the net, while tubers formed within the peat layer and could be harvested without skin damage. Each box had about twelve plants either of Majestic (MJ), King Edward VII (KE) or Pentland Crown (PC) grown from Scottish virus-tested stem-cutting (VTSC) 'seed'. These cultivars were chosen as being among the most popular maincrop potatoes in the U.K., and because they differed in susceptibility to common scab.

Microscopic methods

As hand- and freezing microtome-sections of fresh or fixed material were found inadequate, wax embedding and sectioning on a rotary microtome was employed. Tissue was fixed in formol acetic

alcohol with less than the usual concentration of acetic acid (50% ethyl alcohol : 93 ml, glacial acetic acid : 2 ml, commercial formalin : 5 ml), dehydrated in an ethyl alcohol-n-butanol series (Jensen, 1962) and embedded in Paraplast (Shandon Scientific Co.), a paraffin wax-based embedding medium, melting at 56-57°C. As potato tissue proved difficult to embed, the extended procedure of Pratt & Whetmore (1951) was mostly used. Serial sections 10 µm thick were cut and mounted on slides using Haupt's adhesive (Jensen, 1962). As a routine histological stain, safranin, crystal violet, fast green and orange G (Conant's method, Johansen, 1940) gave consistently good results and was the most satisfactory of various procedures attempted. Stained sections were mounted in neutral Canada balsam to make permanent preparations. Cell contents were not usually well-preserved, but this was of relatively little importance.

Definition of tuber internodes

A tuber is an underground stem which grows by the separation of 'eyes' from the apical bud and by the expansion of tissue so formed. Each eye is a stem node consisting of a scale leaf and axillary bud, and is positioned on a phylotactic spiral (Artschwager, 1924). If a tuber is traversed from stolon to apex, progressively younger lenticels are encountered as more recently formed tissue is reached. Because of the importance of lenticel age in scab infection, the position of lenticels has been described relative to the eyes, which were numbered consecutively from the stolon attachment ('heel') end, unless otherwise stated. Tissue between consecutive eyes is an internode and the first internode is normally between the stolon attachment and the first eye (see Fig. 1). If eye 1 is at the stolon attachment, internode 1 will

be between eyes 1 and 2. The exact limits of the internodes cannot be defined, especially near the apex of larger tubers where the internodes are small, but by plotting lines on the tuber surface a reasonable approximation can be made for the older internodes. Thus in Fig. 1, the line between internodes 2 and 3 runs around the tuber linking eye 2 and a point equidistant between eyes 1 and 3. An eye was said to have 'separated' from the apical bud when tissue was visible between them (i.e., a separation of about 1 mm).

When comparing lenticel development on tubers of different sizes it is often necessary to describe internode distances from the apical bud (i.e. in the opposite direction to that defined above). When this has been done, the internode nearest the apical bud (the most recently separated) is described as A - (minus) 1, the second from the apical bud as A -2 and so on.

C. FORMATION AND DISTRIBUTION OF LENTICELS

Anatomical changes

Stolons, very young tubers, and the youngest tissue of growing tubers, bear stomata which are changed to lenticels as the tuber grows. Stomata are anatomically similar to those on leaves, with guard cell ledges projecting over the stomatal aperture, but they are larger (Table 2) and become raised above the level of the epidermis by periclinal division of cells in the first two sub-epidermal cell-layers below the substomatal cavity. A stolon stoma is shown in Plate 1, while in Plates 2-6 successively older lenticels from serial transverse sections of a young PC tuber are shown. Plate 2 from a section close to the apex shows the guard cell initials in the epidermis, while in

Fig. 1. The marking and numbering of tuber internodes

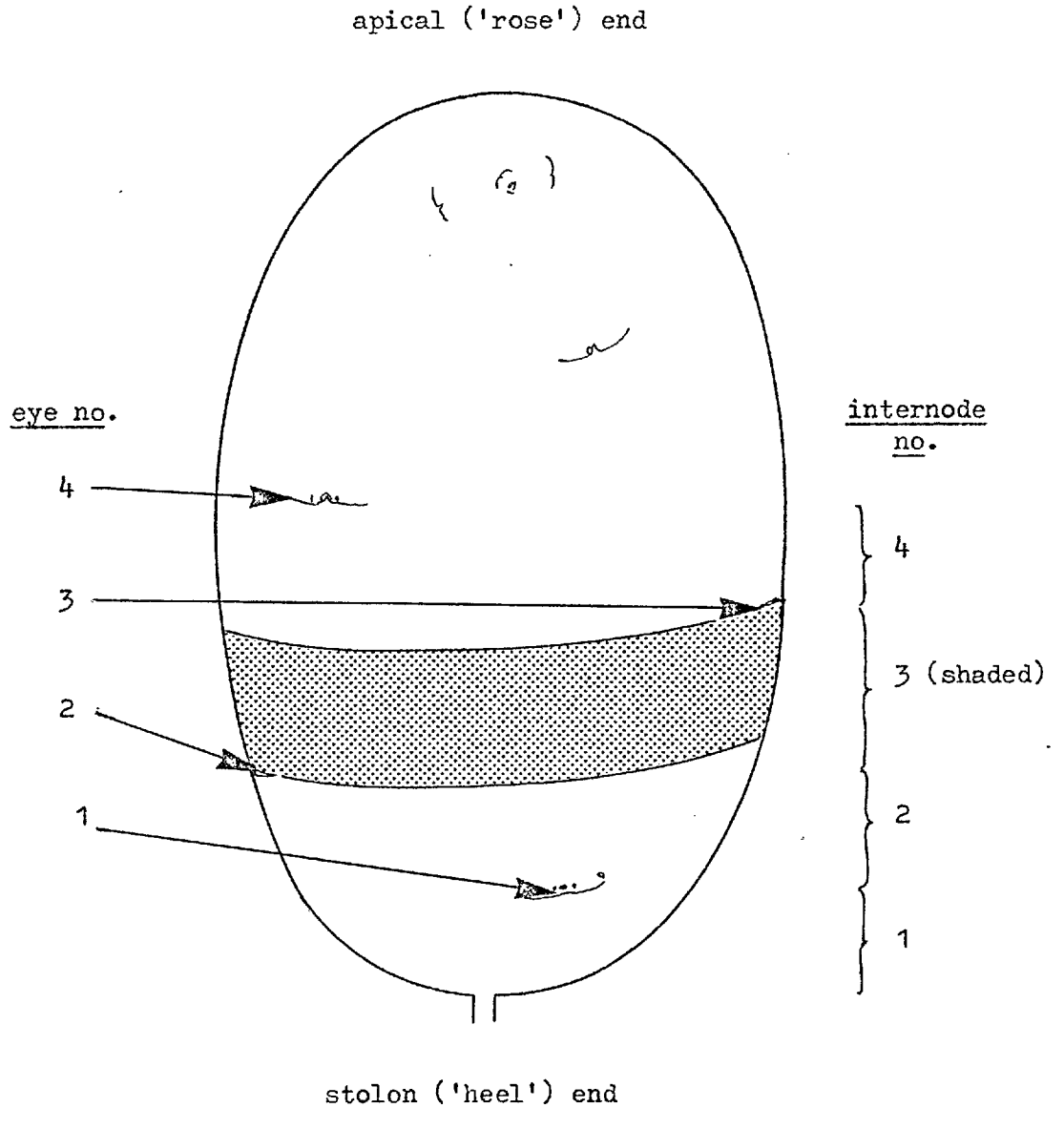


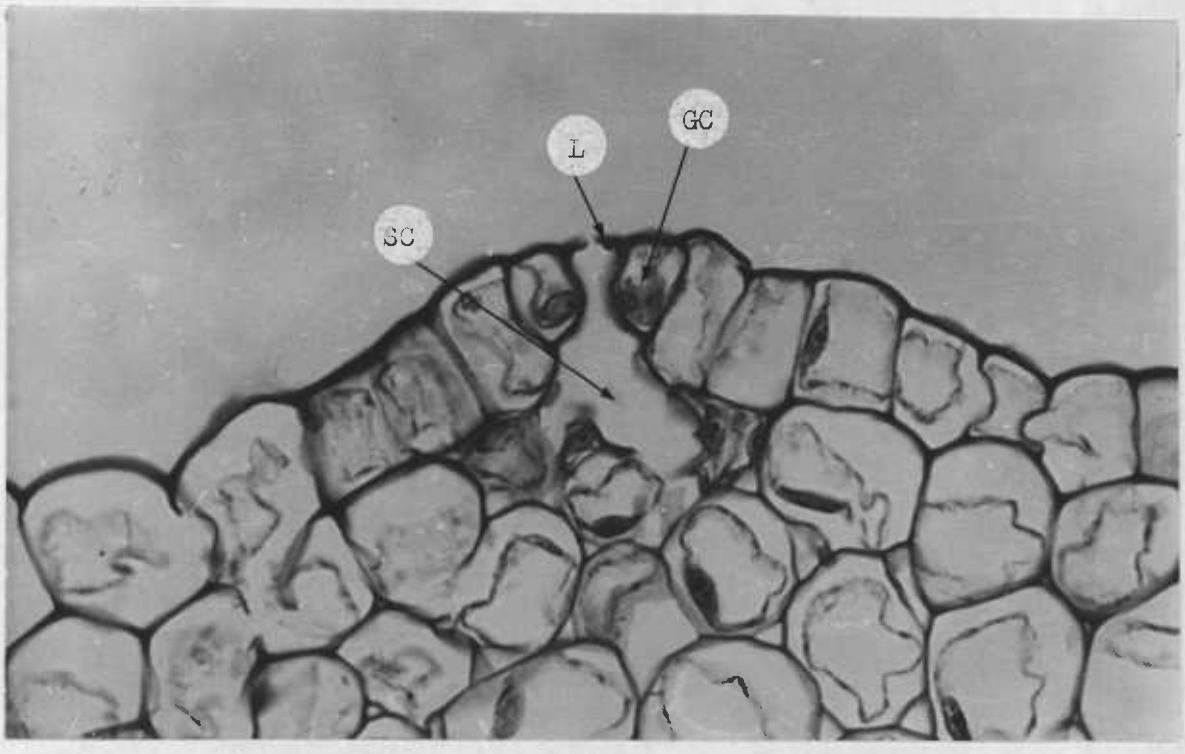
Table 2. The size of potato stomata on the lower leaf surface and on stolons

Cultivar	Mean Stomatal size* (µm)	
	On leaves	On stolons
MJ	40.1 x 24.8	53.6 x 37.6
PC	41.6 x 27.1	72.9 x 55.9
KE	37.2 x 24.8	69.9 x 47.7
S.E. (means)	±1.87 x 0.88	±2.29 x 2.25

*Length x max. width across two guard cells; ten measurements at random on epidermal strips.

Plate 3 the youngest stoma with separated guard cells already has considerable cell division in the subepidermal layer. Division continues and in successively older structures (Plates 4-6) the guard cells are raised above the general surface layer. Division does not become restricted to a single layer of meristematic cells at this stage, but the orientation is such that files of filling cells are produced. At the same time, division in and below the epidermis (see Plate 4 for an early division) results in the development of a periderm several cells thick. Eventually the old guard cells are shed (Plate 7) and suberin is deposited in the walls of the outermost filling cells, and as the tuber swells the lenticels usually become more open, saucer-shaped structures, with filling cells much less obviously arranged than in younger lenticels (Plate 8). A zone capable of meristematic activity continues across the lenticel and may produce proliferation (see this chapter section D page 33) or a cork layer continuous with the outer phellem (Plate 9). This meristematic zone is not anatomically distinct but joins to the phellogen at the outer edges. Lenticel development followed a

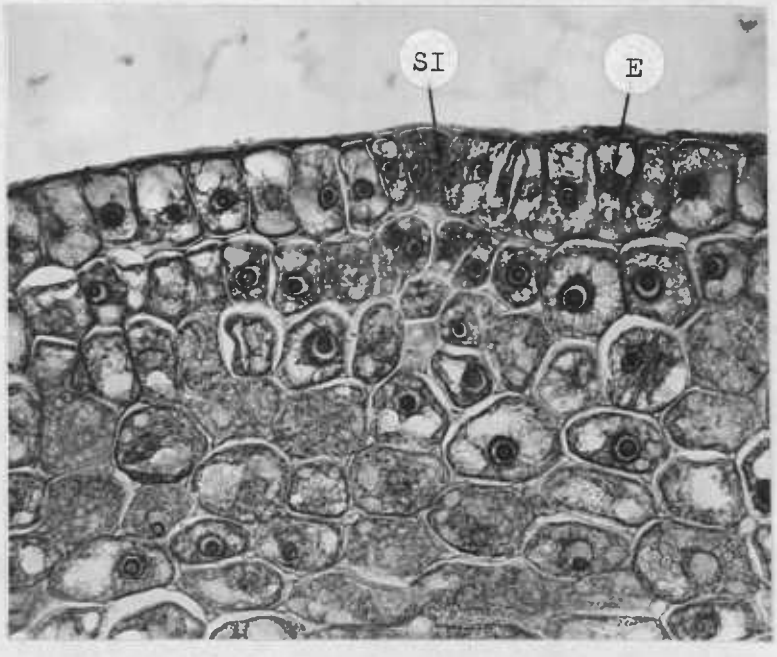
Plate 1. Stoma from TS stolon (PC) x390



PLATES 2-6. Lenticel development (PC):

Successively older structures in vertical section

Plate 2. x390



E	Epidermis	SC	Substomatal cavity
GC	Guard cell	SI	Stomatal initial
L	Ledge of guard cell		

Plate 3. x390

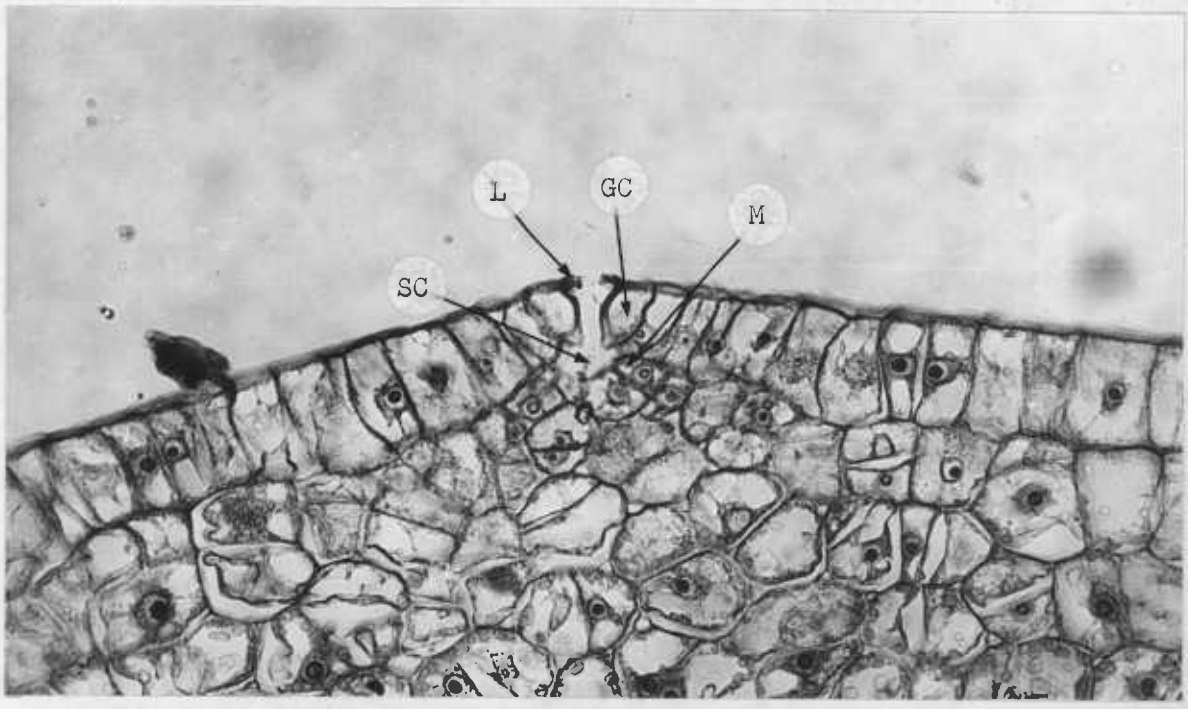
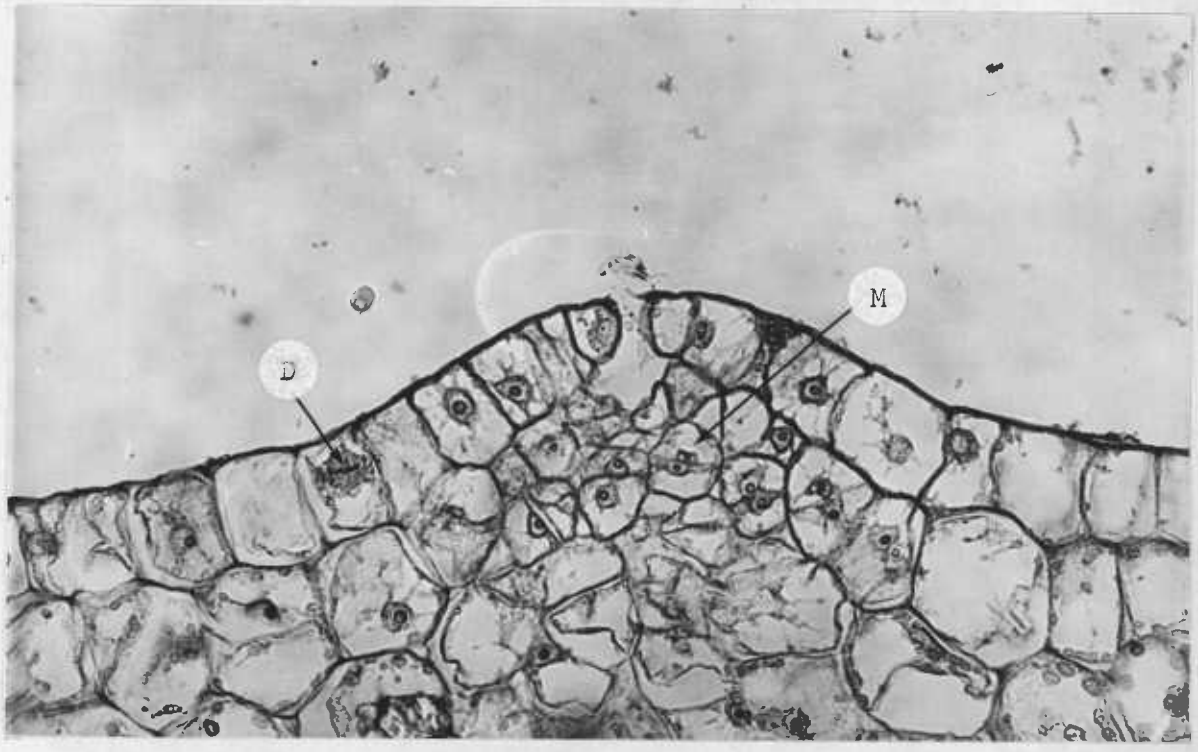


Plate 4. x390



D Dividing cell
GC Guard cell
SC Substomatal cavity
L Ledge of guard cell
M Meristematic region

Plate 5. x390

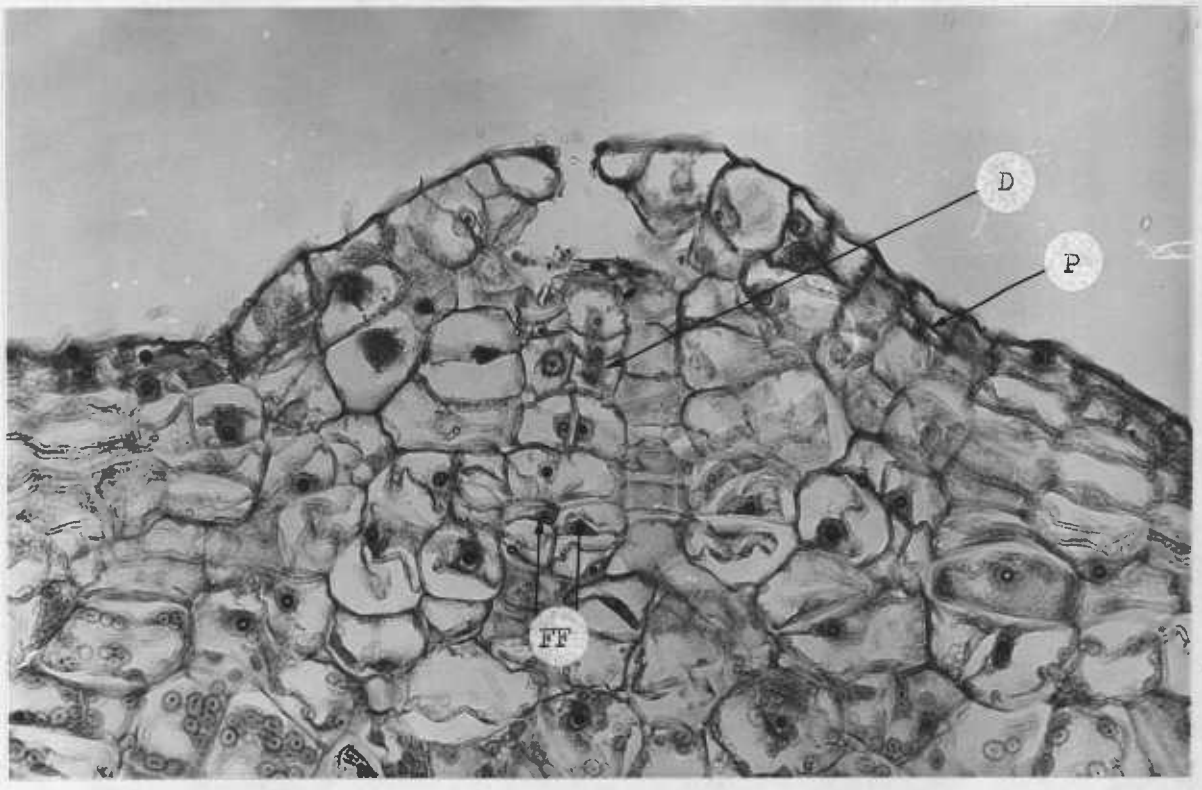


Plate 6. x390

D Dividing cell
FF Files of filling cells
GC Guard cell
P Periderm

Plate 7. MJ Lenticel with unsuberized filling cells VS x200

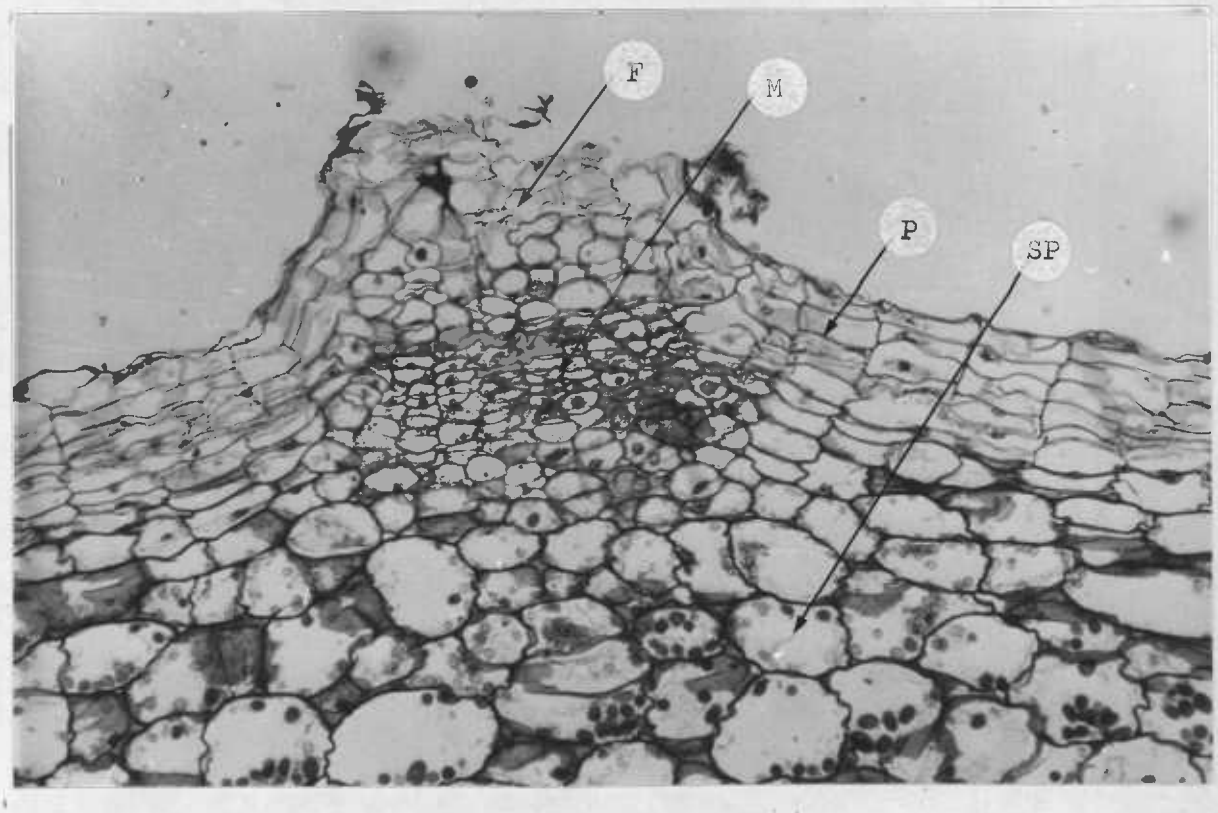
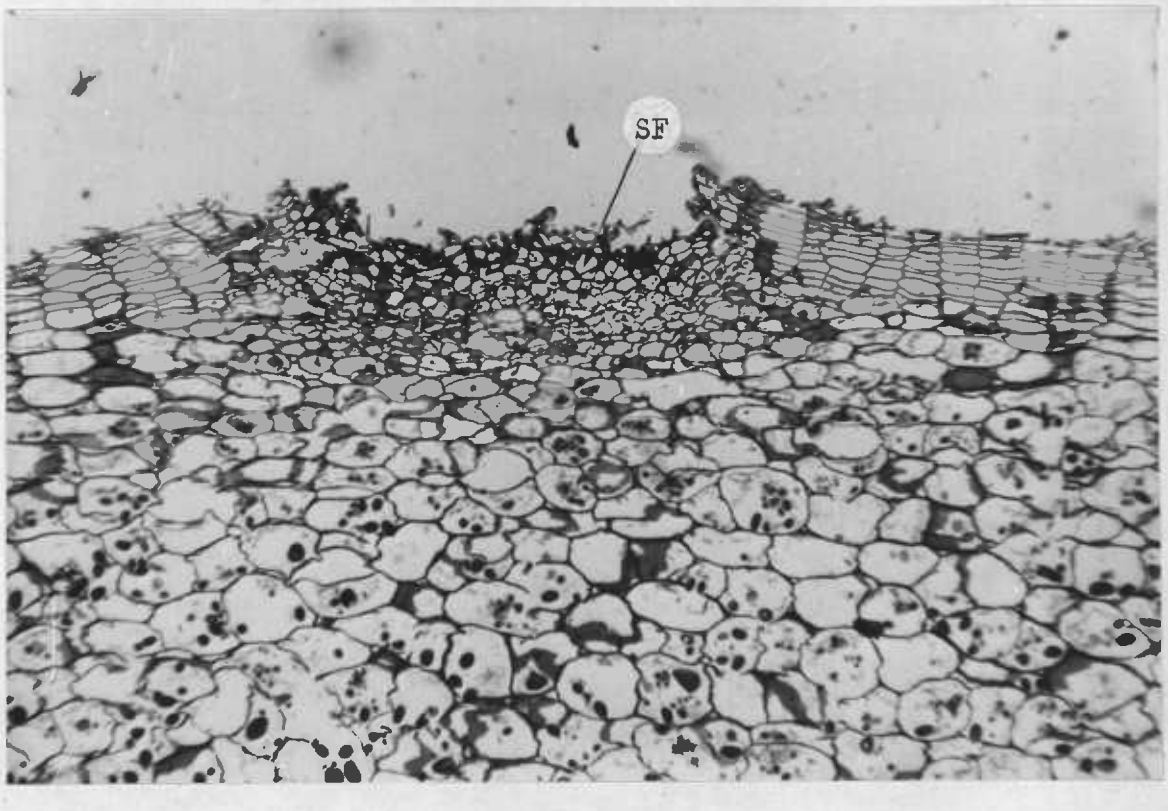
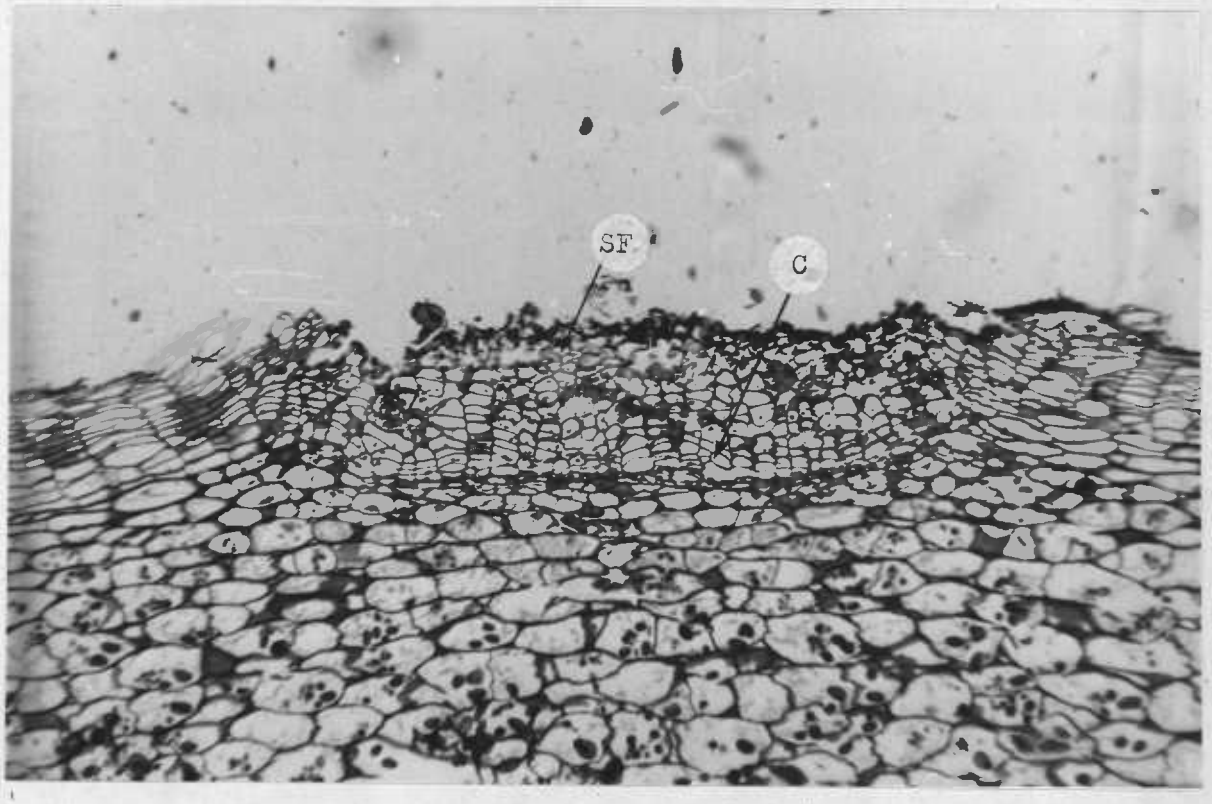


Plate 8. MJ Lenticel with suberized filling cells VS x80



F Unsuberized filling cells
M Meristematic region
SP Starch parenchyma
P Periderm
SF Suberized filling cells

Plate 9. KE Lenticel with cork barrier below filling cells VS x80



C Cork barrier below lenticel
SF Suberized filling cells

similar sequence in the three cultivars examined.

Morphological changes

The change of stomata to lenticels was also followed by observation of the tuber surface using a dissecting microscope and the scanning electron microscope. With the former, guard cells are seen to be raised on small humps which are larger on older tissue and give way to crater-like structures from which the guard cells have been lost, and which contain dark-coloured filling cells.

Several attempts at scanning electron microscopy were made. Sometimes freshly-harvested material was directly coated with gold

in vacuo and sometimes the material was infiltrated with polyethylene glycol (Idle, 1971) prior to coating. Detail was not generally good, perhaps because of changes during specimen preparation and perhaps because of the natural irregularity of the tuber surface, but the features noted by other methods were confirmed. Stomata on the youngest part of a small tuber are shown in Plates 10 and 11, while the lenticel of Plate 12 had already lost its guard cells. Plate 13 shows an older lenticel, only very little raised above the tuber surface, and illustrates the irregular aspect of the surface. Critical point drying did not improve specimen appearance.

Attempts were made to replicate fresh surfaces using Cellofas (Eckert & Caveney, 1970), polystyrene (Chapman, 1967) or nail varnish films to obtain an impression of the surface, and then to make 'positives' with a second type of film, which were then coated with gold and examined. Although these methods gave good results on leaf surfaces, films tended to stick to the tuber surface and very little detail was preserved.

Lenticel formation relative to internode position

Because lenticel age appears to be important in determining scab susceptibility, the development of lenticels relative to their position on the tuber surface (which indicates their age if the rate of tuber eye separation is known) was studied using tubers from the peat boxes during 1971. Tubers were carefully washed in running tap water, measured, and the number of internodes counted. The number of internodes on which stomatal guard cells were visible was recorded from observation with a dissecting microscope, and pieces of the various internodes were cut out and fixed for microscopy. Sections

PLATES 10-13. Scanning electron microscope photographs of the tuber surface (specimens coated with gold, except in Plate 11)

Plate 10. Stoma on Maris Piper tuber x1150

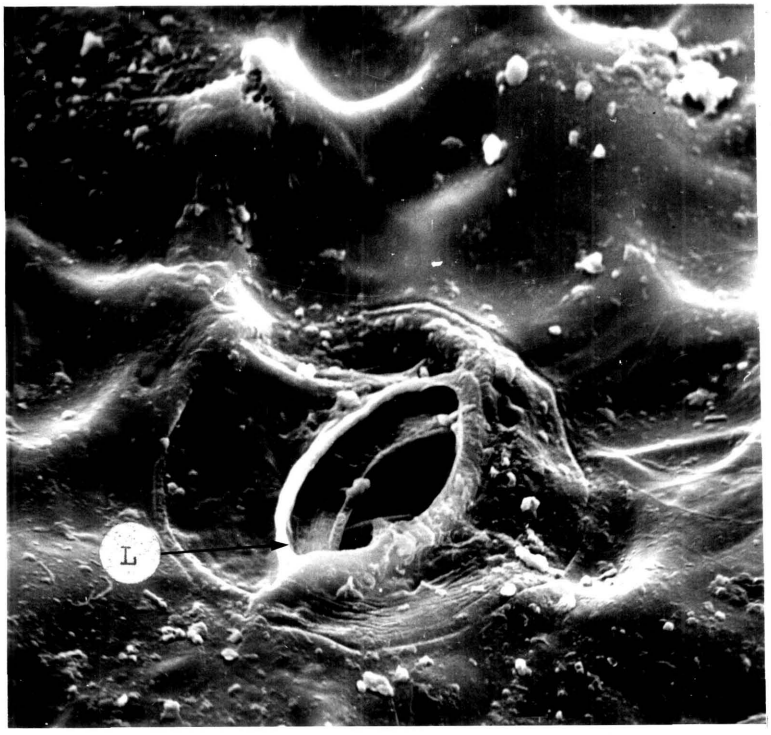
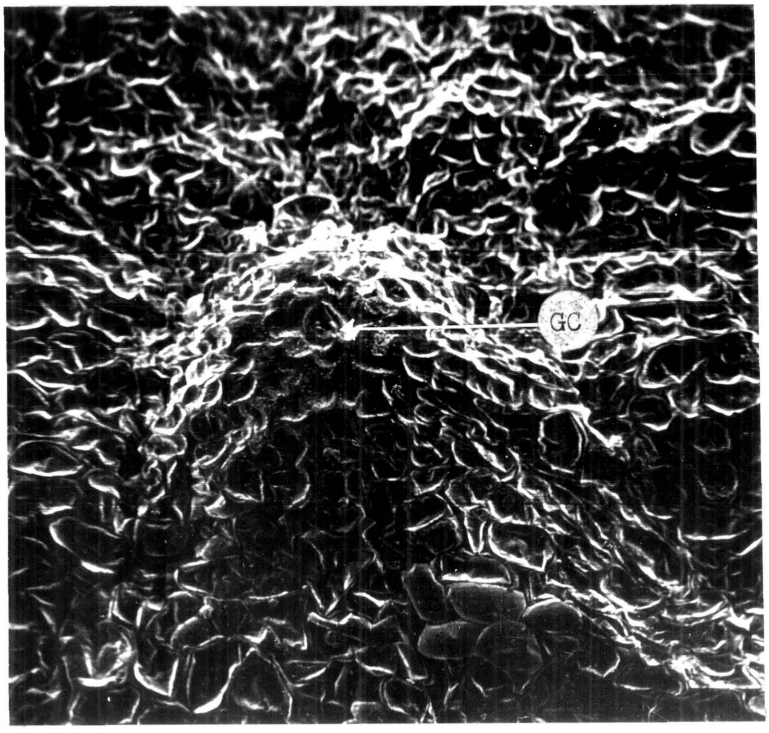


Plate 11. Raised stoma (KE) PEG infiltrated x125



GC Guard cell L Ledge of guard cell

Plate 12. MJ Lenticel with shed guard cells PEG infiltrated x220

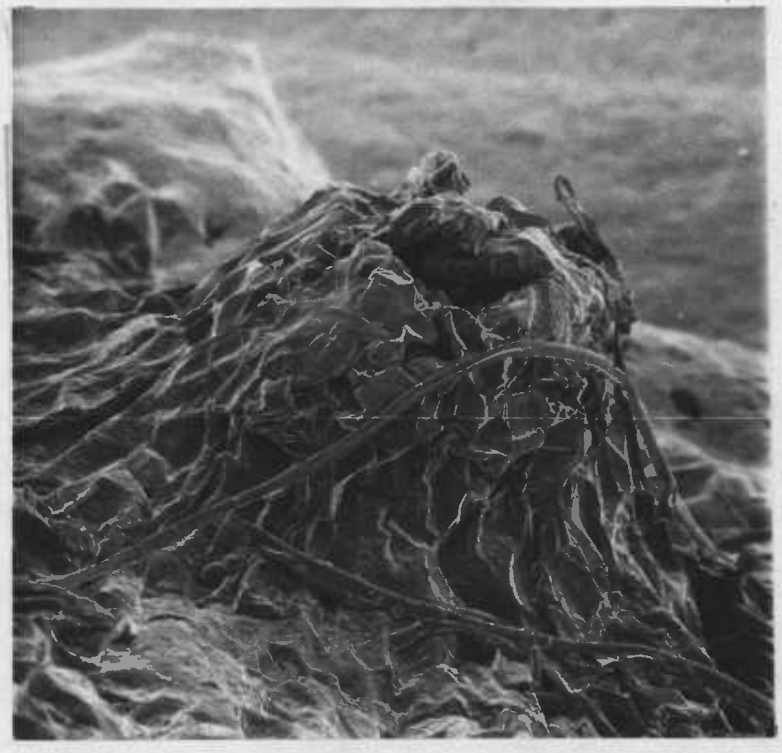
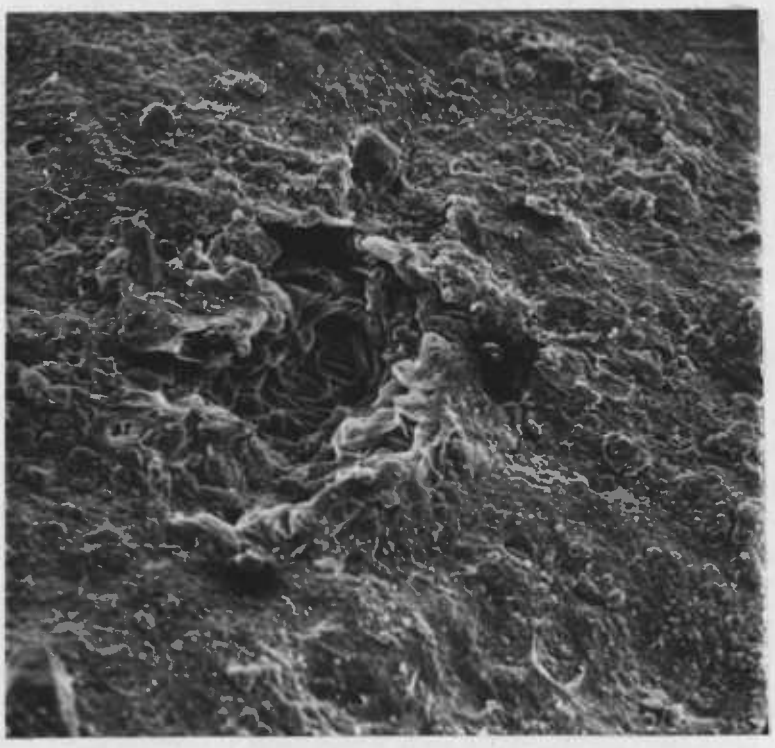


Plate 13. Older lenticel (cv. Maris Piper) x210



at right angles to the tuber surface were examined for the presence of stomatal initials, stomata and lenticels, and the suberization of the filling cells was assessed according to the intensity and extent of the red stain (safranin) in the cell walls. This method was in reasonable agreement with studies of suberization by other methods (see section D, this chapter page 36). Median vertical sections of several lenticels were examined from each internode and allocated to one of the following categories:

0 Suberization none or slight and irregular

1 Suberization evident but not continuous across the walls of the outer filling cells

2 Suberization of walls of outer cells faint but perhaps continuous when viewed at x1000 magnification

3 Suberization obvious and continuous at x400 magnification and below

Stomatal initials were frequent on tubers with only 1-2 internodes, and often extended over the apical half of the youngest internode (A -1), before the guard cells separated. On larger tubers, A -1 became increasingly difficult to define and the occurrence of initials could not therefore be prescribed.

Table 3 summarizes the observations on the occurrence of stomata on tubers of different sizes. Most tubers had the youngest 1-2 internodes (A -1, A -2) bearing stomata, but there was longer persistence on some tubers. Loss of stomatal guard cells appeared to be more erratic and may sometimes have occurred later in PC than in KE or MJ.

The observations on suberization are summarized in Table 4. Each figure represents the mean score from several lenticels from

Table 3. The distribution of stomata on growing tubers of different cultivars

Cultivar	Tuber size*	No. of tubers examined	Number of tubers with stomata present on: A -1 only	A -1 & 2	A -1, 2 & 3	A -1, 2,3&4
	1	0	0	-	-	-
	2	9	4	5	-	-
	3	17	7	10	0	-
MJ	4	13	2	11	0	0
	5	6	3	3	0	0
	6	3	0	3	0	0
	7	2	0	2	0	0
	Total	50	16	34	0	0
	1	4	4	-	-	-
	2	11	0	11	-	-
KE	3	14	5	4	5	-
	4	13	2	11	0	0
	5	6	2	4	0	0
	6	2	0	2	0	0
	Total	50	13	32	5	0
	1	1	1	-	-	-
	2	4	1	3	-	-
	3	11	1	6	4	-
PC	4	9	2	6	0	1
	5	2	0	1	0	1
	6	3	0	1	1	1
	7	1	0	0	1	0
	8	1	1	0	0	0
	Total	32	6	17	6	3

* Number of internodes

Table 4. Progress of 'lenticel' suberization on growing tubers of different sizes*

Tuber size (total number of internodes)	Internode number (from apical bud) A -1	A -2	A -3	A -4	A -5	A -6	A -7	A -8
1	0,0	-	-	-	-	-	-	-
2	0,0,0	0,0,0	-	-	-	-	-	-
3		0,0,½,½	0,0,0	-	-	-	-	-
4		0	0,1,1½	½,1,1,2	-	-	-	-
5		1,1½	0	2,2½	1	-	-	-
6				1½,1	2	3	-	-
7				1½	2	3		-
8								3,3,3
9				1½	3,3		3	
Mean	0.0	0.4	0.4	1.5	2.2	3.0	3.0	3.0

* Each figure is the mean suberization score of several lenticels from this internode; results from three cultivars included in the one table (see text for scoring system)

one piece of tissue, and the results from the three cultivars have been included in the same table as each shows the same pattern. The stomata and lenticels on A -1, A -2, and A -3 were little suberized, but on A -4 and A-5 increasing suberization was evident, forming a 'complete' layer by A -6.

The further development of lenticels on the same three cultivars was recorded from field samples in 1971 and 1972 and these results are reported in chapter 5C (page 108).

Lenticel distribution

The number and density of lenticels on different internodes of tubers of varying size was investigated to see if secondary lenticels (those not developing from stomata) appeared as tubers grew and whether lenticel number was similar on different internodes. MJ tubers from the peat boxes were carefully washed and cut into internode pieces. The size of each internode and the number of lenticels it bore was recorded, although apical internodes of larger tubers had to be ignored, because of their small size.

Table 5 shows the mean number of lenticels (or in a few cases stomata) in the different internodes, and Table 6 the lenticel density for the same data. Internode 1, nearest the stolon, has been omitted as it represents a variable part of an internode

In Table 7 the number of lenticels on each internode, irrespective of tuber size has been calculated. The number of lenticels on a particular internode did not increase as the successively larger tubers were examined, indicating that secondary lenticels were not formed, and consequently lenticel density decreased. Later formed internodes (those nearer to the apex) had progressively fewer lenticels, but since they expanded less, lenticel density remained

Table 5. Lenticel numbers on the different internodes of MJ tubers of differing size

Tuber size (total no. internodes)	No. of tubers examined	Internode number from stolon						
		2	3	4	5	6	7	8
		Mean no. of lenticels						
2	1	56						
3	1	60	47					
4	3	50	36					
5	4	63	61	35				
6	6	60	47	31				
7	6	36	36	28	19			
8	4	41	38	30	18	20		
9	2	24	28	23	14	14	8	
10	1	19	10	8	8	10	3	11
11	2	25	32	31	31	19	17	10

Table 6. Lenticel density on internodes of MJ tubers of differing size

Tuber size (total no. internodes)	Internode number from stolon						
	2	3	4	5	6	7	8
	Lenticel density mm^{-2}						
2	0.65						
3	0.87	0.80					
4	0.23	0.56					
5	0.13	0.23	0.40				
6	0.09	0.17	0.43				
7	0.06	0.06	0.12	0.24			
8	0.04	0.05	0.08	0.16	0.36		
9	0.04	0.05	0.06	0.07	0.32	0.10	
10	0.03	0.04	0.02	0.03	0.04	0.03	0.12
11	0.03	0.03	0.03	0.04	0.05	0.06	0.07

Table 7. Lenticel numbers on internodes of MJ tubers

Internode no.	Number of internodes examined	Mean number of lenticels	Coefficient of variation %
1	30	33.2	49.5
2	30	45.7	37.8
3	28	40.5	37.1
4	24	28.9	34.0
5	14	18.9	51.9
6	7	16.3	54.5
7	4	11.2	84.8
8	3	10.0	17.3

larger than on the older parts of the tuber. Small samples of the later-formed internodes contribute to their large variability.

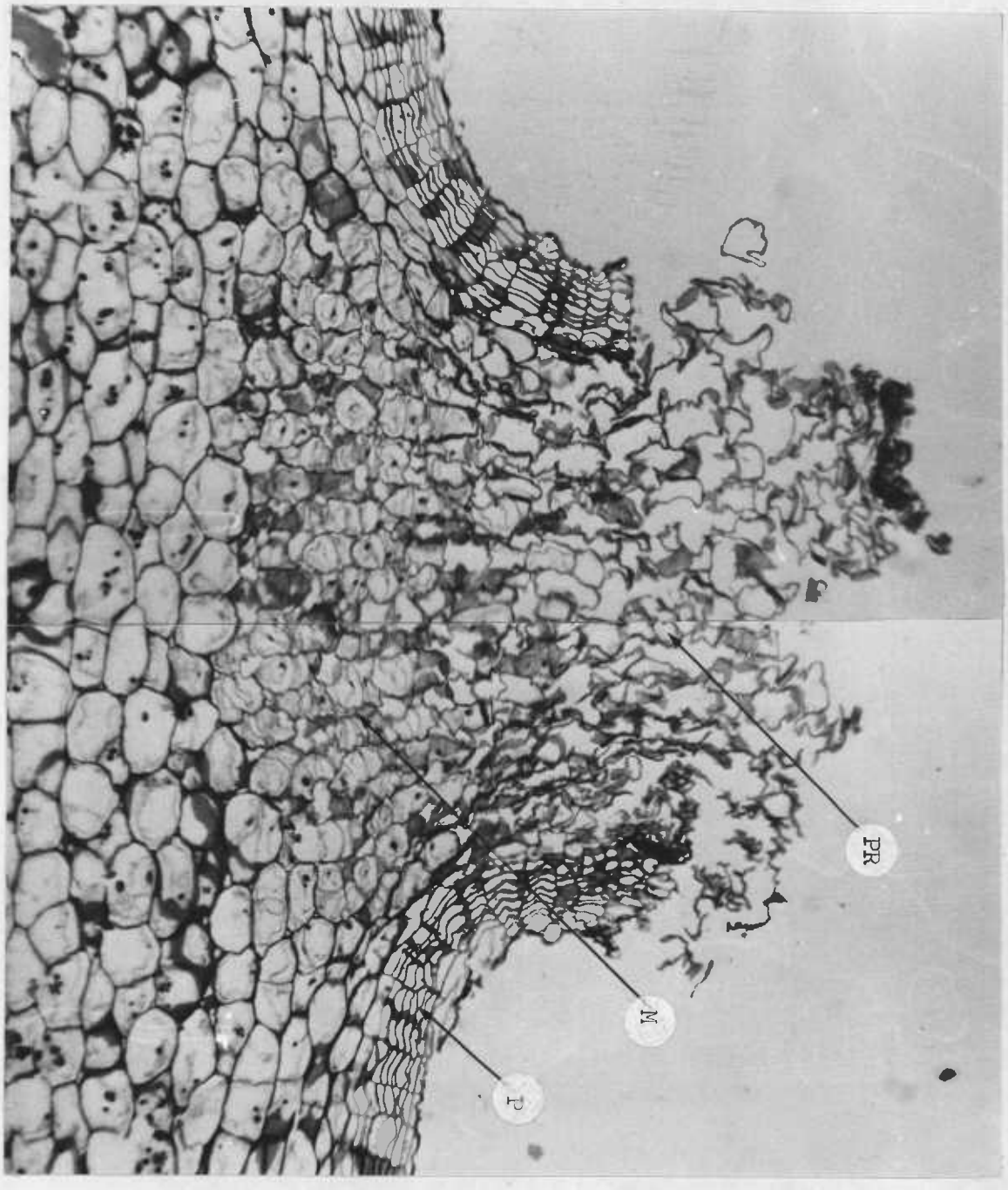
The density of lenticels on the youngest tuber internodes is of the same order as stomatal density estimated from epidermal strips of stolons (0.41 mm^{-2}) but is much less than that of the lower leaf surface (98.7 mm^{-2}).

D. RESPONSE TO WET AND DRY CONDITIONS

Anatomical changes

Vertical sections through lenticels which had proliferated in the field, or which had been induced to proliferate in the laboratory (see next subsection), were studied. The proliferated tissue visible to the naked eye was composed of large thin-walled cells and was produced from tissue at the edge of the cortex underlying the lenticel (Plate 14). Cell division appeared to take place within a more or less saucer-shaped zone, and was not confined to a single layer of meristematic cells. Division was not only under the

Plate 14. Proliferating lenticel (MJ) VS x80



M Meristematic region
P Periderm
PR Proliferated filling cells

Plate 15. Lenticel dried after proliferation (MJ) VS x80

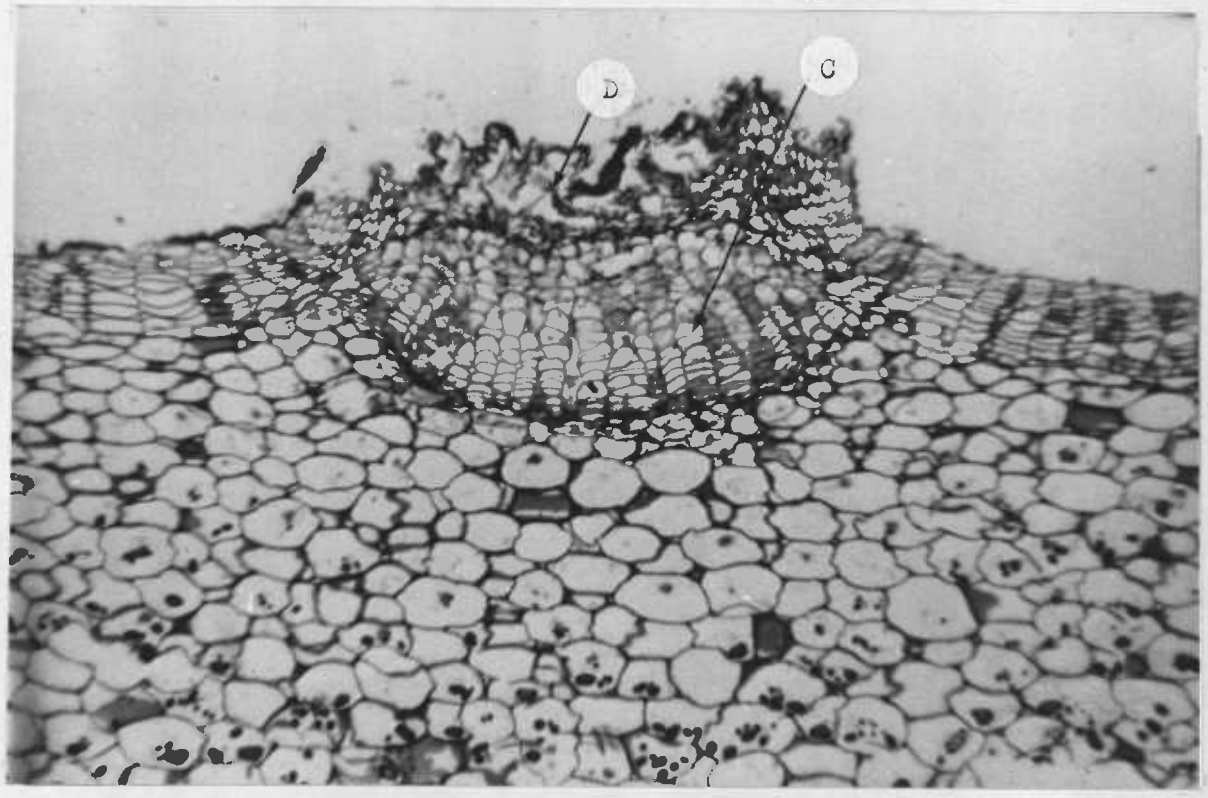
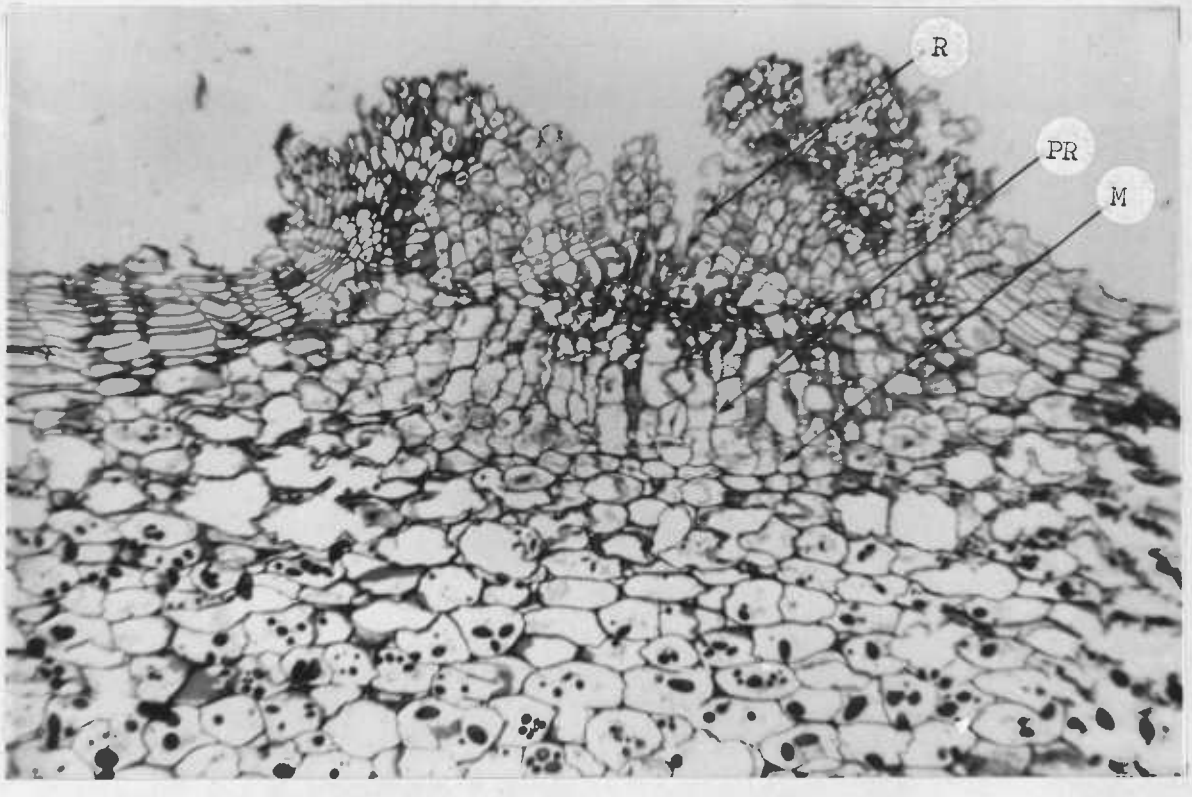


Plate 16. Reproliferating lenticel (KE) VS x80



C Cork barrier below lenticel
D Dead proliferated cells
R Ruptured cork barrier
M Meristematic region
PR Proliferated filling cells

existing lenticel, but also beneath the surrounding periderm, so that the lenticel opening was enlarged by proliferation. Division was more frequent in the centre of the lenticel, so that the proliferated tissue often split into four segments. When proliferated lenticels were dried, the outermost proliferated tissue collapsed, suberin was deposited in the walls of filling cells beneath the collapsed tissue, and eventually a cork-barrier was formed beneath the lenticel in the region where cell division had been occurring (Plate 15).

The deposition of suberin in the walls of filling cells was investigated more closely using MJ tubers from peat boxes in August 1972. Ten tubers were wrapped in wet paper towelling, incubated for 12 days at 15°C to induce proliferation, and were then incubated unwrapped at 15°C. At intervals, lenticels were sampled, fixed, embedded and sectioned. Three slides were produced for each lenticel, one of which was mounted unstained in a non-fluorescent mounting medium, one was stained for 24 h in sudan IV (saturated solution in 70% ethanol), washed in water and mounted in 50% aqueous glycerol, while the third was stained by the usual Conant method, The stained slides were examined for the presence of red-staining cell-walls, while the unstained slides were examined by incident light ultra-violet fluorescence microscopy. For the latter, the exciting filters UG1 (2 mm) and BG38 (4 mm) were used with 400 and 430 nm supression filters, when a light blue fluorescence was emitted from suberized cell walls (Mader, 1954). In each of the staining methods, suberization was recorded on a scale 0-3 (as in section C, page 28) and the results are summarized in Table 8.

The methods gave slightly different results, but clearly indicated a complete suberin layer over the walls of outer filling

cells 5 days after removal to dry conditions. By this time some lenticels showed the beginnings of a cork barrier below the filling cells, which was itself beginning to suberize after a further 2 days.

Table 8. Suberization of proliferated lenticels allowed to dry (mean score)

Days from drying	No. lenticels examined	Suberization detected by: Fluorescence microscopy	Sudan IV staining	Conant staining
0	5	0.0	0.0	0.0
1	7	0.9	0.0	1.1
3	7	1.3	0.4	1.9
5	9	2.9	3.0	3.0
7	9	3.0	3.0	3.0

Proliferation in laboratory experiments

Methods. Fox et al. (1971) reported that lenticels could be proliferated by wrapping tubers in wet paper towelling, and this method was used in these studies to investigate proliferation under various conditions. Tubers were washed, dried, and then about three-quarters of the surface was wrapped in a double layer of absorbent paper towelling moistened with distilled water, leaving the 'rose' end exposed. This method produced proliferation where the tuber had been wrapped, but only rarely did proliferation appear restricted to a particular part of the tuber that was covered. In some experiments, a few tubers were lost by soft-rotting, although tubers wrapped in this way seemed less liable to rot than if the tubers had been completely wrapped in towelling. The wrapped tubers were placed in damp chambers made from wooden seed boxes lined with Polythene sheet and wet paper towelling and these boxes were wrapped in black Polythene sheet to

exclude the light. At intervals, sometimes up to 9 weeks after wrapping, each tuber was examined and an estimate made of the proportion of lenticels in each of the following categories:

0 No proliferation

1 Lenticels swollen but proliferated tissue not yet showing, or lenticels flush with tuber surface but showing fresh white parenchyma

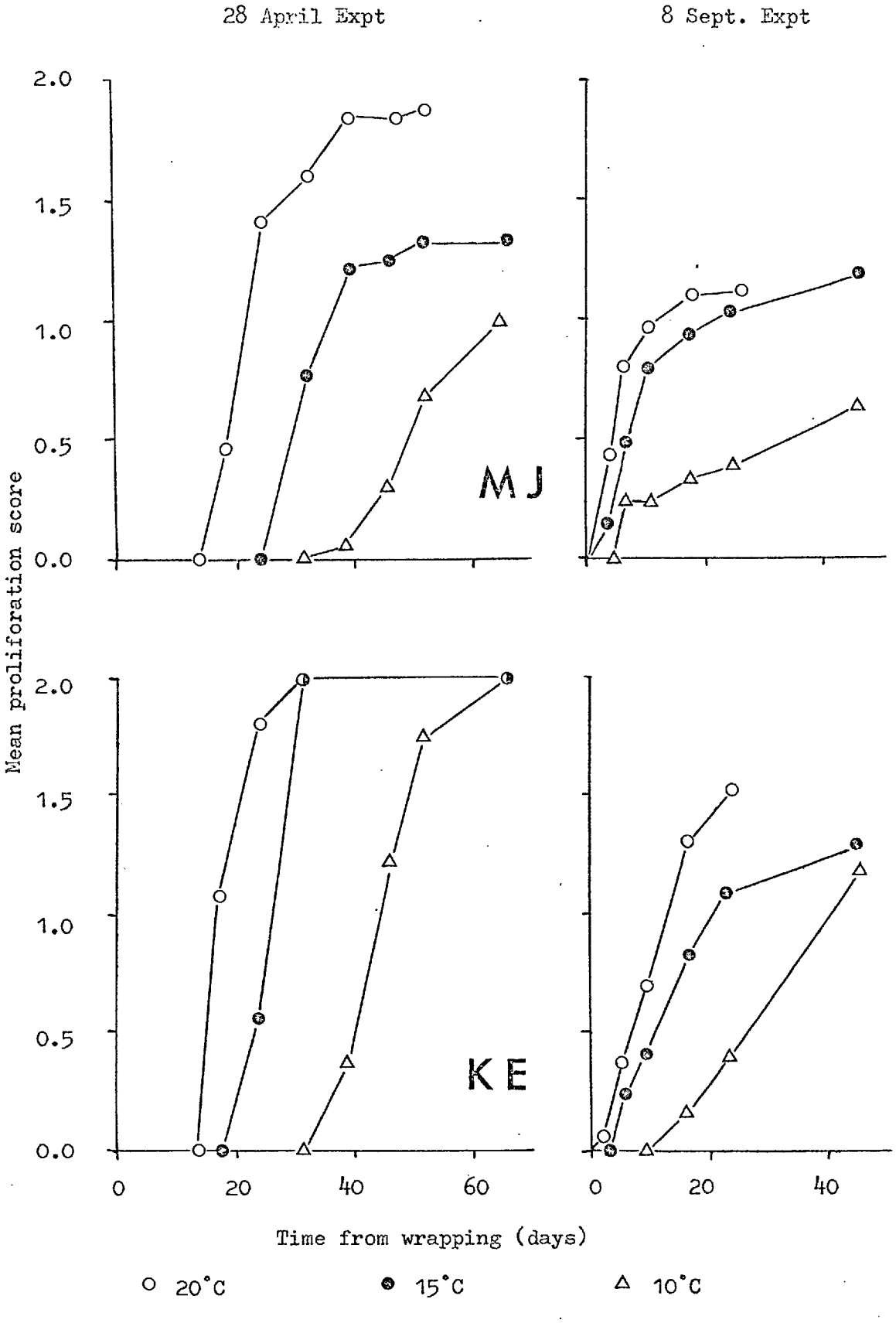
2 Lenticels well-proliferated with extruded white callus-like tissue

In most experiments, lenticels with slight proliferation were recognized as a separate category (score $1\frac{1}{2}$). Since there were differences between lenticels and between tubers in the time taken to proliferate and in the rate of production of proliferated tissue, a mean score was calculated to represent each sample date and treatment, usually based on ten tubers.

Effect of temperature. In two experiments started on 28 April 1972 on stored tubers and 8 September 1972 on freshly harvested tubers, proliferation on KE and MJ at different temperatures was compared. No proliferation occurred in 66 days at 5°C whereas at 10, 15 and 20°C the proliferation score increased more quickly and proliferation began sooner after wrapping as temperature increased, at least in the first experiment (see Fig. 2 where mean scores have been plotted against time). Proliferation started earlier in the second experiment than the first but the scores increased more slowly and tended to stop at a smaller value, while in both experiments proliferation scores of KE eventually exceeded those of MJ at all temperatures.

Effect of sprouting. In experiments on stored tubers, proliferation often seemed to appear earlier on sprouting tubers than on non-sprouting ones of the same cultivar. This was investigated in two experiments

Fig 2. The effect of temperature on proliferation of KE and MJ in two experiments



started on 19 November 1971 and on 7 January 1972, in which tubers of KE, MJ and PC had eyes excised to prevent sprouting or had a small slice cut from the 'heel' end to induce sprouting. In the second experiment some tubers were also both cut and de-eyed, although the MJ ones soft-rotted and yielded no data. The mean proliferation scores shown in Table 9 indicate that proliferation started earlier and scores increased faster in those tubers allowed to sprout. The cut and debudded treatment of the 7 Jan. experiment shows that wounding may have contributed to, but did not totally account for, this result. These conclusions are supported by each of the three cultivars although these differed from one another (see section below on cultivar differences).

Reproliferation. After one experiment, begun on 19 August 1971 and run for 18 days, tubers with proliferated lenticels were allowed to dry for 7 days and the tubers were then re-wrapped and incubated in the usual way. After 2 weeks, proliferation of lenticels appeared on some KE tubers (lenticels had initially proliferated within 5 days of wrapping in this trial) but even after 28 days, activity was not resumed on PC or MJ. Sections of reproliferating lenticels were examined (Plate 16 page 35 shows an early stage) and showed that proliferation re-started in cells beneath the cork barrier formed when the lenticels dried.

Effect of cultivar and tuber age. In various experiments, differences between cultivars and especially between experiment dates were noted. From graphs of proliferation scores plotted against time, the time taken to reach a mean score of 1.00 was estimated as a parameter for comparative purposes. These results are shown in Table 10 for various experiments in 1971/2 (at laboratory temperature of about 20°C) and 1972/3 (at 15°C). On tubers of increasing age, proliferation became progressively 'slower' (i.e. longer time to

Table 9. Lenticel proliferation on sprouting and de-eyed tubers (mean scores)

Experiment & cultivar		Treatment	Time from wrapping (days) 3	6	10	14	18	24*	31	40	45
Expt 1	KE	sprouted	0.45	0.71	1.14	1.17	1.54	1.76	1.86	-	1.99
		de-eyed	0.10	0.14	0.15	0.15	0.25	0.29	0.49	-	1.16
	MJ	sprouted	0.00	0.05	0.08	0.10	0.19	0.29	0.65	-	1.38
		de-eyed	0.00	0.00	0.00	0.03	0.03	0.07	0.08	-	0.50
	PC	sprouted	0.00	0.00	0.00	0.05	0.05	0.14	0.90	-	1.28
		de-eyed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-	0.00
Expt 2	KE	sprouted	0.00	0.05	0.21	0.66	1.52	1.78	1.86	1.90	-
		de-eyed	0.00	0.00	0.02	0.29	0.44	0.83	1.08	1.17	-
		cut & de-eyed	0.00	0.01	0.03	0.22	0.55	0.94	1.00	1.33	-
	MJ	sprouted	0.00	0.02	0.06	0.06	0.17	0.59	1.02	1.19	-
		de-eyed	0.00	0.00	0.00	0.06	0.10	0.21	0.28	0.29	-
	PC	sprouted	0.00	0.00	0.00	0.06	0.21	0.69	0.81	1.06	-
		de-eyed	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
		cut & de-eyed	0.00	0.00	0.02	0.02	0.07	0.13	0.20	0.32	-

* 25 days for KE and MJ in experiment 2

reach score of 1.00) in both seasons, but was always 'slower' in 1972/3 than in the previous season. KE always proliferated 'faster' than MJ or PC in 1971/2 but this effect was not found in the second season. The difference between seasons is unexplained but may have resulted from differences in growing conditions influencing lenticel physiology and the difference in temperatures.

Table 10. Effect of cultivar and tuber age on time (days) to reach a mean proliferation score of 1.00

Date	Cultivar		
	KE	MJ	PC
21 July 1971	<2	7	-
19 August	4	18	7
19 November*	9	38	34
7 January 1972*	16	31	38
28 April (@ 15°C)	26	36	-
26 July 1972	19	12	>47
8 September	22	22	-
1 January 1973	>41	>41	c.78

* deliberately sprouted tubers

In 1972/3 data were limited by much soft-rotting, but results suggested that differences between cultivars may not have been as reproducible as appeared at first. Further observations on the proliferation of different cultivars were made on field samples, and are reported in chapter 5C (page 108).

E. LIQUID UPTAKE

Dye penetration and lenticel anatomy

An attempt was made to test the permeability of lenticels to dye

solutions in the hope that this would yield information on lenticel structure and be a useful indicator of susceptibility to disease as in apple fruit lenticels (Clements, 1935; Edney, 1956). In exploratory tests an aqueous solution of safranin O was used, but for most of the work a 1% aqueous solution of fluorescein (sodium salt) was employed. Tubers were immersed in the dye solution for different periods depending on the experiment, then washed, dried and examined. With safranin, tubers had to be cut vertically through a lenticel to detect penetration, while fluorescein was more sensitive and could be followed by its fluorescence in ultra-violet light either without cutting the tuber, or by taking a thin peeling and observing from the underside.

After immersion for a few hours, some lenticels on most tubers had been visibly penetrated by dye to varying extents. On several occasions, lenticels which were clearly penetrated and those which were not, were cut out, fixed, embedded, sectioned and stained. Serial vertical sections of the lenticels were examined to relate anatomy to dye penetration, but no consistent relationship was evident. Some lenticels which were penetrated had cork barriers formed beneath, but since the cork of the periderm does not allow dye penetration it must be assumed that the barriers of these lenticels were incomplete or damaged. Conversely, only some of the non-penetrated lenticels had cork barriers.

The effects of proliferation on lenticel permeability was studied in several experiments, with rather variable results. Two experiments in November 1971 used KE tubers grown at Rothamsted (Whittlocks field from VTSC 'seed') and stored at +2°C since harvest on 16 September. In both experiments ten tubers per treatment were either wrapped in wet paper towelling to induce proliferation or stored at 73% r.h. over

saturated ammonium chloride and potassium nitrate solution (McLean & Ivimey Cook, 1952). Incubation was in the dark at laboratory temperature for 8 days (4 Nov. experiment) or 14 days (23 Nov. experiment), and in both experiments some tubers were lost by soft-rotting. Tubers were soaked in fluorescein solution for 4 h, washed, examined under ultra-violet light without cutting or peeling, and the number of lenticels penetrated and the extent of penetration of each lenticel was recorded. In both experiments proliferation on the wrapped tubers was very variable in degree and number of lenticels proliferating between replicate tubers. The results (Table 11) showed large variation, especially in the second experiment, and only in the earlier one was there a difference between treatments in the number of lenticels penetrated, but not in the size of the area penetrated. It appears that a proliferated lenticel is more permeable to a dye solution, and this was investigated further in the following experiment.

Table 11. The effect of proliferation of lenticels on their permeability to dye solution

Date.....	4 Nov.		23 Nov.	
	Mean no. lenticels penetrated per tuber	Mean size penetrated area (mm)	Mean no. lenticels penetrated per tuber	Mean size penetrated area (mm)
Wrapped tubers	45.0	1.49	16.5	2.35
73% r.h.	28.8	1.50	11.0	1.09
S.E. (means)	± 4.75	± 0.270	± 3.38	± 1.337

Liquid uptake and tuber turgidity

Lenticel structure may influence penetration of liquids through these openings, but water uptake may also depend on the physiological

condition of the tuber and may enter structures other than lenticels. This was investigated in an experiment using MJ tubers freshly-harvested from peat boxes on 16 August 1972. The tubers were washed, blotted dry, numbered and weighed and then allocated to one of three treatments: (a) storage at 52% r.h. over saturated sodium bisulphate solution (McLean & Ivimey Cook, 1952) in the dark, (b) storage at 100% r.h. over distilled water in the dark, or (c) wrapped in damp towelling to induce proliferation. Ten tubers were allocated to each treatment and the experiment run for 8 days, after which they were removed, blotted dry and reweighed. A few tubers had rotted and were discarded, while the remainder were soaked in fluorescein for $3\frac{1}{2}$ h, washed in tap water, blotted dry and weighed for a third time. Each tuber was then examined under ultra-violet light and the number of lenticels penetrated by dye, and the diameter of each penetrated area (to the nearest mm) recorded. The total number of lenticels on each tuber was also recorded.

Tubers stored at 52% r.h. lost weight during storage, whereas those from other treatments remained more or less constant, or even gained in weight. There were some differences in uptake during soaking (Table 12), but although the tubers stored at 52% r.h. tended to gain more, differences were not of an order corresponding to previous weight losses. The results have been related to tuber fresh weight as larger tubers tended to change weight to a greater extent.

Table 12. Liquid uptake by tubers previously stored under different conditions

Storage treatment	Mean weight loss in store (mg/g f.wt)	Mean weight uptake in soak (mg/g f.wt)	Mean % lenticels penetrated (angles)	Mean size penetrated (mm)
52% r.h.	59.024	1.434	12.18	1.50
100% r.h.	1.343	0.414	8.00	1.73
Proliferated	14.992 (gain)	0.166	18.23	1.94
S.E. (means)	± 5.3338	± 0.2541	± 2.967	± 0.121

Surprisingly, dye penetration showed an opposing trend to weight changes with more lenticels penetrated and to a greater extent in the tubers from the proliferated treatment. Thus dye penetration seems to be related to lenticel condition rather than tuber turgidity.

The distribution of permeable lenticels

In several experiments, tubers were soaked in dye, washed, cut into internode pieces and the size of each piece recorded. The total number of lenticels was counted, and the piece was then peeled and the number of penetrated lenticels recorded. For each internode piece, the percentage of lenticels permeated and the curved surface area of the internode was calculated. Apical internodes were difficult to define and were usually ignored.

On three occasions a total of fourteen KE (from store at the Potato Marketing Board Experimental Station, Sutton Bridge, Lincs.) and six PC tubers were examined after soaking for 4 —4½ h. The proportion of lenticels penetrated was generally greater than from other experiments, probably because peeling was more sensitive to small amounts of penetration than direct examination (mean % penetrated = 62.9% for KE and 57.7% for PC). There were obvious differences between tubers in the proportion of lenticels penetrated, so for comparison between internodes, the percentage of lenticels penetrated on each internode was expressed as a proportion of the mean percentage penetration for that tuber, to give a 'relative penetration index'. The results of this analysis, classified by internode (Table 13) indicate that a smaller proportion of lenticels were penetrated in the internodes nearer the apex, at least in KE where the sample size was sufficiently large to detect the difference. The internodes nearer to the apex are those with a greater density of lenticels (see Table 6 page 32),

Table 13. Lenticel penetration on different internodes of stored tubers
(Mean relative penetration indices)

Internode number from stolon	Cultivar PC	KE
1	1.046	1.013
2	1.155	1.049
3	1.155	1.098
4	0.979	0.988
5	0.874	0.833
S.E. (means)	± 0.0611	± 0.0352

which suggests that where lenticels are more crowded, fewer are open to dye penetration (and presumably to gas exchange), at least on tubers such as these, which have stopped growing. This relationship was confirmed by a correlation of percentage lenticels penetrated (angular transformation) and the reciprocal of lenticel density ($r = 0.54$ for KE and 0.50 for PC with $P < 0.001$ and < 0.01 respectively).

F. DISCUSSION

In attempting to understand the function and disease susceptibility of lenticels of potato tubers, it is essential to realize that on a growing tuber, lenticels are of differing ages and hence differ structurally. In the developmental studies described in this chapter, lenticels sampled for observation have been described in relation to the internodes on which they are situated, as a means of indicating lenticel age. It is therefore necessary to recognize several problems in this procedure, as these may explain some of the variation in the results and will limit the precision with which conclusions may be drawn.

Since a tuber does not grow by the discontinuous formation of internodes, division into these units is an artificial process and

there is no exact boundary between one internode and the next. Not only is the position of the internode boundary subject to error, but lenticels on either side of this division, although situated in different internodes, differ little in age, while within any one internode, all lenticels are not at exactly the same stage in development. A further possible source of error is the variable relation between age and internode separation. The tubers on a single plant are mostly initiated over a 2 - 3 week period (Milthorpe & Moorby, 1966) and do not all grow at the same rate (Moorby, 1967; Gray, 1973) although there is little precise information, and none in relation to internode separation. Thus it is not certain that lenticels sampled from a particular internode on different tubers are of exactly the same age, even if these tubers come from the same plant. In addition, the average rate of internode formation, which is at first fairly constant, decreases as the tuber ages and eventually ceases (Lapwood & Hering, 1970). Because of these factors, the difference in age between lenticels on a tuber surface cannot be exactly inferred from the number of internodes which separates them. It must also be remembered that only a small proportion of lenticels on an internode can generally be sampled for anatomical study.

Despite the uncertainties introduced by these considerations, a general pattern of lenticel development appeared from the data. All lenticels seemed to develop from stomata which arose on each internode as it separated from the apex (there being no evidence of secondary lenticels), and structures with stomatal guard-cells generally occupied the first two internodes from the tuber apex. In dry situations, suberin was then progressively deposited in the walls of the outer filling cells, so that by the fifth internode from the apex, microscopic examination showed a clearly-stained layer across the

lenticel. The changes have generally been followed only in the first-formed internodes (numbers 1 to about 5 from the stolon), since later ones are smaller and difficult to define with fewer lenticels which makes study difficult, and these internodes also expand less than the earlier ones and so are of less economic importance in scab infection (Lapwood & Hering, 1968). The development studies confirmed and extended the observations of Fellows (1926) and their significance in relation to scab infection is discussed in chapter 3E (page 74).

Wrapping in wet paper towelling proved to be a convenient method for studying proliferation, and conditions could be kept fairly uniform between different experiments. The presentation and assessment of results involves some reduction in information, and when comparing mean proliferation scores it should be remembered that these are composite estimates influenced by the proportion of lenticels which had proliferated, the extent to which proliferation had progressed, and differences between replicates in the trial.

The earlier proliferation and more rapidly increasing proliferation score on sprouted as compared to de-eyed tubers sheds some light on the physiology of proliferation, since it is clear that the physical presence of growing sprouts, and not just the stimulus to sprout, accelerates proliferation. This suggests that growth substances produced by the sprout may directly influence the lenticel meristem or may act indirectly through changes in tuber metabolism. Wounding may also slightly accelerate proliferation as Zeck (1957) had suggested.

In a number of proliferation experiments there was no evidence that lenticels on some internodes were more prone to proliferate than others, but there was a more gross effect of tuber age which may be important in pathology. The 'slower' proliferation of older tubers (indicated in Table 10 as the time taken to reach a score of 1.00) may

reflect a slower rate of cell division but is largely explained by the length of the 'latent period' between tuber wrapping and the first appearance of proliferation. Thus mature, and especially stored, tubers required a long period in wet conditions before proliferation began, while immature tubers proliferated more readily. This supports the results of Zeck (1957), rather than those of Fox et al. (1971). It therefore seems unlikely that lenticels would proliferate late in the growing season or in store, except under unusually wet conditions. The results in 1971/2 show that stocks of different cultivars may differ consistently in the time taken to reach the same proliferation score, since in five trials MJ was always 'slower' than KE, but the 1972/3 results suggest that this difference may not be simply a cultivar characteristic. Thus although more extensive work on several stocks of each cultivar in several seasons would be needed to clarify this question, the ability of lenticels to proliferate appears to be influenced by season and growing conditions. The relevance of proliferation to infection by Erwinia carotovora and Phytophthora infestans is discussed in chapter 4, and observations on the occurrence of proliferation in the field, in chapter 5C (page 108).

Proliferated lenticels allowed to dry, develop suberin and cork barriers in a manner and at a rate similar to wound-healing (Artschwager, 1927; Radatz, 1967). The change in moisture conditions used was rather drastic, and in the less extreme field conditions a cork barrier was not invariably formed after proliferation stopped (see chapter 5C, page 110). At least some lenticels could proliferate again if returned to wet conditions. In lenticel development from stomata, the rate of suberization has been described relative to internodes but appears to be a little slower than on drying proliferated lenticels, since about two internodes form each week on the young growing tubers used in

this study.

Since tuber age and environmental conditions influence their structure, classifications of lenticels such as those of Wutz (1955) are rather unsatisfactory. Lenticels on mature tubers normally have suberized filling cells (Salix type), but may or may not have a cork barrier later, typical of the Sambucus type (see chapter 5C, page 108). Lenticels are neither 'transverse' nor 'longitudinal' (compare Wetmore 1926a & b). Anatomical investigations are time-consuming and destructive, so it would be convenient to detect lenticel anatomy by other tests like dye penetration. Lenticels show great variation in the extent to which they will be penetrated by dyes (as shown by Wigginton, 1973), and this was related to the structure of the lenticel rather than tuber turgidity, but there was no direct relationship with the presence or absence of a cork layer in contrast to the results of Clements (1935) on apple fruits. In chapter 5B (page 99), observations on lenticel anatomy and dye penetration on tubers sampled throughout the growing season are recorded, and this is related to infection by bacteria and the blight fungus.

Chapter 3: SCAB INFECTION

A. INTRODUCTION

General

Common scab is a more or less superficially blemishing disease of growing potato tubers, causing a reduction in saleable rather than total yield. The visible lesion comprises the corky material produced by the host in response to infection and is rarely more than a few millimetres deep. On the basis of lesion morphology, many different types of scab have been recognised, but since this is mostly an expression of host susceptibility and pathogen virulence (Labruyère, 1971), the procedure is of doubtful value, although there may be a good reason for recognising russet scab, with its less discrete lesions and different relation to soil moisture, as a separate disease (Harrison, 1962).

The actinomycete which causes common scab is generally considered to be Streptomyces scabies, but streptomycete taxonomy is confused and it is possible that other species may also be involved (Millard & Burr, 1926; Corbaz, 1964; Labruyère, 1971), and perhaps especially with russet scab. Study of the pathogen, especially population studies of soil isolates, is confused by the difficulty of identifying S. scabies and the existence of non-pathogenic isolates. After an extensive study of morphological and serological relationships, Labruyère (1971) concluded that although some tests, like melanin production narrow the investigation, only a pathogenicity test (which is difficult and time consuming) will conclusively identify the pathogen. S. scabies is a normal soil inhabitant and is most frequent on light, dry soils and on land recently limed or ploughed from long-term grass (Lapwood, 1973).

Streptomycetes may also parasitise sugar and garden beets, mangel (Millard & Beeley, 1927), turnip, radish, carrot and sweet potato (Person & Martin, 1940), although the relationship of such diseases to S. scabies is not always clear. Only some isolates from mangels were pathogenic to potatoes and appeared to be S. scabies (Millard & Beeley, 1927), while Hooker (1949) was able to infect roots of a wide range of crop plants including soybean, wheat, radish and garden beet with some isolates of S. scabies pathogenic to potatoes.

S. scabies does not penetrate intact skin and although some workers report infection through wounds (Thaxter, 1891; Schaal, 1934) it is typically an invasion of the lenticel, a process which has been studied anatomically by Jones (1931) and Labruyère (1971). The extent of the lesion will depend on the rate of growth of the pathogen down the middle lamellae of the cell walls, on the speed of host response in producing one or more cork barriers until the infected area is confined and on the swelling of the tuber after infection. The variation in the susceptibility of lenticels on a single tuber was a principal reason for much of the work described in chapter 2 of this thesis, and is discussed in detail below. Potato cultivars differ in their susceptibility to scab and there have been several attempts to connect resistance with anatomical or physiological factors, mostly in the hope of finding a simple indicator of scab susceptibility for the potato breeder. Johnson & Schaal (1952) claimed that resistance was related to the quantity of chlorogenic acid in the tuber skin or to its oxidised products after infection (Schaal & Johnson, 1955). These theories have been refuted by Emilsson (1953) and Holm & Adams (1960), but none of these workers examined concentrations in the critical region (i.e. lenticels of the appropriate age) and the case for or against the involvement of

these, or other, substances is inconclusive. A similar criticism applies to other work (Longrée, 1931; Darling, 1937) which suggested that scab-susceptible cultivars had loose-structured and more slowly suberizing lenticels. The findings of Cooper et al., (1954) that cultivars with living periderm cells were more resistant to infection than those with many dead cells, is difficult to understand in relation to the infection process although such a relationship was generally confirmed, but with some exceptions, by Emilsson & Heiken (1956) and McKee (1958). There is clearly a need to understand cultivar susceptibility in relation to the changing susceptibility of individual lenticels, and the study of lenticel development on KE, MJ and PC (chapter 2C, page 17), three cultivars differing widely in scab susceptibility, was designed to facilitate this and is discussed in section E (page 74) of this chapter.

Infection of potatoes is dependent on dry soil and it has been recognised for many years that moist soil conditions will prevent scab (Sanford, 1923), but it is only more recently that commercial irrigation regimes for the protection of 'seed' (Labruyère, 1971) and ware crops (Lapwood et al., 1973) have been established. The reasons for the effects of soil moisture on this disease are discussed in more detail below.

Lenticel susceptibility

Fellows (1926) was the first to realise that scab infection was more severe on tubers which were growing rapidly at the time of inoculation than on those which were growing slowly or not at all, and that infection was usually confined to an area near the apex of the tuber. By transplanting growing plants with measured and tagged tubers to infected soil, Richardson (1952) was able to confirm that

infection occurred only on the growing regions of expanding tubers and Hooker & Page (1960) achieved similar results by direct inoculation of attached tubers growing in a dark enclosure above soil level. These workers realised that infection occurred through lenticels or the stomata which preceded them, but did not critically define the limits of scab susceptibility in terms of lenticel anatomy. Fellows (1926) believed that stomata were infected on the basis of unstained vertical sections which showed blackening of the middle lamellae of cell walls below the aperture (on artificially inoculated tubers), but could not detect the pathogen in any of these sections or reproduce the symptoms on detached tubers. He suggested that the resistance of older lenticels was due to suberization of the filling cells (see also Jones, 1931) but with only circumstantial evidence. Hooker & Page (1960) covered tubers with ink and then inoculated those showing evidence of further growth and obtained infection only on tissue "a week or less in age" at the time of inoculation, but the relation to lenticel development was not examined. The fine dimensions of the actinomycete mycelium growing in the middle lamella of the cell wall is a problem in detecting the recently infected structure, and Labruyère (1971) was unable to detect infection before the first macroscopic symptoms developed, which was always at a lenticel. Procedures used for staining S. scabies mycelium in lesions include carbol auramine which fluoresces in ultra-violet light (Richards, 1943), a modified Gram's technique (Hutchins & Lutman, 1941) and toluidene blue (Shoemaker & Riddell, 1954), but no account of their successful use has been published. Tests with these and other stains are reported in section B of this chapter (page 58).

An indirect approach to the definition of lenticel susceptibility has been possible from field experiments designed to control scab by

irrigation. Lapwood & Hering (1968 & 1970) imposed brief infection periods on plants otherwise kept under wet conditions and showed that in 5, 10 and 15 day infection periods, respectively 4, 5 and 6 internodes became infected and that at harvest the infected internodes were nearer the apex the later the infection period relative to tuber growth. When the position of scab infection was related to tuber internode numbers and to tuber growth it was apparent that the structures on each internode must have been susceptible only for a period of 10 - 15 days, which was the time taken for 3 –4 new internodes to form at that stage of tuber growth. The analysis was extended by Lapwood & Adams (1973) to infection conditions briefly interrupted by rain, which showed that the susceptible internodes appeared to be the third and fourth from the apex only. At the same rate of growth, this would correspond in time to a period slightly shorter than had been deduced from the previous work, and suggests that the stomata (present on the youngest one or two internodes, see chapter 2C of this thesis, page 25) were not infected. Work designed to test this theory and identify the susceptible period more accurately is described in this chapter (section C, page 61).

Effect of soil moisture

Several investigators have sought to understand why scab infection is decreased in wet soils. Changes in soil water potential have complex effects on the soil environment, the host, the pathogen and other micro-organisms, all of which might influence disease development and which are reviewed by Lapwood & Adams (1974, see this thesis page 143). The susceptibility of a single lenticel is not permanently altered by a change in soil moisture, but it is possible that lenticel proliferation in the critical internodes might prevent

infection by displacing the invaded tissue as mentioned by Labruyère (1971). There is no evidence that established infection is eradicated in wet soils.

Most investigators highlight the decrease in numbers of S. scabies which can be isolated from wet as compared to dry soil (e.g. Labruyère, 1965). It is unlikely that this is a direct effect on the pathogen since it will grow (e.g. in shake culture) or even infect potato tubers (Barker & Page, 1954) in wet conditions in the absence of other micro-organisms. The effects of moisture on soil aeration were not thought to be sufficient to account for disease changes by Dippenaar (1933) who could not increase scab by aerating moist soil, and in pot experiments, Labruyère (1971) could not decrease scab with CO_2 and N_2 even at concentrations which adversely affected the host. Sanford (1926) was probably the first to show that the growth of S. scabies was decreased by certain bacteria and since then several authors have attributed the scab control in wet soil to population increases of antagonistic bacteria. Such changes in bacterial populations do occur, but it is difficult to prove their controlling influence on the growth and infection of S. scabies. Dippenaar (1933) attempted to do this by burying glass slides 'seeded' with S. scabies in autoclaved and non-autoclaved soils at several moisture contents, but since similar numbers of bacteria and fungi developed in the two soil treatments and S. scabies grew less in both autoclaved and non-autoclaved wet soils, the data do not support the antagonism he claims. In a comparison of irrigated and 'dry' soils, Labruyère (1971) found little difference in the total actinomycete populations, but the proportion of tyrosinase positive isolates, and especially pathogenic S. scabies, was less in wet soil, suggesting that other actinomycetes as well as

bacteria might be involved in an antagonistic relationship. Lewis (1970) isolated from lenticels of tubers grown in wet or dry soils, and obtained more bacteria from those in wet soil and more actinomycetes from those in dry soil, while the frequency of joint isolation was less than predicted, suggesting an interaction between the two groups of organisms. However, Lewis was cautious in his interpretation since the frequency of isolating S. scabies was much less than might have been expected from subsequent infection and although antagonism was suggested, he had no evidence for antibiosis (Lewis, 1962).

Antagonism by other micro-organisms has also been used as an explanation of scab control which is sometimes achieved by green manuring (Millard & Taylor, 1927). Some workers consider the principal antagonists to be other actinomycetes (e.g. Labruyère, 1971) and Orellana (1947) even obtained isolates of S. scabies from a single scab lesion which were mutually antagonistic in vitro. Weinhold & Bowman (1965), however, found Bacillus subtilis to be the main antagonistic organism isolated from a green-manured soil in which scab incidence had been decreased, and attributed its activity to antibiosis.

The nature of scab control in wet soil still awaits clarification and section D of this chapter (page 69) describes a few preliminary experiments on the growth of S. scabies in relation to wet soils and antagonism.

B. STAINING STUDIES

For an adequate study of host-pathogen interaction, a sensitive technique for staining the mycelium of S. scabies in the host tissue is required. Such a technique would allow the early stages of infection to be recorded and the susceptible structures to be determined,

while perhaps helping in the study of the ways in which differences in scab susceptibility between cultivars are expressed. On several occasions scab lesions cut from naturally infected or inoculated tubers (see chapter 3C) were fixed, embedded in Paraplast, sectioned (see chapter 2B, page 15) and then stained by various methods, including those recommended for scab lesions (page 55). In most sections, mycelium of S. scabies had grown and sporulated inside some of the outer cells of the lesion, and thus the efficacy of the following staining procedures was easily established.

Conant's quadruple stain (Johansen, 1940) stained the intracellular mycelium a pale green but it could not be distinguished within the cell walls elsewhere in the lesion because these were stained darker green, or where suberized, red.

Modified Gram's stain (Hutchins & Lutman, 1941) gave intense violet-stained intracellular mycelium, but cell walls were also stained heavily.

The fluorescent carbol auramine stain of Richards (1943) was tested on several occasions. Examination using the fluorescence microscope showed some staining of cell walls within the scab lesion compared with unstained controls, but intracellular mycelium was only very faintly fluorescent. A smear preparation of S. scabies from a laboratory culture did not appear to be stained by the procedure. Some variations on the procedure were attempted by staining in recommended (0.1%) and double strength carbol auramine for the recommended 4 min or for 10 min and decolourising for 3 min either once or in two changes (recommended) of destaining solution. In no preparation was there an improvement in staining of the mycelium or evidence that the more intense staining of the cell walls was due to the presence of S. scabies within them.

Table 14. Fluorescent dyes used for staining scab lesions

Dye	Concentration (w/v) and solvent
Acridene orange (BDH; C.I. 46005)	1% aqueous
Calcofluor white RWP conc (Cyanamid 18447)	1% in absolute ethanol
Dichloro (R) fluorescein (BDH)	1% in absolute ethanol
Fluoresceine OP (L.B. Holliday)	Saturated (<1%) in absolute ethanol
Fluorescent yellow FGPN (L.B. Holliday)	Saturated (<1%) in acetone
Lanasol (Cole & Wilson)	1% aqueous
Oil colour 7G (Fine Dyestuffs & Chemicals)	Saturated (<1%) in absolute ethanol
Pontacyl brilliant pink B (DuPont)	1% aqueous
Rhodamine 6GD base (L.B. Holliday)	Saturated (c. 1%) in absolute ethanol
Uvitex SWN conc (CIBA)	1% in absolute ethanol

Several other fluorescent dyes (Table 14) were used by staining for 30 min and then washing briefly in the stain solvent or in the solvent acidified with dilute hydrochloric acid. Preparations were then rinsed in water, mounted in glycerol and examined by incident light fluorescence microscopy. Several dyes (acridene orange, lanasol and pontacyl brilliant pink B) stained the periderm, and uvitex stained the starch grains, but in no case was there differentiation between the host tissue and mycelium of S. scabies. Although the pathogen is acid-fast and the host tissue not (Richards, 1943), the acid wash did not improve the preparations.

Toluidene blue (Shoemaker & Riddell, 1954) stained mycelium satisfactorily and although cell walls were also stained, mycelium was detected in a wall sectioned tangentially. Increasing the staining time beyond 2 h did not increase the intensity of the stain in

mycelium or host tissue. This stain appeared the most useful of those tried, but no procedure was found suitable for detecting the extent of mycelium in the middle lamellae of the cell walls of the lesion.

C. SUSCEPTIBILITY STUDIES

Inoculation of detached tubers

Although most investigators have found it necessary to use growing tubers when studying infection by S. scabies, Lawrence (1956) was able to obtain limited lesion development on young detached tubers, and methods similar to his were used to try and define the position of susceptible 'lenticels'. Throughout the studies a Dutch isolate of S. scabies (Dr. R.E. Labruyère, strain S40) normally producing a deep scab lesion, was used.

In a preliminary experiment, tubers of MJ (very susceptible to scab) and PC (a much more resistant cultivar) 3 to 30 mm in diameter were harvested from glasshouse-grown plants and inoculated by dipping in a blended culture of S. scabies. This was prepared by filtering a 9 day shake-culture in 100 ml potato dextrose liquid medium and homogenizing the mycelium in about 100 ml glass-distilled water. A similarly-produced homogenate of an autoclaved culture served as a control. After inoculation, the tubers were covered in moist sterile horticultural grade Vermiculite in plastic sandwich boxes and incubated in the dark at 25°C. Seven and 14 days after inoculation, the tubers were examined and their size, internode number and position of scab lesions recorded. No infection developed on controls, but lesions appeared on some inoculated tubers of both cultivars (Table 15). Infection was not severe enough to define the scab-susceptible internodes, but indicated that on small tubers this might be a

practicable method, although in view of the infection of PC tubers its relation to infection in the field might be questioned.

Table 15. Position of scab infection on the internodes of detached tubers (preliminary experiment)

Cultivar MJ		Cultivar PC	
Tuber size*	Position of scab lesions †	Tuber size*	Position of scab lesions †
2	-		
3	-	2	-
3	A -1,2,3	4	-
3	A -2,3	5	A -2,3,4
4	-	5	A -2,3,4
4	-	5	A -2,3,4,5
6	A -1,2,3	5	-
6	A -1,2,3	8	-
6	-		
7	-		
8	A -6		

* Total number of internodes
† Internodes affected, numbered from tuber apex.

In a more extensive experiment (summer 1971) MJ, KE and PC tubers harvested from peat boxes (see chapter 2B, page 15) were used. Up to ten tubers of each cultivar were carefully harvested on each of several dates, washed, their number of internodes recorded and then inoculated by dipping in a blended 12 -15 day old shake-culture of S. scabies in 100 ml Czapek-Dox liquid medium, as modified by Rogers (1968). Incubation in sterile moist Vermiculite as before was for at least 11 days, after which the position of scab lesions (internodes affected) was recorded. The results are summarized in Table 16 which

Table 16. Position of scab infection on detached tubers, summer 1971 experiment

	Cultivar								
	PC		MJ		KE		MJ & KE		
Total no. tubers of all sizes inoculated...	32		38		50		88		
Total no. tubers infected.....	0		10		10		20		
Internode no. (from apex)	I*	N†	I	N	I	N	I	N	I/N
A -1	0	32	4	38	2	50	6	88	0.07
A -2	0	32	6	38	2	49	8	87	0.09
A -3	0	31	5	33	4	43	9	76	0.12
A -4	0	22	3	24	2	34	5	58	0.09
A -5	0	13	1	15	1	21	2	36	0.06
A -6	0	10	0	11	0	11	0	22	0.00
A -7	0	7	0	4	0	7	0	11	0.00
A -8	0	4	0	2	0	3	0	5	0.00
A -9	0	1	0	1	0	1	0	2	0.00

*I = Number of tubers with lesions on this internode
†N = Total number of tubers with this internode present

illustrates that few tubers were infected and lesions were confined to the younger internodes of those tubers that were infected. Cultivar differences reflected their accepted field susceptibilities, but this method did not give sufficiently frequent infection to define the internodes with susceptible lenticels.

Glasshouse experiment

In this experiment, tubers of known size and internode number growing in a dark air chamber above soil level were inoculated by surrounding them with Vermiculite soaked in a culture of S. scabies

(see Dykstra, 1956).

To produce these tubers, eye plugs 24 mm in diameter were removed from scab-free MJ tubers on 15 March 1972 using a cork borer and were placed in moist Vermiculite in sandwich boxes to sprout. On 4 April, six sprouted plugs were transferred to each of five polythene-lined wooden seed boxes which had been half-filled with nutrient 'Eff' compost. Each box was covered with black polythene sheet (in which slits were made for the growing shoots) and a layer of damp polystyrene foam about 20 mm thick to prevent the temperature rising excessively. On 15 May, when the first tubers had formed in the space between the compost and polythene, the tubers growing in one box were inoculated. A second box of tubers was inoculated on 22 May, the third on 25 May and the remaining two on 2 June. For each box, ten 7-day petri dish cultures of S. scabies (S40) grown on potato dextrose agar with added 0.7% peptone were blended thoroughly in 150 ml distilled water and the liquid thoroughly mixed into 250 g Vermiculite previously moistened with 200 ml distilled water. A further 50 ml water used to rinse out the blender was added to the Vermiculite which was then placed carefully around the tubers and filling the seed box. The black polythene was not replaced. Tubers were tagged and their size and number of internodes recorded before inoculation. After 5 days, the box was flooded with water and the individual plants carefully separated in running water. The tagged tubers were remeasured, and freshly formed ones tagged and the plants were then repotted individually in 'Eff' compost to allow further growth. After 16 days the plants were again washed out, the tubers measured and scab infection recorded.

From a total of ninety-five tubers inoculated, only eight were definitely and eleven possibly scab-infected and even so there was never more than a few lesions on any tuber. The position of lesions,

backdated to be the internode number at the end of the 5 day inoculation period is shown in Table 17. With so little infection it is impossible to define exactly the number and position of scab-susceptible internodes, but the results are not inconsistent with the theory of Lapwood & Adams (1973), that at any time internodes A -3 and A -4 are susceptible.

Table 17. Position of scab infection on attached tubers in glasshouse experiment

Backdated internode number (from apex)	Numbers of this internode exposed	Number of tubers with lesions on this internode		
		Definite	Possible	Total
A -1	95	0	1	1
A -2	93	1	2	3
A -3	83	3	5	8
A -4	65	6	8	14
A -5	46	5	6	11
A -6	28	1	2	3
A -7	17	0	1	1
A -8	4	0	0	0
A -9	2	0	0	0

Field experiment, 1971

An experiment on a scabby site at Woburn, Beds., aimed to study lenticel susceptibility to *S. scabies* by controlling soil moisture (and hence infection) at particular stages of tuber growth and to relate these moisture changes to the position of scab lesions that developed. The scab-susceptible cultivar Maris Piper was planted in School Field on 12 April and first tubers were found on 26 May when two blocks, each of three plots 4 rows x 4 plants separated by guard areas of crop, were marked. Within each block plots were randomly

allocated to be:

(a) continuously dry under a transparent Polythene cover,

(b) dry (covered) for 2 weeks, wet (by hand watering when necessary) for 3 weeks and dry for a further 2 weeks or

(c) wet, dry and wet, inversely to (b).

In each plot a porous pot tensiometer (Gallenkamp) was sited in the ridge top between plants, with its bulb centre 6 in (about 100 mm) below the soil surface, and six further tensiometers were positioned in the surrounding crop. The tensiometers in the guard area were read three times weekly, and the plot ones once a week. Each week, six plants taken at random from the guard area were dug to assess tuber development (internode number on the five largest tubers of each plant). On 14 July when the experiment was concluded, the four central plants from each plot, and four plants from the guard area exposed to natural weather throughout, were carefully lifted and the five largest tubers from each plant used to assess which internodes carried scab infection. The tuber development sample of 7 July had also been scored for the position of infection.

The rainfall, soil moisture tension in each plot and the mean tuber size is shown in Fig. 3. The weather was dull and wet for much of the experiment and covered plots dried only slowly. Severe infection of some plants by Rhizoctonia solani resulted in severed stolons and hence variable and delayed tuber growth. Tubers on 7 and 14 July were often severely scabbed, especially those from the continuously dry treatment. The other treatments and guard samples generally showed a scab-free area near the stolon attatchment and the extent of this area is shown to be about three internodes in Table 18. Because of the ineffectiveness of the applied treatments, scab infection conditions (when tension exceeded about -30 cm Hg, see Lapwood, 1966) were

Fig. 3. Rainfall, soil moisture changes in different treatments and tuber growth, Woburn, 1971 (arrows indicate dates when regimes were changed)

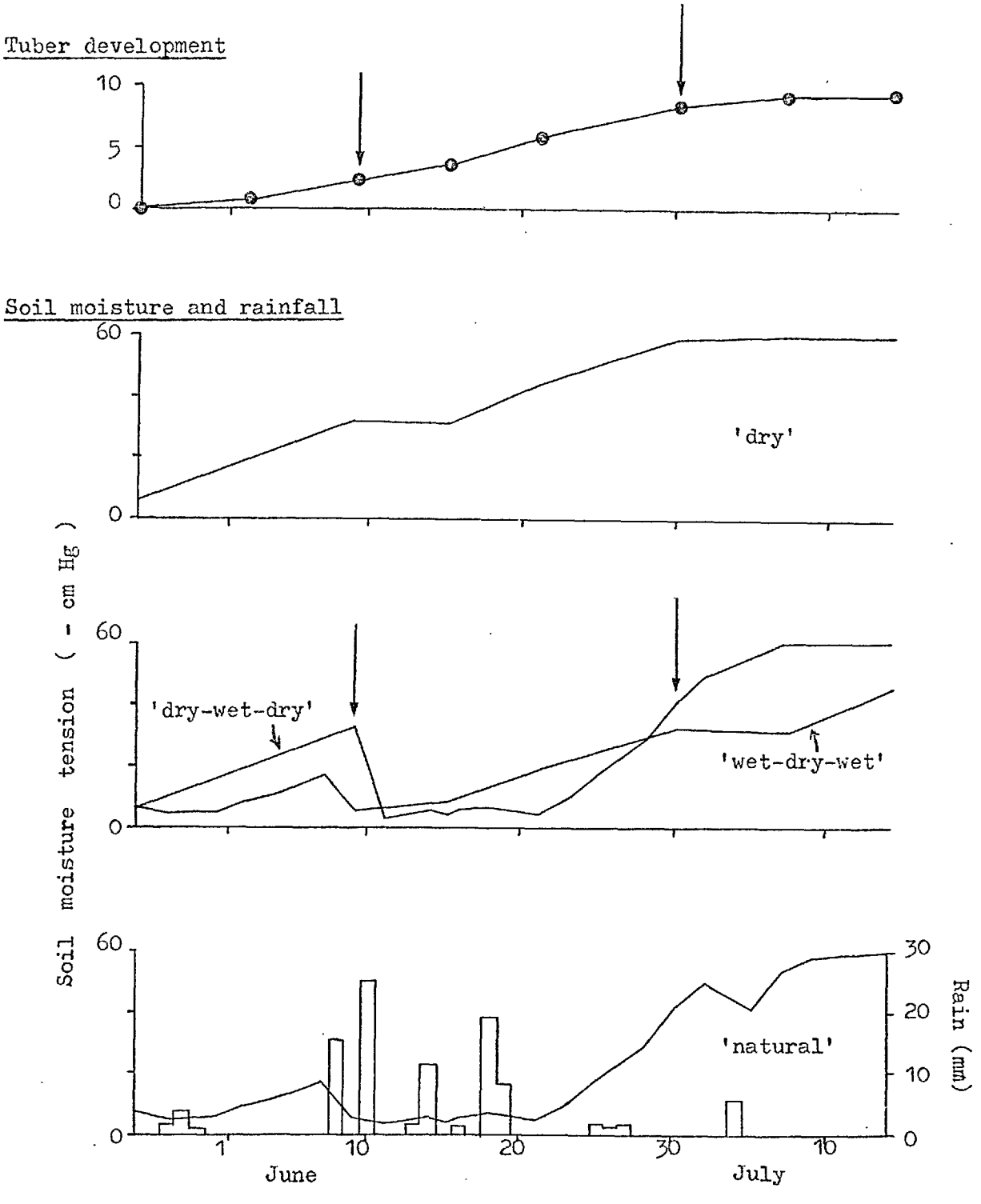


Table 18. Scab infection of tubers exposed to different watering regimes in 1971 field experiment

Treatment and date of sample	Number of tubers examined	Numbers of tubers with scab-free area	Mean extent of scab-free area (no. of internodes)	
Dry (14 July)	40	7	0.2 ± 0.09	
Wet-dry-wet (14 July)	40	19	2.4*	Mean 2.8 ± 0.24
Dry-wet-dry (14 July)	40	34	2.6*	
Natural (7 July)	30	30	3.9*	
Natural (14 July)	20	16	2.6*	

* Do not differ significantly at $P = 0.05$

reached on the 'dry' treatment by about 9 June and on all others, including the 'natural' by 28 June, after which infection conditions probably continued. On these dates, tubers had on average three and eight internodes respectively (estimated from the graph). Thus when tubers with three internodes entered scab infection conditions, all their lenticels were subsequently infected, but when tubers had eight internodes at the time S. scabies could infect, the lenticels on the oldest three internodes proved to be resistant to infection.

Despite the several sources of error in this experiment, including the erratic tuberisation, the poor control of soil moisture, and the inherent variability of the tensiometers, the data indicate that lenticels on the youngest five internodes were, or became susceptible to scab, agreeing with the more accurate data from Great Hill Bottom, Woburn (Lapwood & Adams, 1973).

D. SOIL MOISTURE AND THE GROWTH OF *S. scabies*

Growth on seeded slides buried in soil

The experiment used a method similar to that described by Dippenaar (1933) to determine the effect of different soil moistures on the germination and growth of *S. scabies* on slides seeded with the pathogen. Unused glass microscope slides were cleaned by rinsing in hot Teepol in glass-distilled water and thoroughly washed in hot, and then cold, distilled water. After standing overnight in 5% acetic acid, the slides were rinsed in distilled water, stood in fresh distilled water for 24 h, rinsed in distilled water, rinsed in 'Analytical Grade' acetone, air dried and autoclaved at 121°C for 15 min (a cleaning procedure recommended by the glass manufacturers: Deverall, pers. comm.). The slides were each seeded with three drops of a suspension of *S. scabies* (S40) spores in sterile distilled water washed from a 14 - day old 25°C slope culture on asparagine glucose agar (Gregory & Vaisey, 1956), and allowed to air dry in a sterile transfer room. On 25 September 1972, soils at three different moisture contents were taken from the area of the 1972 Lenticel Study (Rothamsted Garden Plots) described in chapter 5 (page 99) and sampled into Polythene bags which were quickly sealed. 'Dry' soil was taken from an area underneath the Polythene covers where tensiometers were indicating a soil moisture drier than -60 cm Hg tension and 'wet' soil from a watered plot with a tension of about -10 cm Hg, while an 'intermediate' soil came from the 'natural' plot where tensiometers were reading about -30 cm Hg. In the laboratory, each soil was quickly distributed to two 400 ml glass beakers which were sealed with aluminium foil and weighed. One beaker of each soil was autoclaved twice on succesive days at 121°C for 1 h, while the other beaker was

untreated. All beakers were reweighed and, where necessary, the weight loss made up with sterile filtered pond water. The soil from each of the six beakers was distributed to fill each of four sterile petri dishes and one 'seeded' slide placed face-downwards onto each soil surface. The petri dish lid was secured with adhesive tape to keep the slide in contact with the soil and the four replicate dishes were placed in a sealed Polythene bag at laboratory temperature (about 20°C) for 8 days. Five samples of each autoclaved soil were transferred to 10 ml sterile distilled water and, after shaking and settling, drops were spread onto nutrient agar plates which were incubated at 25°C for the duration of the experiment to check the effectiveness of the autoclave treatment. Soil samples were also taken to assess the percentage moisture on a dry weight basis. At the end of the experiment the slides were carefully removed, dried, stained for 1 min in carbol fuchsin, rinsed in distilled water, dried and examined. In the area of each drop, the presence of actinomycete filaments, fungal hyphae and colonies of bacteria was each assessed on a scale 0 (none) to 4 (abundant).

On two of the nutrient agar plates prepared from the 'dry' sterile soil, one small bacterial colony developed but elsewhere there was no growth. The growth scores on the slides are shown in Table 19, which indicates the abundant germination and growth of S. scabies in all the autoclaved soils irrespective of moisture content, while growth in the non-sterile soil was much less and decreased as soil moisture increased. Bacteria and fungi showed an opposite moisture response to the actinomycete. A few fungi invaded the autoclaved soil, but did not appear to interfere with the growth of S. scabies. The pathogen sporulated freely on the slides from the 'wet' autoclaved soil, but only sparingly on those from the 'intermediate'

and not at all on those from the 'dry' soil. No sporulation was seen on slides in the non sterile soils. Thus the experiment indicated that the growth of S. scabies was not inhibited by moisture as such, but only in the presence of other micro-organisms, especially bacteria.

Table 19. Growth of micro-organisms on 'seeded' slides

Soil moisture†	Growth (% maximum score*) Autoclaved soil			Non-autoclaved soil		
	A	B	F	A	B	F
'Wet' (21.2%)	100	0	17	25	37	33
'Intermediate' (16.8%)	98	0	10	40	19	27
'Dry' (9.0%)	98	0	2	62	0	12

* Maximum score for each organism and soil = 48; A = actinomycete hyphae, B = bacterial colonies, F = fungal hyphae

† Moisture % dry weight

The effects of Bacillus subtilis on the germination and growth of S. scabies spores

These preliminary experiments tested the effects of B. subtilis, a soil bacterium likely to be an antagonist (Weinhold & Bowman, 1965), on the germination of S. scabies on glass slides.

Slides were cleaned as in the previous experiment and each 'seeded' with three drops of a suspension of S. scabies prepared in 1% MJ potato extract in filtered rain water (McKee, 1964) by washing from a petri dish culture on asparagine glucose agar.

In the first experiment, a suspension of B. subtilis was prepared in sterile distilled water from a 24 h nutrient agar slope culture and two slides were prepared for each of the following treatments:

(1) S. scabies air-dried

(2) S. scabies air-dried followed by B. subtilis air-dried

(3) S. scabies air-dried and rewetted with sterile distilled water

(4) S. scabies air-dried and rewetted with B. subtilis suspension

(5) S. scabies drops not dried

(6) Mixed drops of S. scabies and B. subtilis

The slides were incubated in dark damp chambers at 25°C for 4 days. Drops dried during the course of the experiment, and the slides were stained in rose bengal and carbol fuchsin (Dippenaar, 1933) before examination. There was a little germination of S. scabies in treatment (1), and abundant in treatments (3) and (5) but none in the presence of B. subtilis (see Plates 17 and 18).

In the second experiment, S. scabies was dried onto all slides, and the drops rewetted with a nutrient broth culture of B. subtilis, the supernatent from such a culture, or sterile nutrient broth, each at five dilutions in sterile distilled water. The culture of B. subtilis was a 48 h shake culture at laboratory temperature (about 20°C), and this was used undiluted and at 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4}. The supernatent was prepared by centrifuging a portion of the same culture at 8,000 g for 10 min, and repeating the centrifugation on the first supernatent. Both this and the sterile broth were diluted similarly to the suspension. Additional slides were unwetted or wetted either with filtered rain water or sterile or non-sterile distilled water. Two slides, each with three drops of S. scabies, were used for each treatment and these were incubated in dark damp chambers at 25°C for 4 days, dried, stained in carbol fuchsin for 1 min, washed in distilled water, dried and examined. The germination and growth of S. scabies was recorded on a scale 0 (none) to 4 (abundant) for each drop. The concentration of the bacterial suspensions, determined by haemocytometer counts (3 drops x 10 counts) of the one-tenth dilution, was estimated

Plate 17. Germination of S.scabies in absence of B. subtilis x 1950

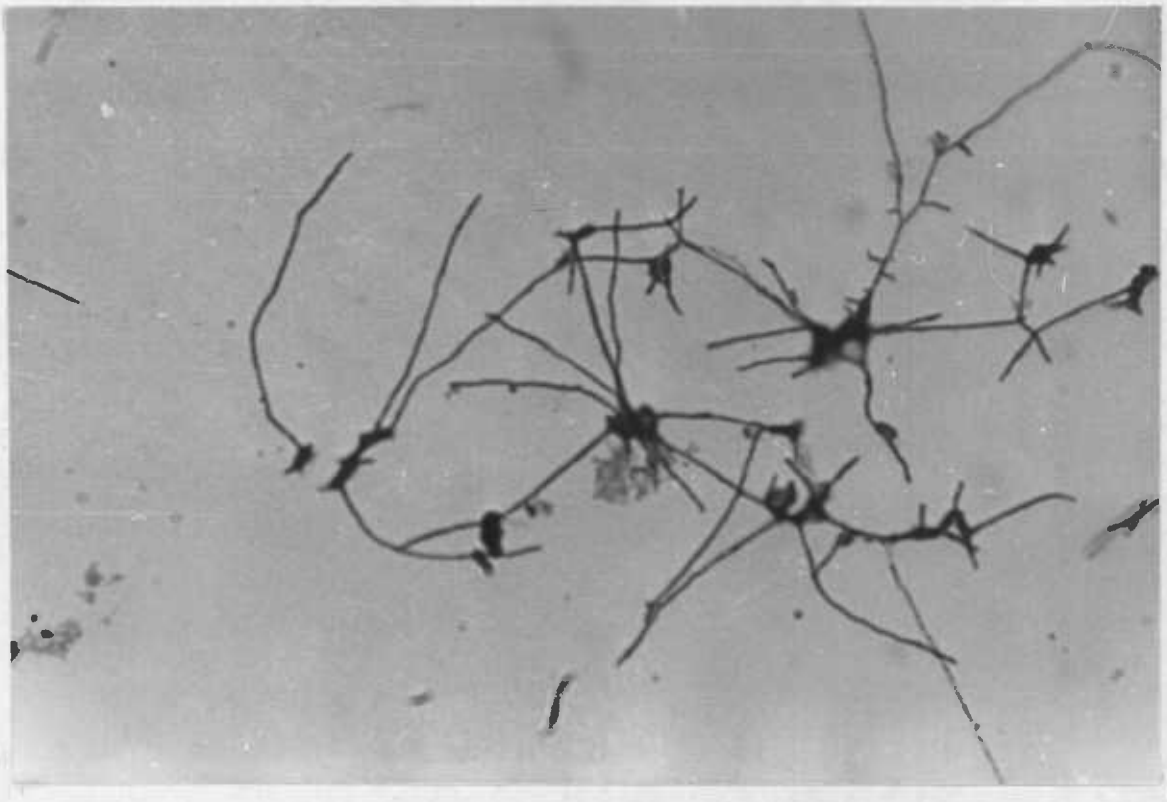
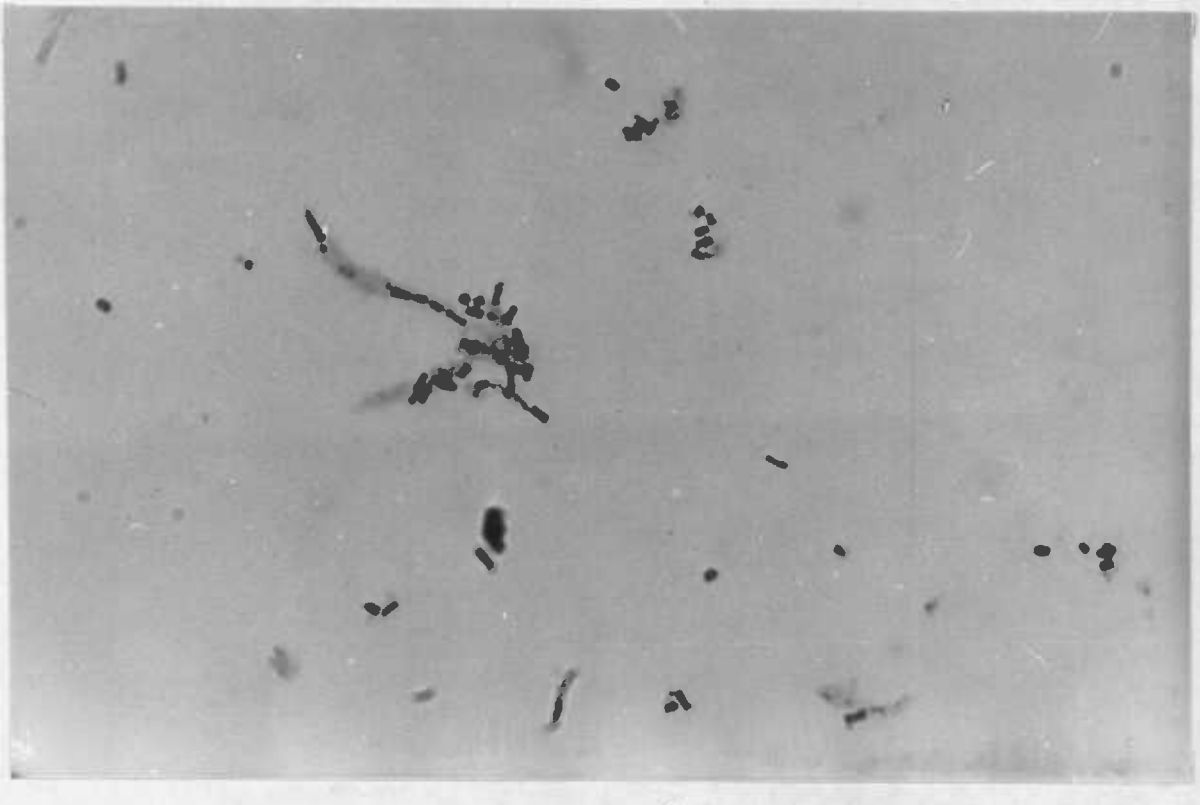


Plate 18. Ungerminated S. scabies in the presence of B. subtilis x 1950



as 2×10^8 down to 2×10^4 cells ml^{-1}. The results (Table 20) confirm the inhibitory effect of B. subtilis on the germination of S. scabies and show that this effect extended to the supernatent of the culture, indicating antibiosis. The effect was reduced by dilution. Unwetted drops hardly germinated and some of the results are a little erratic owing to drying of some of the drops during the course of the experiment. This confirms previous indications that water as such does not prevent germination or growth of the pathogen.

Table 20. The effect of B. subtilis and a culture supernatent on the germination and growth of S. scabies

	Total score (6 replicates, max. score 24)				
	Undiluted	10^{-1}	10^{-2}	10^{-3}	10^{-4}
B. subtilis	0	0	11	16	17
Supernatent	0	10	10	20	12
Sterile broth	23	23	24	21	24

Controls: Sterile and non-sterile distilled water, both 24

Filtered rain water 18

Unwetted 4

E. DISCUSSION

Lenticel susceptibility

A critical definition of the tuber internodes which carry structures susceptible to S. scabies has not yet been achieved. Direct inoculation of detached or growing tubers resulted in little infection, possibly owing to the isolate used, since a recent pathogenicity test on a different isolate gave more frequent infection. At present, therefore, the most accurate results are the indirect ones of Lapwood & Adams (1973), which indicated that susceptibility was restricted to those lenticels on the third and fourth internodes from the apex at any

one time, and in view of the errors inherent in equating internode differences with age (see chapter 2F, page 47), the other results are not inconsistent with this. The results of chapter 2 indicated that the youngest two internodes (A -1 and A -2) were likely to carry stomata, the (susceptible) A -3 and A -4 had lenticels with incompletely suberized filling cells, while on older and resistant internodes (A -5 and older), suberization appeared complete across the outer walls of the filling cells in dry soils.

Resistance of well-suberized lenticels is understandable, in view of the resistance of unwounded periderm to infection, even if autoclaved (Hooker & Page, 1960), and was postulated by Fellows (1926) and Jones (1931). Lack of infection of stomata may be inherent resistance or may more probably reflect the smaller 'target area' and hence low probability of infection. Other workers have not related their findings to internodes, which makes comparisons difficult. Thus Hooker & Page(1960) sprayed tubers with Indian ink, allowed them to grow for a further week, and then inoculated those showing apical growth (un-inked tissue). Lesions later developed only on the un-inked parts of tubers, which were described as "a week or less in age". At normal rates of tuber growth, this would correspond to the youngest two (stomatal) internodes, but would depend whether the ink cover extended to the earliest internodes as defined in this thesis. Only Fellows (1926) claimed to have directly detected infection of stomata (by blackening in the cell walls), but if so (and it is not conclusive) it could be attributed to the inoculation procedure, in which a suspension of S. scabies was forced under pressure into soil containing growing tubers and which could have forcibly injected the tubers with inoculum. Differences between cultivars in the rate of lenticel development (relative to internode number) in comparable conditions could not be

detected, especially in the rate of suberization over the critical internodes (see chapter 2C, page 30). Although slight differences might be demonstrated in large samples, there can have been no differences of sufficient magnitude to account for the differences in scab susceptibility between cultivars, in contrast to the theories of Longrée (1931) and Darling (1937).

Effect of soil moisture

There is little evidence that the effects of soil moisture on the host would be sufficient to explain scab control in wet soils. A comparison of tubers growing in wet and dry soils showed little difference in the rate stomata changed to lenticels (Adams & Lapwood, unpublished), and proliferation in wet soil would expose unsuberized tissue (which is anticipated to be susceptible) at the soil-tuber interface and this would rapidly suberize when the soil dried (see chapter 2D, page 36). Lenticels, whether proliferated or not, might leak more metabolites into wet than dry soil, which could inhibit scab infection or alter the microflora to be unfavourable to the growth of S. scabies. The former is a possible explanation for Lawrence & Barker's (1963) results with sterile tubers, when increased infection was obtained by washing in water or 40% methanol prior to inoculation.

Most workers consider that scab control in wet soil is related to smaller populations of S. scabies in these conditions, which is attributed to antagonistic effects of other micro-organisms. My experiment, using slides 'seeded' with S. scabies in sterile and non-sterile soils of differing moisture contents supports the theory of antagonism in wet soil, since germination and growth was greatly decreased compared to dry soil, but not if the soil was sterilized. Park (1960) suggested that antagonism may operate by competition

exploitation or antibiosis, and my results with Bacillus subtilis, like those of Weinhold & Bowman (1965), showed an antibiotic effect, although the concurrent operation of competition and/or exploitation is not excluded.

Chapter 4. INFECTION BY BACTERIA OR THE BLIGHT FUNGUS

A. INTRODUCTION

Soft-rotting bacteria may infect young tubers through the stolon attachment and especially through wounds, either caused 'naturally' (Harper et al., 1963) or during and after harvest. Smith & Ramsey (1947) first highlighted the role of the lenticel as another entry point for the bacteria, following the discovery of rots around lenticels of washed tubers, which had been stored under warm moist conditions. They were able to reproduce the symptoms on freshly-harvested tubers soaked in a suspension of Erwinia carotovora, and it is to this soft-rot coliform group of bacteria that most tuber rotting is generally attributed (Boyd, 1972). However, the possibility that other pectolytic bacteria (eg. Clostridium spp., Pseudomonas spp.) may also be involved should not be overlooked (Lund & Nicholls, 1970; Pérombelon, 1972; Lapwood & Legg, 1973).

In the U.K., Erwinia carotovora vars atroseptica or carotovora can be detected in most 'seed' stocks. The former seems to be more frequently isolated both from 'seed' stocks and from rots in commercial stores, and also causes the blackleg symptom in potato crops. However, the factors influencing the expression of blackleg symptoms are poorly understood. Despite widespread contamination of 'seed', blackleg incidence in the crop is usually small and is unrelated to subsequent progeny contamination (Pérombelon, 1972). The bacteria do not overwinter in soil (Logan, 1968) and the main source of infection is the breakdown of the mother tuber, releasing bacteria into the soil. Lenticels of growing tubers may be infected in soil (Lapwood & Hide, 1971) or after harvest especially if tubers are pressure-washed or transported

in a current of water ('flume') as at some packing stations. Under these conditions contaminated soil or rotting tubers may release bacteria into the water, which is often recycled and thus becomes a source of infection to subsequent loads of tubers. The packaging of tubers in Polythene bags may provide an ideal environment for the rotting of tubers so infected (Scholey et al., 1968). The spread of bacterial rotting in store might also be caused by tuber breakdown and the infection of adjacent tubers through lenticels.

Moist conditions after inoculation are necessary for rots to develop from lenticels (Smith & Ramsey, 1947) and in drying conditions such rots become arrested forming sunken 'hard rot' lesions (Logan, 1964; Bétencourt & Prunier, 1965). Apparently healthy tubers may carry latent infections, at least partly associated with lenticels, and by testing in warm moist conditions, Pérombelon (1972) was able to induce most tubers of several Scottish potato 'seed' stocks to rot. The factors involved in 'triggering' latent infections are only partly understood. Wet conditions appear essential for rots to develop and rotting is enhanced in atmospheres with depleted oxygen (Lund & Nicholls, 1970; Lund & Wyatt, 1972).

In view of the possible importance of lenticel infection at different times before and after harvest, it is surprising so little is known about the susceptibility of lenticels to bacterial penetration, and the factors which govern subsequent latency or rot development. Bétencourt & Prunier (1965) noticed rots associated with proliferated lenticels and suggested that tuber infection would be more frequent in wet, poorly-aerated soils when lenticels would be most likely to proliferate and would be less suberized. Fox et al., (1971) reported that lenticels of tubers stored at relative humidities less than 80% were often blocked with secondary periderm and were not infected when

tubers were soaked in a suspension of Erwinia carotovora var. atroseptica, whereas at 100% r.h., lenticels sometimes proliferated and infection and rotting occurred. This latter work using very few lenticels from stored tubers provides the only quantitative data on lenticel susceptibility, which deserves further study.

Late blight (caused by Phytophthora infestans) is the most intensively studied disease of potatoes, if not of all crops. The air-borne sporangia of the pathogen are responsible for the spread of the disease on the leaves and stems of the plant, which may rapidly reach epidemic proportions in suitable weather conditions. Tubers may become infected before harvest by rainwater washing sporangia into the soil, or at harvest if there is living infected haulm. Infection occurs only through wounds, eyes or lenticels (Lacey, 1967). Relatively few lenticels become infected in most laboratory inoculations (Lapwood, 1967), making studies of any change in their resistance difficult. Their susceptibility appears to decrease as the tuber ages (Zan, 1962; Lacey, 1967)and on one occasion Lacey noted infection particularly associated with proliferated lenticels. Zeck (1957) advanced evidence that proliferation increased the susceptibility of lenticels of mature tubers to blight in a direct inoculation, although detailed results are not presented and it is therefore not possible to assess the degree to which this might affect tuber susceptibility in practice.

In the field both the bacterial and fungal pathogens discussed here spread in wet conditions, which favour tuber decay by bacteria (the release of which into the soil may lead to the infection of other tubers) and the sporulation of P. infestans on the haulm (from which spores may be washed into the soil to infect developing tubers). The literature also suggests that wet soil may predispose the lenticels to infection by both pathogens by causing proliferation, but little is

known about lenticel susceptibility after harvest. In the experiments described here, factors influencing the susceptibility of lenticels to bacterial penetration were studied especially in relation to relative humidity and proliferation in an attempt to assess the importance of lenticels as infection sites during growth and storage of the potato tubers. Lenticel infection by blight in relation to proliferation was also studied in a few experiments. The effects of tuber age on lenticel susceptibility both to bacteria and to the blight pathogen were studied in the experiment described in chapter 5B (page 99).

B. MATERIALS AND METHODS: BACTERIA

The bacteria used, which all cause soft-rotting when inoculated into potato tubers, were:

Bacillus polymyxa (Prazmouski) Migula NCPPB 611

Erwinia carotovora (Jones) Bergey et al., var. atroseptica (van Hall) Dye NCPPB 1277

E. c. var. carotovora (Jones) Dye Logan strain 362/23

E. c. var. aroideae (Townsend) Graham & Dowson Logan strain 963/8

Pseudomonas marginalis (Brown) Stapp NCPPB 667 & 1187

Ps. viridiflava (Burkholder) Dowson NCPPB 1474

Stock cultures were maintained on nutrient agar slopes and 48 h cultures grown at 25°C on nutrient agar flats in 4 oz (114 ml) medicine bottles were used for experiments. Suspensions were prepared in 0.85% saline in glass-distilled water, and their concentrations estimated by measuring the turbidity using a nephelometer previously calibrated with bacterial suspensions, the concentrations of which had been determined by haemocytometer counts.

Tubers to be inoculated were carefully washed in running tap

water to remove surface dirt, dried and soaked in the bacterial suspension, or in saline. After inoculation the tubers were carefully but thoroughly washed in tap water to remove the bacteria, dried, and the tubers of each treatment placed in a Polythene bag with a little sterile distilled water to wet the tuber surfaces and maintain a humid atmosphere. The bag was sealed and left in the dark at laboratory temperature overnight for rots to develop from penetrated lenticels, after which the tubers were air-dried and left at laboratory temperature for 24 h. 'Hard rot' lesions developed as lenticel rots became arrested, as shown by Smith & Ramsey (1947), and could be counted and measured. Bacteria probably did not spread over the surface of tubers in the Polythene bag as no pectolytic bacteria could be detected in the water sampled at the end of the bag incubation.

The number of lenticels infected varied greatly between tubers from the same stock and also with tubers of different ages (see chapter 5B, page 105). In some experiments lenticels were induced to proliferate by wrapping the tuber in wet paper towelling or incubated at 100% r.h. for a period prior to inoculation to increase susceptibility to infection. Because of the variation in procedure and susceptibility of tubers used, results of experiments on different dates were not always comparable.

C. GENERAL FACTORS INFLUENCING BACTERIAL INFECTION

Bacterial genus and species

In three experiments, the ability of the different bacteria to penetrate lenticels and cause rots was compared. In the first, five tubers each of MJ and KE were soaked for 3 h in suspensions of either *E. c. atroseptica*, *E. c. carotovora*, *E. c. aroideae*, *Ps. marginalis*, *Bs. polymyxa* or saline. Rots developed only from the *Erwinia*-inoculated

tubers, but as bacterial concentration was not determined, no comparisons between bacterial variety could be made. These results were confirmed in a second experiment using ten MJ tubers soaked for $3\frac{1}{2}$ h in either E. c. atroseptica, Bs. polymyxa, Ps. marginalis, Ps. viridiflava or saline.

In the third experiment, only E. c. vars atroseptica and carotovora were used, on tubers of PC, KE and MJ incubated at 100% r.h. at laboratory temperature for 8 days from harvesting on 31 July 1971. Bacterial suspensions were both adjusted to concentrations of 1.2×10^8 cells ml^{-1} and ten tubers of each cultivar were soaked in each suspension or in saline for $3\frac{1}{2}$h. The results (Table 21) showed no difference in the ability of the bacterial varieties to penetrate lenticels and cause rots. The greater susceptibility of MJ was probably because lenticels had proliferated extensively on this cultivar and not on the others. No rots appeared on the control tubers.

Table 21. Lenticel infection by E. c. atroseptica, and E. c. carotovora on different potato cultivars

Cultivar	Bacterial variety		
	atroseptica	carotovora	Total and S.E. (means)
	Lenticel rots per tuber (mean no.)		
KE	11.2	11.3	11.3
MJ	55.8	71.1	63.5 ± 3.73
PC	2.7	13.3	8.0
Total	23.2	31.9	
S.E. (means)	± 3.05		

Immersion time

The number of rots developing after different immersion times was investigated in two experiments with E. c. atroseptica on MJ and KE.

Tubers harvested on 10 August 1971 were stored at 100% r.h. for 7 days (first experiment) and those harvested on 6 September 1971 at 100% r.h. for 9 days (second experiment), before soaking in suspensions respectively of 4×10^8 and 2.7×10^8 cells ml^{-1}. No differences between cultivars appeared in this experiment, but when the data were bulked they showed a considerable difference between experiments which can only be associated with the longer incubation at 100% r.h. in the second experiment (Table 22). The results of the second experiment indicated that times of immersion longer than 2 h did not increase the number of rots developing.

Table 22. Lenticel infection after different immersion times

Immersion time	Lenticel rots per tuber (mean no. from ten tubers) Expt 1	Expt 2
0 min	0.1	0.0
1 min	0.0	-
2 min	0.2	-
5 min	0.1	-
15 min	1.0	9.1
1 h	2.7	11.7
2 h	-	25.7
3 h	10.5	21.2
5 h	-	22.1
S. E. (means)	± 0.86	± 3.68

Inoculum concentration

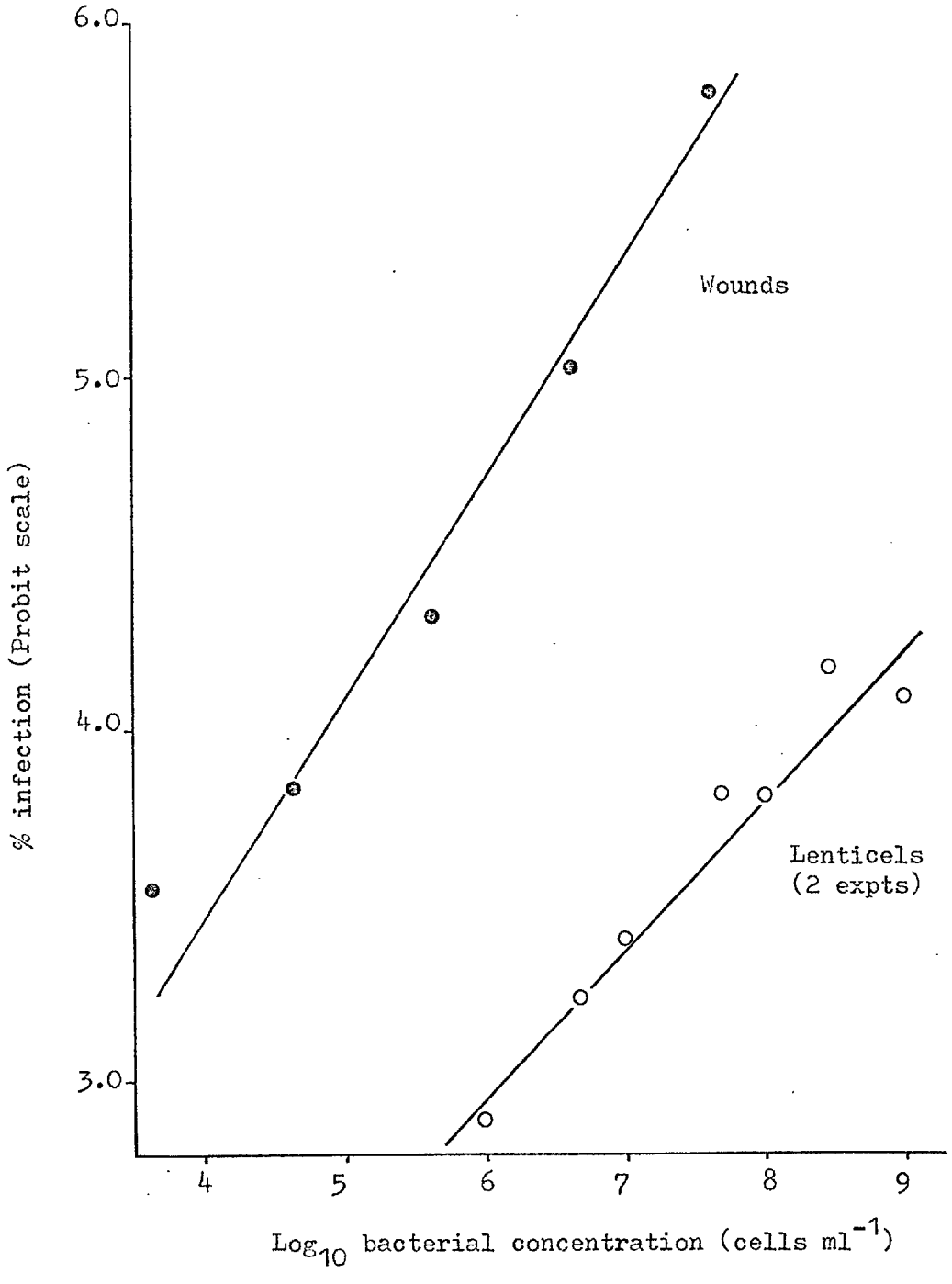
Two experiments in May 1971 compared infection on KE tubers following 4½ h immersion in different suspensions of E. c. atroseptica. Ten tubers were soaked either in one of four suspensions of differing

concentration or in saline. In the first experiment, tubers were 'Mids' discarded (because of their small size) from the packing line at the Potato Marketing Board Experimental Station, Sutton Bridge, Lincs., and the controls were quite heavily infected with soft-rotting bacteria, while in the second experiment VTSC 'seed' tubers were used and controls were uninfected. The results (Table 23) show that more lenticels were infected as the concentration of inoculum was increased, and since total numbers of lenticels were also recorded the percentage of lenticels infected could also be calculated. After subtracting the control value in the first experiment, means at each concentration were converted to probits and plotted against bacterial concentration (Fig. 4). The two experiments were then found to give comparable results and under these conditions, the concentration needed to produce 20% lenticels infected was estimated as 7.1×10^8 cells ml^{-1}. KE 'Mids' were the only stock of tubers which gave infected controls in the bacterial experiments and this contamination may have come from the packing line 'flume'.

Table 23. The effect of inoculum concentration on lenticel infection

Expt 1: 'Mids'		Expt 2: VTSC 'seed'	
Inoculum cells ml^{-1}	Lenticel rots per tuber	Inoculum cells ml^{-1}	Lenticel rots per tuber
0	14.0	0	0.0
5×10^5	13.8	10^6	2.3
5×10^6	15.9	10^7	7.3
5×10^7	23.9	10^8	14.6
5×10^8	33.4	10^9	21.5
S.E. (means)	$\pm$ 2.51		$\pm$ 3.68

Fig. 4. Infection of small wounds and lenticels by bacteria at different concentrations



Infection of pricked tubers (lenticels v wounds)

Infection of small wounds was compared with that of lenticels in an experiment using PC tubers (stored at +2°C) in May 1971. The ten tubers used at each of five inoculum concentrations of E. c. atroseptica were each pricked at ten sites using the point of a $2\frac{3}{4}$ in (70 mm) darning needle, protruding about 2 mm through a cork. Wound sites were chosen to be away from lenticels or other wounds and were marked with a Text Mark pen before the tubers were immersed in the bacterial suspensions for $3\frac{1}{2}$ h and then treated in the usual way (page 81).

No lenticels became infected at any concentration, but the percentage of pricks infected and the mean rot diameter increased with the concentration of the suspension. The results are shown in Table 24 and are compared with lenticel infection in Fig. 4. Ten tubers which had been similarly wounded were soaked in 1% fluorescein for $3\frac{1}{2}$ h and all pricks were visibly penetrated by dye on examination under ultra-violet light, with a mean diameter of 3.77 ± 0.87 mm.

This experiment shows that the wounds were more easily penetrated than lenticels (1.3×10^5 cell ml^{-1} infected 20% of wounds as compared to 7.1×10^8 in the lenticel experiment).

Table 24. Infection of small wounds by bacteria at different concentrations

Inoculum cells ml^{-1}	Nos. rots developing Untransformed (100 max)	Angles	Mean rot size (mm)
0	0	0.00	-
4×10^3	5	8.19	1.20
4×10^4	12	15.64	1.25
4×10^5	25	28.72	1.80
4×10^6	51	45.28	2.35
4×10^7	79	67.20	2.63
S.E. (means)		± 4.446	± 0.153

Infection after partial immersion in suspension

The experiments so far described have assessed infection after complete immersion of tubers in a bacterial suspension. While this may indicate the susceptibility of the lenticels, tubers in soil or perhaps in a 'flume' may be only partially immersed, and penetration of liquids into the tuber may be less under these conditions. This was investigated using small KE tubers from a store at +2°C which were wrapped in wet paper towelling for 7 days prior to inoculation on 6 October 1971. Tubers were then assigned at random to the following treatments:

(a) Half-immersed in a suspension of E. c. atroseptica (3×10^8 cells ml^{-1}) for $3\frac{1}{2}$ h (ten tubers)

(b) Half-immersed in 1% fluorescein for $3\frac{1}{2}$ h (ten tubers)

(c) Totally immersed in E. c. atroseptica (five tubers)

(d) Totally immersed in 1% fluorescein (five tubers)

Half-immersion was achieved by placing the small tubers 'rose' end upwards in plastic egg-box halves after filling the wells with bacterial suspension or dye. Inoculated tubers were treated as previously, but lenticel infection was recorded only on the 'heel' end half of each tuber, irrespective of whether it had been partially or completely immersed. Penetration of lenticels by fluorescein was also estimated on the 'heel' half only.

Table 25. Dye penetration and bacterial infection of lenticels of partially and totally immersed tubers

Immersion treatment	Dye penetration: Lenticels penetrated %	Dye penetration: Lenticels penetrated Angles	Dye penetration: Size of area (mm)	Bacterial infection: Lenticels infected %	Bacterial infection: Lenticels infected Angles	Bacterial infection: Size of rot (mm)
Partial	8.74	15.22	2.78	21.07	25.85	2.52
Total	34.16	34.40	3.18	39.33	38.46	2.27
S.E.D.(means)		± 7.640	± 0.157		± 5.973	± 0.211

The results (Table 25) show that some lenticels were still penetrated by dye or infected by bacteria when tubers were only partially immersed, but that the percentage of lenticels affected was less than on totally immersed tubers.

D. TUBER PRETREATMENT AND BACTERIAL INFECTION

Proliferation, and storage at different relative humidities

In several experiments infection was compared after different relative humidity pre-treatments to investigate further the effects reported by Fox et al. (1971) (see this chapter, section A, page 79). In three experiments, tubers harvested in July or August were stored for 2 weeks in the dark at laboratory temperature (about 20°C) over distilled water or sulphuric acid solutions, which produced relative humidities of 100, 75, or 50%. The results of the inoculations are shown in Table 26. Tubers soaked in saline were uninfected on all dates.

Variation within treatments was large, but pretreatment at different relative humidities did not appear to influence either the number of lenticels infected or the diameter of rots. The very large number of MJ lenticels infected from the 20 July experiment is unexplained, but shows that the lenticels of tubers from different sources may vary greatly in their susceptibility.

In two further experiments, using ten KE tubers per treatment, the effect of lenticel proliferation as well as r.h. was investigated. Relative humidities of 52 and 73% were maintained over saturated salt solutions (see chapter 2E, pages 44 & 45). A seed box lined with Polythene and damp Airoporena (a foamed polyurethane sheet supplied by Progress Mercantile Co. Ltd., London E.C.1) provided conditions of 100% r.h., while in a similar box, tubers were wrapped in wet paper

Table 26. Lenticel infection of tubers stored for 2 weeks at different relative humidities before inoculation

Harvest date.....	1 July 1971		20 July 1971				3 August 1971			
Tuber source.....	Peat boxes*		Rothamsted: Garden Plots				Rothamsted: Garden Plots			
Inoculum†.....	3×10^8 (6 h)		3×10^8 ($3\frac{1}{2}$ h)				4×10^8 ($3\frac{1}{2}$ h)			
Tubers used (No. and cultivar)....	7 MJ		4 MJ		6 KE		5 MJ		5 KE	
Relative humidity	Rots per tuber (mean no.)	Rot size (mm)	Rots per tuber (mean no.)	Rot size (mm)	Rots per tuber (mean no.)	Rot size (mm)	Rots per tuber (mean no.)	Rot size (mm)	Rots per tuber (mean no.)	Rot size (mm)
100%	13.4	1.71	95.5	1.33	10.5	1.41	9.6	1.19	1.6	1.25
75%	16.3	1.15	66.0	2.52	23.0	1.93	12.8	1.23	9.0	1.18
50%	19.7	1.16	50.8	2.23	14.5	1.79	7.8	1.03	20.0	1.31
S. E. (means)	±5.80	±0.023	±24.56	±0.032	±7.65	±0.021	±5.23	±0.012	±6.94	±0.008

* See chapter 2B (page 15)

† E. c. atroseptica concentration (cells ml^{-1}) and immersion time

towelling to induce proliferation (see chapter 2D, page 37). Incubation was at laboratory temperature in the dark, for 14 days (1 Sept. tubers) or 13 days (tubers harvested on 16 Sept. and stored at +2°C until 12 Oct.) before inoculation. The results (Table 27) indicate that more and larger rots developed when lenticels were proliferated, as compared to the three r.h. treatments. Tubers of 1 September at 52% r.h. developed more rots than those stored at 100% or 73%, but rots were very small and restricted to the immediate region of the lenticel. Otherwise, relative humidity appeared to have no effect on lenticel susceptibility. Controls were again uninfected. Some lenticels from additional tubers of each treatment in the experiment on 1 September tubers were sectioned and examined microscopically (see chapter 2B, page 15). All lenticels, except those from the wrapped treatment, were well-suberized and some from each r.h. treatment had a cork barrier sealing the lenticel.

Table 27. Lenticel infection of KE tubers either stored at different relative humidities or proliferated before inoculation

Harvest date.....	1 Sept 1971		16 Sept 1971	
Tuber source..... (Rothamsted fields)	Garden Plots		Whittlocks	
Inoculum (cells ml^{-1})...	1.3×10^8		1.6×10^8	
Pretreatment	Rots per tuber (mean no.)	Rot size (mm)	Rots per tuber (mean no.)	Rot size (mm)
Proliferated	13.8	3.04	38.1	4.26
100% r.h.	4.1	1.95	2.3	3.13
73% r.h.	3.9	1.23	10.2	2.82
52% r.h.	13.3	1.20	6.3	2.25
S.E. (means)	± 2.81	± 0.092	± 3.31	± 0.065

Proliferation on half the tuber surface

The experiments described above showed that proliferation increased the susceptibility of lenticels to infection and this was further investigated in two experiments in which tubers were partially covered in wet paper towelling so that lenticels would proliferate on part of the tuber only. Infection of lenticels was then compared on the proliferated and non-proliferated parts of each tuber, thus eliminating the variation between tubers.

In the first experiment KE, MJ and PC tubers which had been stored at +2°C from harvest on 16 September 1971 were incubated at laboratory temperature in the dark from 27 November for 21 days, after one side (from 'rose' to 'heel' end) of each tuber had been covered in wet paper towelling. In the second experiment, KE tubers which had been harvested on 2 November 1972 and stored at +2°C until 2 January 1973 were wrapped as before and incubated in the dark at 20°C for 29 days. The wrapped and non-wrapped parts of each tuber were marked with a Text Mark pen, and the tubers were then soaked for $3\frac{1}{2}$ h in a suspension of E. c. atroseptica (7×10^7 cells ml^{-1}) or in saline, followed by the the usual procedure. Most proliferated lenticels were found on the wrapped halves of tubers and were frequent on KE (both experiments), less so on PC and very sparingly on MJ. The total numbers of lenticels and the number infected were recorded on each half of each tuber. The ratio I = % lenticels infected on wrapped half of tuber: % infected on unwrapped half was calculated for each tuber.

The results (Table 28) show that on KE (both experiments) and on PC, more than twice as many lenticels were infected on the wrapped half of the tuber than on the unwrapped half, whereas on MJ, where there was very little proliferation, the infection did not differ significantly between halves. Controls were uninfected.

Table 28. The effect of proliferation on the susceptibility of lenticels to infection

Cultivar (and expt no.)	Numbers of tubers tested	Mean I*	Probability I>1.000
MJ (1)	19	1.170 ± 0.1337	0.3 - 0.2
PC (1)	10	2.360 ± 0.4237	0.02 - 0.01
KE (1)	20	2.300 ± 0.3861	0.01 - 0.001
KE (2)	20	2.015 ± 0.4501	0.05 - 0.02

* I = % lenticels infected on wrapped half of tuber : % infected on unwrapped half

E. PROLIFERATION AND BLIGHT INFECTION

In two experiments, the effect of proliferation on lenticel susceptibility to P. infestans was investigated using the general inoculation method of Lacey (1967). KE tubers were carefully harvested from peat boxes (chapter 2B, page 15) on 24 August and 24 September 1971, and some were wrapped in wet paper towelling to induce proliferation (see chapter 2D, page 37) and incubated in the dark at laboratory temperature for 14 days (24 Aug. harvest) or 10 days (24 Sept. harvest). The remaining tubers were incubated unwrapped for similar periods.

For inoculation, the tubers were placed on damp Airoporena in the bottom of wooden seed boxes lined with Polythene. Boxes were placed on the floor and a zoospore-sporangial suspension or distilled water (controls) atomized over the tubers. Fresh sporangia of near similar age were produced for each experiment, from infected leaves placed in a cool damp chamber overnight and the suspension in glass-distilled water was stood at 15°C for 1-2 h prior to inoculation to encourage the release of zoosporangia. Ten proliferated and ten non-proliferated tubers from the 24 August harvest, and twelve of each from the second harvest, were inoculated and in both experiments

five proliferated and five non-proliferated tubers were used as controls. Wounded tubers were included in each inoculation treatment to check the infectivity of the inoculum. After inoculation, tubers were covered with a second piece of Airoporena wetted in glass distilled water and the boxes covered with a Polythene flap. Infection of lenticels was assessed after incubation at 15°C for 6 days (24 Aug. experiment) or 10 days (24 Sept. experiment).

Tubers sprayed with distilled water were not infected, but most of those inoculated developed infections from eyes or lenticels, or both. The frequency of lenticel infection was low and there were no significant differences between proliferated and non-proliferated tubers (Table 29).

Table 29. The effect of proliferation on lenticel infection by P. infestans

Tuber pretreatment	Lenticel infections per tuber (mean nos.)	
	24 Aug. harvest	24 Sept. harvest
Proliferated	1.8	1.4
Non-proliferated	0.8	1.2
S.E. (means)	± 0.42	± 0.57

In two further experiments in August and September 1972 an attempt was made to compare susceptibilities of proliferated and non-proliferated lenticels by inoculation of individual lenticels using 6.5 mm filter paper discs soaked in a zoospore-sporangial suspension. Although Lacey (1967) obtained frequent lenticel infection by this method, in these experiments no infection occurred, despite the use of an active zoospore inoculum.

Neither of these methods was particularly suitable for assessing lenticel susceptibility and no differences between proliferated and

non-proliferated lenticels were detected. The effects of soil conditions on the structure and blight- susceptibility of lenticels was investigated in the experiment described in chapter 5B (page 99).

F. DISCUSSION

The susceptibility of tubers to soft-rotting will be governed by several factors, of which the susceptibility of the lenticel to bacterial penetration is only one. Subsequent rot development will be influenced inter alia by tuber turgidity, temperature, oxygen tension (Pérombelon & Lowe, 1971 and 1972) and sugar content of the tubers which varies with tuber age (Webb & Wood, 1972), and such factors, plus the changing susceptibility of the lenticel as the tuber matures (see chapter 5B, page 105) may partly account for the large variability encountered in these experiments. Interpretation of the results, and comparisons with those of other investigators is therefore difficult, but several conclusions nevertheless emerged.

Of the various bacteria which can cause soft-rotting of potato tubers, only Erwinia carotovora varieties invaded lenticels and caused rots under these conditions. Although some infection did occur during a few minutes immersion in a bacterial suspension, the number of lenticels infected increased with immersion time, at least up to 2 h. This contrasts with Smith & Ramsey (1947), who also used freshly-harvested tubers, and obtained little increase in infection with immersion times longer than 2 or 3 min. However, in their experiments, inoculated tubers were transferred to moist incubation conditions apparently without washing, and infection may have continued from a surface film of the suspension.

Progressively more lenticels were infected as the concentration of the suspension was increased, and although lenticels of growing

tubers would have been more susceptible than those tested here (see chapter 5B, page 105), small wounds were much more readily infected than lenticels when tubers were immersed in a bacterial suspension. Since lenticel infection was less on partly, as compared to totally, immersed tubers, these experiments suggest that post-harvest infections of lenticels would be relatively insignificant compared to that of wounds, which inevitably occur at harvest, during 'fluming' and in other post-harvest operations.

Storage of tubers for 2 weeks at different relative humidities did not influence the anatomy of lenticels on mature tubers, nor was their susceptibility to bacterial infection modified. Only wrapping in wet paper towelling to induce proliferation had a significant effect on both the anatomy and susceptibility of the lenticels. Other experiments (see chapters 2D, page 40 and 5C, page 108) confirm the slow reaction of lenticels on mature tubers even to conditions of continuous surface wetness, but this contrasts with Fox et. al., (1971). They reported differences in lenticel anatomy and susceptibility from mature tubers stored for 3 weeks at 100, 80 and 58% r.h., and using samples of only ten lenticels for the infection study. These differences are hard to explain, unless the stock of tubers used by Fox et. al., either behaved very differently to those used here, or was naturally greatly contaminated which would not have been detectable without tubers soaked in water as a control treatment. Proliferation, at least on tubers at or approaching maturity, approximately doubled the susceptibility of lenticels to infection. This could not be because these tubers absorbed more suspension, since the results presented in chapter 2E (page 44) indicated greater water uptake by tubers stored at lower relative humidities. Probably the larger area of tissue exposed to the bacteria, together with the turgidity of the

tissue produced in this way, accounts for the phenomenon.

Whilst proliferation undoubtedly affects the suseptibility of lenticels in laboratory tests, its significance in the epidemiology of the bacterial soft-rot complex is less certain. Lenticels of growing tubers were relatively susceptible to infection and their susceptibility was not influenced significantly by soil conditions (chapter 5B, page 105), while with more mature and stored tubers, conditions are unlikely to be sufficient to produce a significant degree of proliferation. Pérombelon's (1972) observations on widespread stock contamination indicated that rotting in potato stores may be due to the triggering of latent infection, rather than the spread of bacteria between tubers, and my results suggest that lenticel infection is predominantly a feature of growing tubers, thereby supporting his conclusion.

Proliferation has also been implicated in lenticel infection of peach roots by Agrobacterium tumefasciens (Kerr, 1972) and of potato tubers by Phytophthora infestans (Zeck, 1957; Lacey, 1967), but the methods used here for blight were insufficiently sensitive.

Chapter 5: LENTICEL STRUCTURE AND SUSCEPTIBILITY (FIELD STUDIES)

A. INTRODUCTION

In chapter 2, environmental conditions in laboratory experiments were shown to influence lenticel structure. In 'wet' situations, lenticels proliferated, while in 'dry' conditions, suberization and perhaps the formation of a cork barrier, was stimulated. Proliferation predisposed a lenticel to bacterial penetration (chapter 4D, page 89), but lenticels proliferated less readily as they aged (chapter 2D, page 40), and age might also influence lenticel susceptibility to pathogens in other ways. Lenticel proliferation and age may also influence blight infection as discussed in chapter 4A (page 80).

In order to assess the importance of lenticels as infection sites, we need to know how lenticel structure and susceptibility to pathogens are related to tuber age and soil moisture conditions, and this has been attempted in the investigations described in this chapter. In the experiment described in section B different soil moisture regimes were imposed on growing tubers at different stages in their development, and the effect of these conditions on lenticel structure and susceptibility was studied by inoculating harvested tubers with Phytophthora infestans or bacteria (Erwinia carotovora var. atroseptica) or soaking in fluorescein dye. Anatomical studies on lenticels from this experiment (1972) and also from samples in 1971 are reported and discussed in relation to soil conditions in section C (page 108).

B. 1972 FIELD EXPERIMENT

Materials and methods

Layout. A single block consisting of three six-row plots (60 plants per row) was planted on 18 April 1972 using Rothamsted once-grown 'seed' derived from VTSC tubers. Single rows of PC separated the plots and served as guards at the ends of the block, while each plot was planted with two rows each of KE, MJ, and PC as sub-plots which were randomized within each main plot. The first plants emerged on 15 May and first tubers were noticed (MJ) on 1 June. One of the three main plots received only natural rainfall ('natural' plot), whereas predetermined areas of the other plots were kept either 'wet' (by hand watering when necessary to maintain soil at or wetter than -10 cm Hg tension) or 'dry' (by a transparent Polythene cover when necessary) for 3 weeks prior to sampling. Samples were dug at 3-weekly intervals between late June and late September for the laboratory tests described below. Changes in soil moisture were monitored daily in the next sampling area of each plot by three porous pot tensiometers (Gallenkamp) sited as described in chapter 3C (page 66).

Sampling procedure. As the various tests could not all be done on the same day, each sample was spread over a period of three days. On the first day, two plants from each subplot (cultivar/soil moisture treatment combination) were carefully lifted and the five largest undamaged tubers from each plant were used for the fluorescein test. On both the second and third days, four plants (yielding twenty tubers) were harvested from each subplot and inoculated respectively with either *E. c. atroseptica* or *P. infestans*. The five sampling periods were 27-29 June (1), 18-20 July (2), 8-10 August (3), 29-31 August (4) and 19-21 September (5). Some plants from the 'natural' plot were

lifted on 2 October and stored at +2°C for inoculation during the storage period (2 January, fluorescein and 10 January, bacterial tests). The tubers were carefully washed in running tap water and dried on paper towelling before soaking or inoculation, using procedures similar to those of chapters 2 and 4, but detailed below. Care was taken to standardize procedures between the different sampling dates.

Fluorescein soak. Tubers were measured, scored for proliferation (see chapter 2D, page 38), soaked for 3½ h in fluorescein, washed, dried and then examined under ultra-violet light without cutting or peeling (see chapter 2E, page 42), The total number of lenticels, the number penetrated by dye and the size of each penetrated area (to the nearest mm) were recorded for each tuber. A few lenticels from each cultivar/soil moisture treatment combination were then cut out and fixed for microscopic examination (chapter 2B, page 15), the results of which are reported in section C of this chapter (page 108).

Inoculation with bacteria. This followed the method described in chapter 4B (page 81), using E. c. atroseptica in suspensions adjusted to 2×10^7 cells ml^{-1} and soaking for 3½ h. Ten tubers from each subplot were inoculated and ten were soaked in saline as controls. After soaking, each batch of ten tubers was washed, dried and put into a separate Polythene bag with 50 ml sterile distilled water (25 ml on 28 June) for the overnight incubation and after 24 h drying, the number of rots and the size of each (to nearest mm) was recorded for each tuber.

Inoculation with P. infestans. This followed the method of Lacey (1967) described in chapter 4E (page 93), using a suspension of P. infestans whose concentration was estimated using a haemocytometer and adjusted to 45,000 sporangia ml^{-1} (30,000 only on 31 August) prior to incubation at 15°C to stimulate zoospore release. Of the twenty tubers harvested from each subplot, ten were inoculated and ten used

as controls. Each ten tuber batch was placed in a wooden seed box (360 x 220 x 80 mm) prepared as before, the boxes were placed in groups of four on the floor, and 10 ml glass-distilled water atomized over each group of boxes to wet the tuber surfaces. This was followed by 20 ml either of the sporangial-zoospore suspension (giving about 300 sporangia cm^{-2} sprayed surface), or distilled water (controls). The wet Airoporena pieces, which lined the boxes and which had been laid over the tubers, were removed after 1 day and replaced by a single piece over the top of the box, but not touching the tubers. Infection of eyes and lenticels was scored after 5 days (6 days on the first sample) and also after 8 days on the fourth and fifth samples only.

Results

General. Soil moisture tension in the different plots, rainfall, the pre-sample periods and sampling days are shown in Fig. 5, which indicates that the growing season was predominantly dry, with rain in early August and mid-September, corresponding with the latter parts of the third and fifth pre-sample periods respectively. Thus on the first, second and fourth samples, 'natural' plots were virtually replicates of the 'dry' ones, while at sampling on the other occasions soil was as wet as in the 'wet' plots.

Fluorescein. Tubers soaked in late June (1) were so extensively penetrated that numbers of lenticels affected could not be assessed. The data from other samples were examined in various ways; the mean percentage of lenticels penetrated is shown in Table 30, while the angular transformations necessary for statistical analysis are shown in the appendix (Table A1). The percentage of lenticels penetrated decreased as tubers aged and were stored, but results were otherwise

Fig. 5. Relation between rainfall and changes in soil moisture during the different 3 week pre-sampling periods

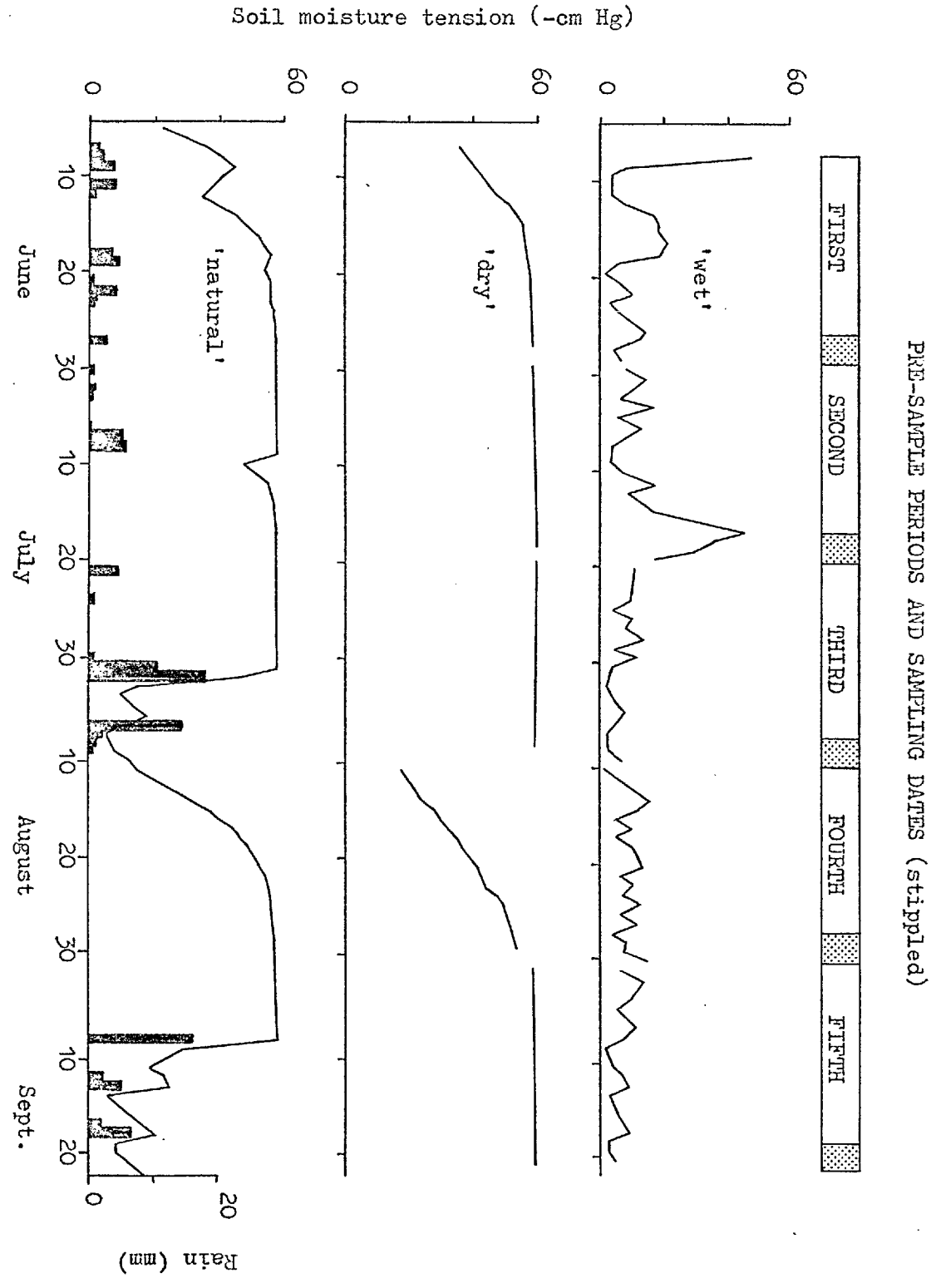


Table 30. Effect of field treatment in predisposing lenticels to fluorescein penetration

	Sample				
	2	3	4	5	Post-harvest
	Mean % lenticels penetrated				
PC wet	24.90	9.18	9.82	9.98	
natural	15.72	11.53	13.34	9.87	
dry	22.78	15.53	12.76	8.22	
MJ wet	34.78	24.45	9.90	15.27	
natural	26.16	19.51	26.69	15.73	
dry	29.56	11.81	20.02	19.94	
KE wet	26.62	15.63	12.23	10.57	
natural	17.65	20.24	12.85	15.74	
dry	31.44	24.11	11.88	10.63	
Cultivar totals					
PC	21.45a	12.03b	12.03b	9.32b	3.17
MJ	30.43a	18.59b	18.87b	17.02b	8.71
KE	25.46a	20.13ab	12.31b	12.08b	4.64
Treatment totals					
wet	28.68c	16.93cd	10.71cd	11.91cd	
natural	19.77c	17.19cd	17.56cd	13.74cd	
dry	27.25c	16.88d	15.00d	13.18d	
Grand total					
	25.54e	17.00f	14.39f	12.91f	5.59

In the lower parts of the table, means followed by the same letter did not differ significantly at p = 0.05 (transformed data). Comparisons are valid vertically and horizontally within, but not between, cultivar, treatment and grand totals. Post-harvest data are excluded from statistical comparison.

Table 31. Effect of field treatment in predisposing lenticels to bacterial infection

	Sample					
	1	2	3	4	5	Post-Harvest
	Mean no. lenticels infected per tuber					
PC wet	34.9	35.4	42.5	6.4	2.8	
natural	22.8	18.2	26.4	12.7	2.3	
dry	42.6	32.0	12.5	12.1	5.4	
MJ wet	28.7	37.2	21.8	13.5	2.3	
natural	28.2	34.9	33.0	15.0	4.0	
dry	34.1	24.4	21.3	13.9	16.0	
KE wet	19.3	21.9	10.4	5.6	3.7	
natural	17.4	23.1	27.1	1.9	2.7	
dry	-	21.3	5.9	7.2	3.9	
Cultivar totals						
PC	33.4a	28.4a	27.1a	10.4b	3.5c	2.1
MJ	30.3a	32.2a	25.4a	14.1b	7.4bc	9.5
KE	18.4a	22.1a	14.5a	4.9bc	3.4bc	4.3
Treatment totals						
wet	27.6d	31.5d	24.9d	8.5e	2.9f	
natural	22.8d	25.4d	28.8d	9.9e	3.0f	
dry	38.3d	25.7d	13.2d	11.1de	8.4df	
Grand total						
	28.50g	27.55g	22.32g	9.81h	4.79j	5.3

See footnotes of Table 30

variable and showed no consistent effect of the soil moisture treatment or cultivar.

The mean number of lenticels recorded per tuber (125.0 $\pm$ 2.38) did not differ significantly between cultivars and soil moisture treatments, or increase between July and September, (samples 2-5) despite a continued increase in tuber surface area.

Bacteria. Owing to the small size of tubers which had developed, no KE 'dry' plot sample was inoculated in June (1). The mean number of infected lenticels per tuber (x) in each treatment and date is shown in Table 31, but for analysis this was transformed to the form $\log_{10}(x+1)$ which is detailed in the appendix (Table A2). Apart from a little infection in June (1), control tubers remained healthy. The most significant effect was the decrease in number of rots as tubers of all cultivars aged, indicating increasing resistance of the lenticels. There seemed no further change after harvest. There was no consistent effect of soil moisture treatment, or of cultivar. The diameter of rots which developed tended to be greater on MJ than KE or PC (Table 32).

Table 32. The effect of cultivar on the size of bacterial rots

Cultivar	Sample 1	2	3	4	5	Total
	% of rots 3 mm or more in diameter					
PC	15.1	39.6	28.3	36.2	28.6	26.9
MJ	32.0	50.0	40.6	52.1	42.1	42.5
KE	12.0	54.0	32.7	35.4	29.1	36.5
Total	21.3	47.5	33.9	43.7	35.7	35.7

Blight. The mean number of infected lenticels per tuber (y) for each treatment and date is shown in Table 33, while for analysis, this

Table 33. Effect of field treatment in predisposing lenticels to blight infection

	Sample				
	1	2	3	4*	5*
	Mean no. lenticels infected per tuber				
PC wet	5.9	0.2	1.3	0.9(1.0)	1.9(2.6)
natural	6.5	0.8	0.5	0.4(0.6)	3.0(3.6)
dry	5.2	2.7	2.1	0.3(0.6)	2.0(2.4)
MJ wet	6.6	2.1	0.9	1.2(1.4)	0.6(1.2)
natural	6.8	2.4	1.5	0.0(0.2)	1.0(1.3)
dry	3.3	3.0	1.8	0.5(0.6)	0.5(0.6)
KE wet	13.2	1.7	1.0	0.6(1.4)	0.6(1.3)
natural	9.9	1.9	3.1	0.0(0.4)	0.5(1.3)
dry	8.3	5.5	5.4	0.0(0.1)	0.3(1.0)
Cultivar totals					
PC	5.9a	1.2cf	1.3cf	0.5(0.7)cf	2.3(2.9)f
MJ	5.6a	2.5de	1.4ce	0.6(0.7)ce	0.7(1.0)ce
KE	10.5b	3.0d	3.2d	0.2(0.6)c	0.5(0.7)c
Treatment totals					
wet	8.6g	1.3h	1.1h	0.9(1.3)h	1.0(1.7)h
natural	7.7g	1.7h	1.7h	0.1(0.4)m	1.5(2.1)h
dry	5.6g	3.7k	3.1k	0.3(0.4)m	0.9(1.3)hm
Grand total					
	7.30n	2.26p	1.96p	0.43(0.70)r	1.16(1.70)s

See footnotes of Table 30

* 5 day score followed by 8 day (in brackets).

Statistical comparisons are based on 5 day scores

was transformed to the form $\log_{10}(y+1)$ which is detailed in the appendix (Table A3). No control tubers became infected. Infection was much more frequent in June (1) than subsequent samples, and bearing in mind the less concentrated inoculum used in late August (4), a decreasing frequency with increasing tuber age was evident. As anticipated, KE was more susceptible than MJ or PC (at least in earlier samples), but there was no consistent effect of soil moisture treatment. Skin cracks were numerous on PC in late September (5), and penetration of these may account for the large number of infections.

The number of eyes infected (summarized in Table 34) increased considerably on the late September sample of all cultivars, but otherwise showed no general trend.

Table 34. Mean number of eyes per tuber infected by P. infestans

Cultivar	Sample				
	1	2	3	4*	5*
PC	0.9	0.3	0.7	0.6(0.7)	2.3(2.3)
MJ	0.7	0.6	0.5	0.2(0.2)	1.1(1.2)
KE	2.3	0.9	1.0	0.5(0.9)	1.4(2.1)
Totals	1.29	0.59	0.73	0.43(0.59)	1.59(1.88)

S.E. (means): Cultivar.sample $\pm$ 0.25 (vertical or horizontal comparisons)

Sample totals $\pm$ 0.145

* 5 day score followed by 8 day in brackets. S.Es refer to 5 day means

Summary. Lenticel penetration by dyes and infection by bacteria decreased as tubers aged, and was not sufficiently affected by soil moisture treatment to be detectable with this degree of replication. If such differences do occur, they must be slight and therefore seem

unlikely to be of great biological significance. The mean number of lenticels affected per tuber on each occasion (bulking cultivars) for the three tests is shown in Fig. 6. Under these conditions, many more lenticels were infected by E. carotovora than by P. infestans, while dyes usually affected even more lenticels.

C. LENTICEL ANATOMY IN 1971 AND 1972

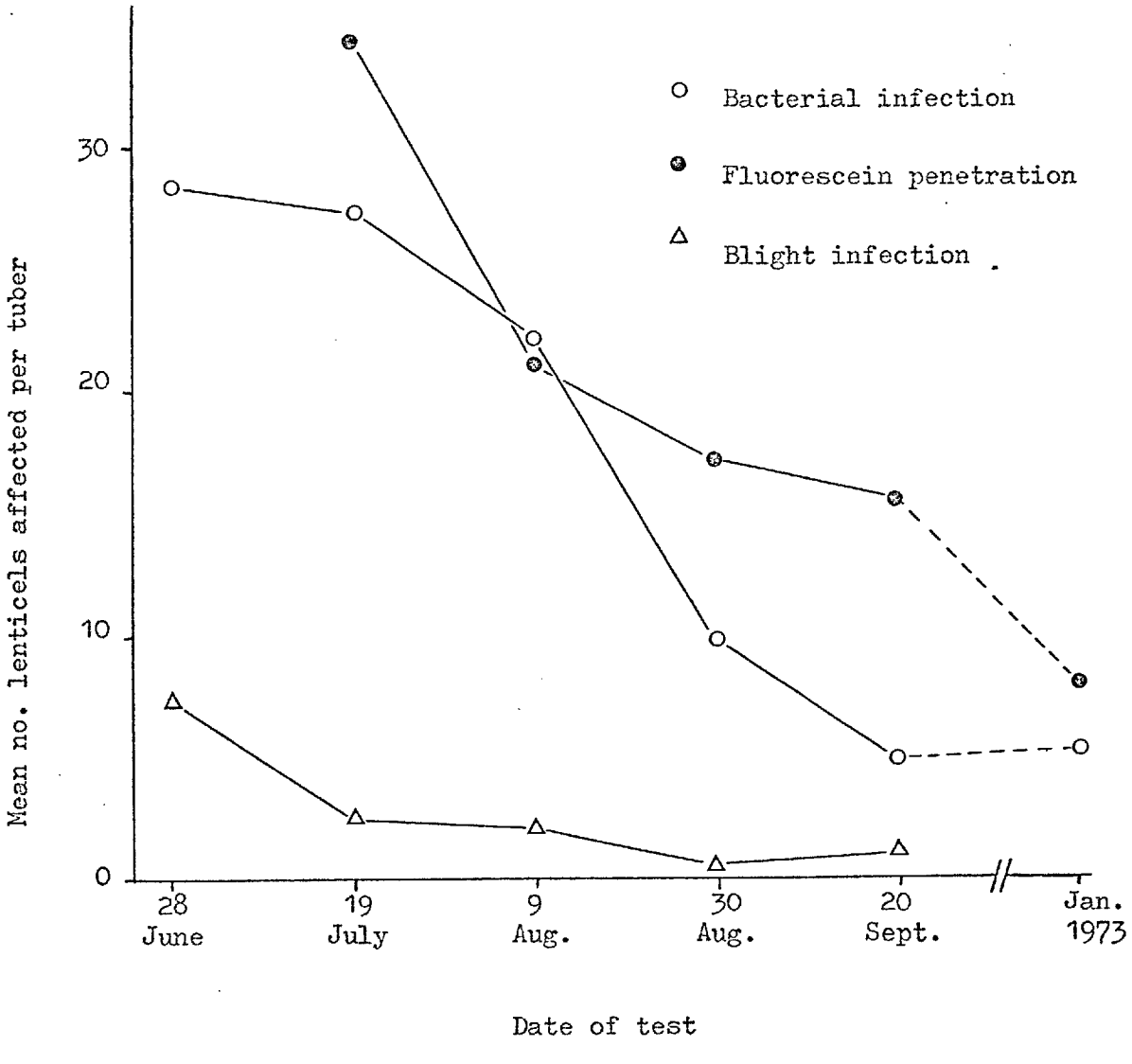
Materials and methods

Sampling in 1972 was from the plots of the experiment described above (section B), while in 1971, a small area of the same three cultivars, also raised from Rothamsted once-grown 'seed' (from VTSC source) was used. At different dates throughout the season, two plants per cultivar were carefully lifted and the five largest tubers from each plant washed and scored for proliferation (see chapter 2D, page 38). A few lenticels were then cut out, fixed, wax embedded, sectioned and examined microscopically after Conant staining (see chapter 2B, page 15). In 1971, the plants sampled had been exposed only to the prevailing weather conditions except for a small area of each cultivar which was kept wet by hand-watering on alternate days from 24 August until plants were sampled on 7 October. In 1972, the samples for the fluorescein dye penetration test (see above, section B) from each of the soil treatments were examined, and additional samples of the 'natural' plot only were made early in the season for scoring proliferation. In both years, soil moisture tensions were estimated using porous pot tensiometers sited as previously described (page 66).

Stained sections were examined and scored for each of the following characteristics:

(a) Proliferation: presence or absence.

Fig. 6. Relation between lenticel penetration or infection and tuber age (data bulked for cultivars)



(b) Cork barrier below filling cells: presence or absence.

(c) Suberization: scored on a scale, 0 (absent), 1 (slight to moderate) or 2 (intense staining) at x75 magnification.

Results

The scores for proliferation and suberization and the proportion of lenticels with cork barriers for each cultivar on each sampling date in both years, are recorded in the appendix (Tables A4-A7). In 1971 and in plots receiving natural rainfall in 1972, there were few significant differences between cultivars and the bulked results have been used to compile Figs. 7 and 8 (1971 and 1972 respectively), which indicate the relation between rainfall, soil moisture tension, proliferation, suberization and the presence of cork barriers.

In 1971, the first samples (June) were from wet soil and were extensively proliferated, but during July, which was predominantly dry, proliferation ceased, filling cells became suberized and by early August some lenticels had cork barriers beneath. The rain of early and mid-August resulted in reproliferation, but although many of the tubers were affected, they did not proliferate extensively and mean scores remained small. In fact, proliferation at this stage involved the production of fresh white parenchyma flush with the tuber surface (score 1) and never the extrusion of callus-like tissue (score 2). This activity of the lenticels was evidenced by a decrease in the suberization of the filling cells and in the presence of cork barriers, which tendency was reversed as the soil dried in late August and September. The irrigation applied from 24 August until 7 October had little, if any, effect on the lenticels (Tables A4 and A5).

In 1972, soil had been wet in May and early June, but by the first samples at the end of June had become quite dry (tension greater

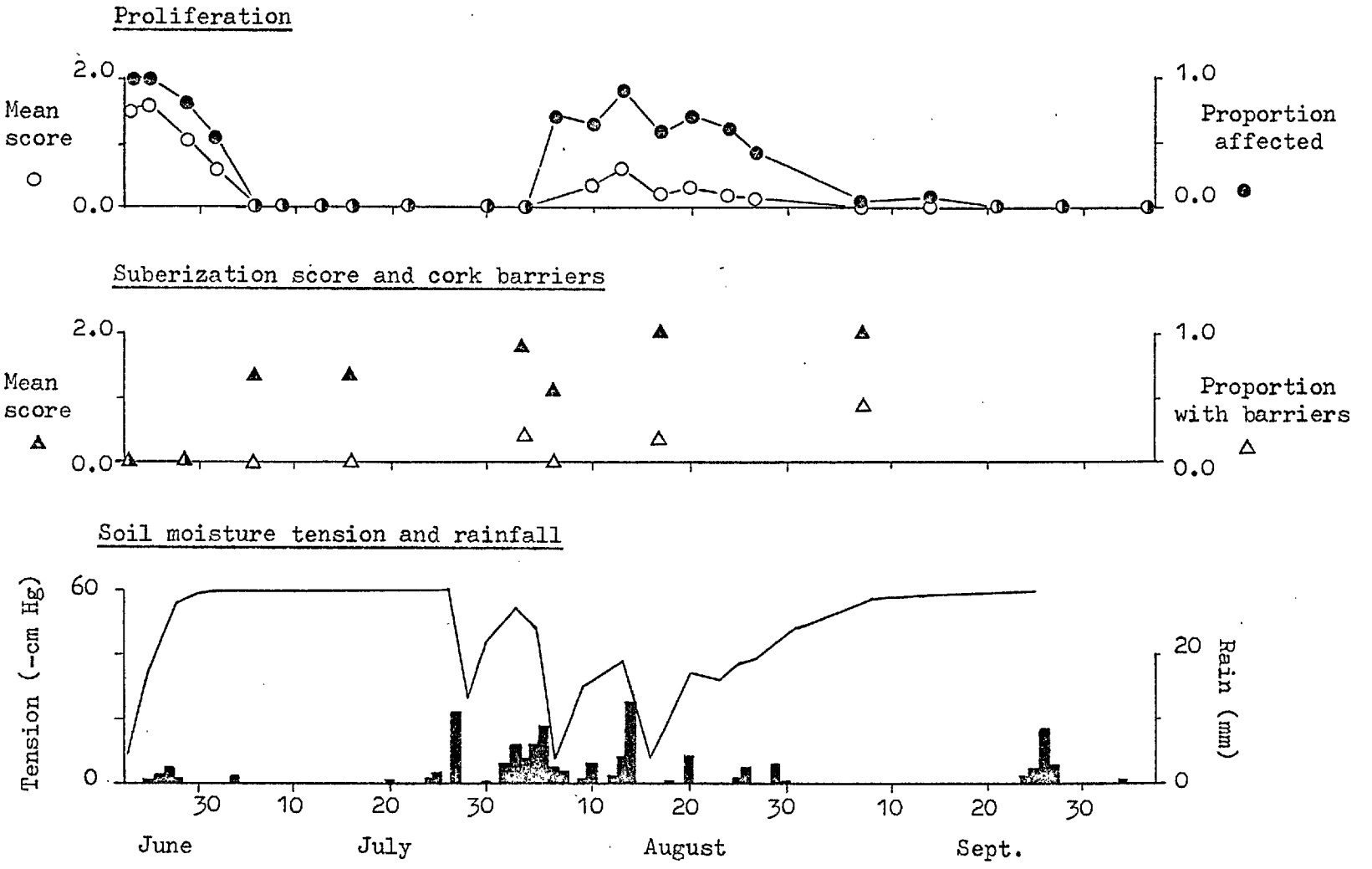


Fig. 7. Relation between rainfall, soil moisture changes and the proliferation or suberization of lenticels, 1971

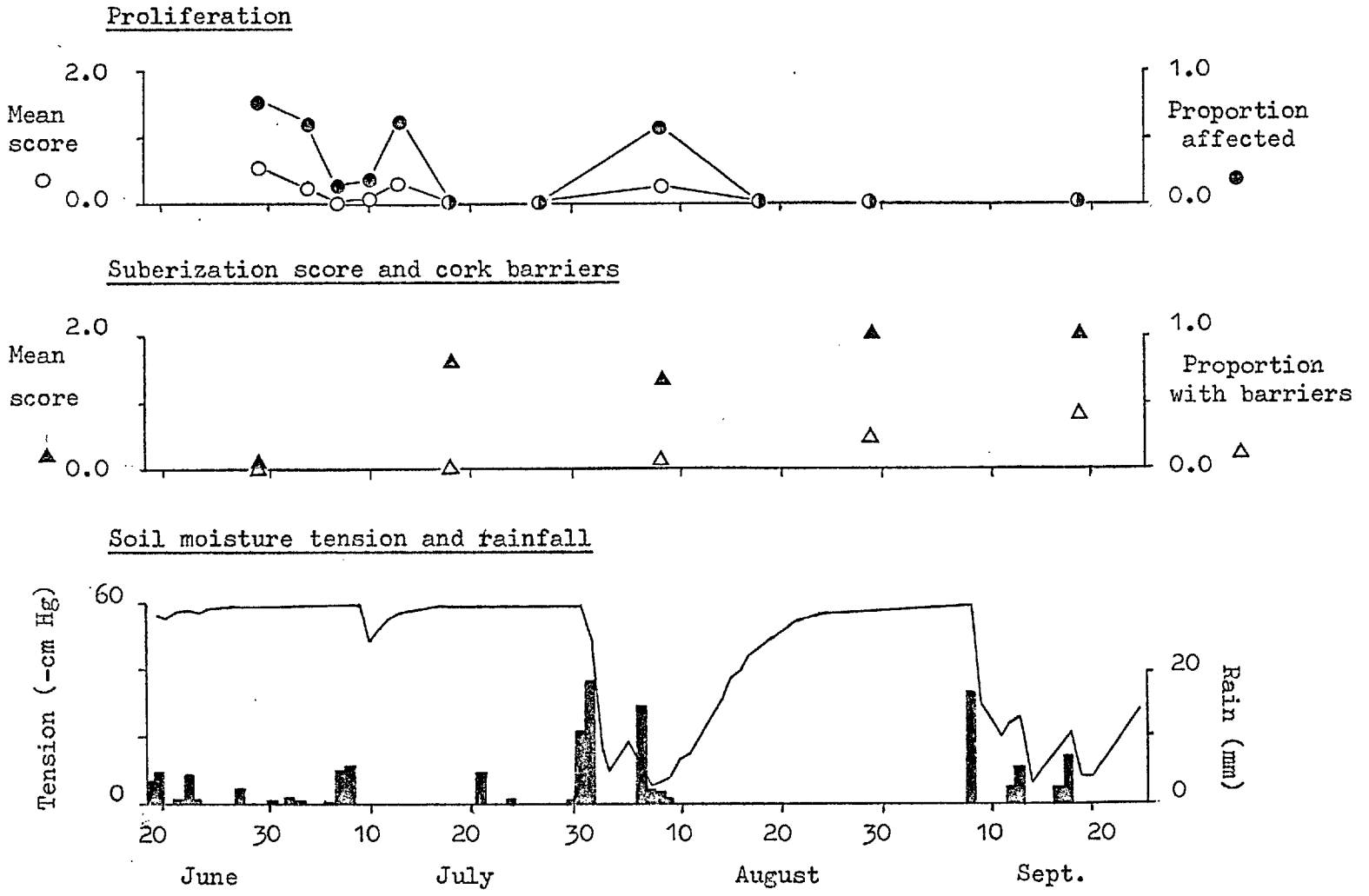


Fig. 8. Relation between rainfall, soil moisture changes and the proliferation or suberization of lenticels, 1972

than -60 cm Hg). Many tubers showed some, but only slight, proliferation which increased following rain on 7 and 8 July and then died away. Much rain in late July and early August kept soils wetter than about -15 cm Hg for about 10 days, and on about half of the tubers sampled some lenticels produced fresh parenchyma, which (as in 1971) never extruded as callus tissue. As a result, the mean suberization score of the filling cells decreased, but in the ensuing dry period suberization increased and cork barriers were developed. The soil again became wet in mid-September, but no proliferation developed, nor did the mean suberization score or the proportion of lenticels with cork barriers decrease.

The soil treatments applied in 1972 influenced lenticel proliferation only up to early August (the first three sample periods). During this time, lenticels proliferated in the 'wet' plots and as a consequence the suberization score decreased, while on the last two samples (late August and mid-September) no proliferation occurred and suberization and cork barriers appeared unaffected.

Thus lenticels proliferated less readily as the season progressed, which agrees with laboratory tests (chapter 2D, page 40) and it seems unlikely that proliferation would be stimulated under field conditions after mid-August. In both years, PC was slightly less responsive to wet conditions (Tables A4-A7), which again agrees with laboratory findings. Cork barriers developed in about half the lenticels examined by September, and in 1972 this was irrespective of soil moisture status.

D. DISCUSSION

Studies of lenticels in the field have largely confirmed the laboratory investigations described in chapter 2. As tubers aged, they proliferated less readily in wet soils (whether resulting from

rain or hand-watering), and 3 weeks during which soil moisture was kept near to field capacity was insufficient to stimulate proliferation in late August and September. Decreasing penetration of dyes as tubers aged was presumably a reflection of increasing suberization and more frequent cork barriers in the lenticels.

Although proliferation was induced in the wet plots during the earlier samples in 1972, subsequent infection by Erwinia carotovora in the laboratory did not differ between samples from different soil treatments. Laboratory tests on older tubers had shown that proliferation could increase lenticel susceptibility (chapter 4D), but either this was not so for young tubers, or the proliferation was insufficiently frequent (or the test insufficiently sensitive) for the difference to be determined. Whatever the reason, it is unlikely that proliferation would significantly affect the susceptibility of young tubers in the soil, and as tubers aged their lenticels would be increasingly unlikely to proliferate. Infection of lenticels decreased as tubers aged, presumably for similar reasons to those decreasing dye penetration. Thus, as is discussed in chapter 4F (page 95), lenticel infection is likely to be a feature of younger tubers, and wet soils are likely to influence the incidence of soft-rotting more by aiding pathogen movement than by affecting lenticel susceptibility.

Many fewer lenticels were infected by Phytophthora infestans than by Erwinia carotovora under these conditions, and numbers infected also decreased as tubers aged, in agreement with Zan (1962) and Lacey (1967), whereas eye infection showed no particular trend as Lacey (1967) and Lapwood (1967) noted. It was difficult to detect small changes in lenticel resistance when so few were infected, but as with E. carotovora, soil moisture differences did not appear to affect the numbers of lenticels infected, and proliferation can hardly be a major factor in

the epidemiology of tuber infection by P. infestans, even though Zeck (1957) claimed that proliferation increased lenticel susceptibility in laboratory tests.

Chapter 6: GENERAL DISCUSSION

When attempting to understand how different conditions influence any plant disease, it is essential to study their effects not only on the pathogen, but also on the host. Thus a knowledge of lenticel anatomy and response to environmental conditions, such as has been gained through these investigations, is essential if the factors affecting the infection of potato tubers by several important diseases are to be understood.

Many earlier investigators obtained inconclusive results from experiments on common scab, often because the relationship of the disease to soil moisture was poorly understood, but also because the changes of lenticel susceptibility with the growth of the tuber were not appreciated. The structures which are susceptible, and hence the time each developing lenticel is susceptible, has at present only been detected indirectly and awaits conclusive evidence from direct inoculation, but this is only a matter of technique now that the principles of internode growth and lenticel development have been established. Not surprisingly, previous attempts to understand differences in the scab susceptibility of cultivars have given confused and contradictory results, but the way should shortly be open to investigate the biochemistry of the lenticels where these differences are expressed, although there may be difficulties in working with such small 'target' areas.

The way in which scab infection is prevented in wet soils cannot be explained solely in terms of lenticel structure, but future work on populations of, and interactions between, micro-organisms must be related to the relatively small part of the tuber surface where infection would be possible at that time.

Although several other diseases are caused by actinomycetes similar or identical to S. scabies, the host-parasite relationship in potato common scab is almost certainly unique. The structures which are infected to cause soil rot (S. ipomoea (Person & Martin) Waksman & Henrici) of sweet potatoes are not known, but lenticels have not been described on this root crop, and there are similar uncertainties about the scab diseases of other roots, including beet and mangels. However, the diseases may be similar in other respects, since infection on sweet potatoes can be controlled by irrigation (Poole, 1925) or by sulphur application (Person, 1946), both of which are effective treatments against common scab of potato (Barnes, 1972).

With the bacterial soft-rots, lenticels are not the only infection sites and these studies suggest they become less important as the tuber matures, while at and after harvest, spread of the disease is largely through wounds. The increasing resistance of maturing lenticels may be related to their structure, but only in general terms. While proliferation increased susceptibility in laboratory tests of mature tubers, conditions are unlikely to be sufficiently severe to stimulate such proliferation and it seems unlikely that this process plays a significant part in the epidemiology of the soft-rotting diseases. Lenticel infection of younger tubers presumably depends largely on the movement of bacteria in soil water to the infection site, but such infections may only occasionally (depending on soil moisture conditions) destroy the tuber immediately. A major deficiency in our present knowledge is an understanding of latent infection and the conditions which can stimulate the resumption of pathogenic activity in store and cause extensive losses.

Laboratory tests were insufficiently sensitive to detect differences in blight susceptibility due to proliferation as few

lenticels became infected, and increasingly so as tubers aged. Such differences are not necessarily discounted, but must be slight and thus lenticel proliferation is unlikely to be important in the epidemiology of tuber infection by Phytophthora infestans.

Several potato diseases other than those studied here are reported to infect lenticels. Skin spot infection (by Oospora pustulans Owen & Wakef.) is favoured by damp conditions, which Allen (1957) suggested could be related to a change in the lenticel filling cells, but this seems unlikely since lenticels were much more susceptible in October than August, while my studies indicate that lenticels would be increasingly less responsive to moisture changes as they matured. Silver scurf (caused by Helminthosporium solani Dur. & Mont.) may also infect lenticels but is a very superficial and slow-developing disease, and as for skin spot, tubers become more susceptible as they mature (Burke, 1938). Spongospora subterranea (Wallr.) Lagerh. causing powdery scab is technically a difficult pathogen to study and while a little is known of its life-cycle and of root infection, tuber infection has not been directly studied. It may occur on young tubers and is most severe in wet soils and especially if the moisture content fluctuates (Kole, 1954). Our knowledge of the host-parasite relationships in these diseases is thus scanty, but now that more is known about lenticel structure and physiology further advances should be possible.

APPENDIX

Table A1. Effect of field treatment in predisposing lenticels to fluorescein penetration - transformed data (angles) (compare with Table 30)

	Sample				
	2	3	4	5	Post-harvest
PC wet	30.41	17.97	17.39	17.35	
natural	23.61	19.46	20.02	18.31	
dry	27.49	21.87	21.03	15.48	
MJ wet	36.96	29.38	17.85	22.84	
natural	30.72	25.96	31.27	23.16	
dry	32.40	19.11	26.58	26.02	
KE wet	31.06	23.36	20.00	18.78	
natural	24.49	26.48	20.74	22.21	
dry	34.50	29.16	20.77	18.53	
Cultivar totals					
PC	27.17	19.77	19.48	16.99	10.25
MJ	33.36	24.81	25.24	24.01	17.16
KE	30.01	26.45	20.50	19.48	12.43
Treatment totals					
wet	22.81	23.57	18.41	19.66	
natural	26.27	24.08	24.01	21.17	
dry	31.46	23.38	22.79	20.01	
Grand total					
	30.18	23.68	21.74	20.28	13.68

S.E. (means), excluding post-harvest data:

Sample date totals $\pm$ 1.165

Sample.Cultivar and Sample.Treatment $\pm$ 2.018

Sample.Cultivar.Treatment $\pm$ 3.496

Table A2. Effect of field treatment in predisposing lenticels to bacterial infection - log.transformed data (compare with Table 31)

	Sample					
	1	2	3	4	5	Post-harvest
PC wet	1.544	1.494	1.633	0.728	0.405	
natural	1.321	1.220	1.377	0.917	0.374	
dry	1.622	1.441	1.014	0.948	0.506	
MJ wet	1.303	1.488	1.306	0.858	0.246	
natural	1.358	1.486	1.487	0.921	0.428	
dry	1.519	1.241	1.276	0.754	1.044	
KE wet	1.270	1.228	0.910	0.703	0.548	
natural	1.226	1.370	1.417	0.358	0.319	
dry	-	1.212	0.744	0.614	0.508	
Cultivar totals						
PC	1.496	1.385	1.341	0.864	0.429	0.424
MJ	1.393	1.405	1.357	0.845	0.573	0.925
KE	1.238	1.270	1.024	0.558	0.458	0.661
Treatment totals						
wet	1.372	1.404	1.283	0.763	0.400	
natural	1.302	1.359	1.427	0.732	0.374	
dry	1.453	1.298	1.011	0.772	0.686	
Grand total						
	1.376	1.353	1.241	0.756	0.487	0.670

S.E. (means), excluding post-harvest data:

Sample date totals ± 0.0630

Sample.Cultivar and Sample.Treatment ± 0.1091

Sample.Cultivar.Treatment ± 0.1890

Table A3. Effect of field treatment in predisposing lenticels to blight infection - log. transformed data (compare with Table 33)

	Sample				
	1	2	3	4*	5*
PC wet	0.801	0.060	0.301	0.258	0.373
natural	0.814	0.198	0.120	0.108	0.482
dry	0.734	0.516	0.436	0.060	0.411
MJ wet	0.834	0.428	0.186	0.294	0.156
natural	0.879	0.453	0.283	0.000	0.246
dry	0.559	0.406	0.341	0.138	0.151
KE wet	1.130	0.323	0.246	0.151	0.156
natural	1.023	0.391	0.543	0.000	0.126
dry	0.949	0.636	0.740	0.000	0.090
Cultivar totals					
PC	0.783	0.258	0.286	0.142	0.422
MJ	0.757	0.429	0.270	0.144	0.184
KE	1.034	0.450	0.510	0.050	0.124
Treatment totals					
wet	0.921	0.270	0.244	0.234	0.228
natural	0.905	0.347	0.316	0.036	0.284
dry	0.748	0.519	0.506	0.066	0.217
Grand total					
	0.858	0.379	0.355	0.112	0.243

* based on 5 day scores

S.E. (means): Sample date totals ± 0.0317

Sample.Cultivar and Sample.Treatment ± 0.0549

Sample.Cultivar.Treatment ± 0.0951

Table A4. Proliferation on sampled tubers in 1971

Sample date	PC Mean Score	PC Nos affected*	MJ Mean Score	MJ Nos affected*	KE Mean Score	KE Nos affected*	Total Mean Score	Total Proportion affected
23 June	1.13	10	1.61	10	1.70	10	1.48	1.00
25	1.09	10	1.80	10	1.80	10	1.56	1.00
29	1.00	10	0.45	4	1.65	10	1.03	0.80
2 July	1.19	10	0.25	2	0.17	4	0.54	0.53
6	0.00	0	0.00	0	0.00	0	0.00	0.00
9	0.00	0	0.00	0	0.00	0	0.00	0.00
13	0.00	0	0.00	0	0.00	0	0.00	0.00
16	0.00	0	0.00	0	0.00	0	0.00	0.00
22	0.00	0	0.00	0	0.00	0	0.00	0.00
30	0.00	0	0.00	0	0.00	0	0.00	0.00
3 Aug	0.00	0	0.00	0	0.00	0	0.00	0.00
6	-	9	-	9	-	3	-	0.70
10	0.14	5	0.59	8	0.28	6	0.34	0.63
13	0.52	9	0.62	8	0.62	10	0.59	0.90
17	0.21	6	0.09	4	0.26	7	0.19	0.57
20	0.31	6	0.16	6	0.45	9	0.31	0.70
24	0.15	6	0.15	5	0.12	7	0.14	0.60
27	0.09	4	0.05	3	0.25	6	0.13	0.43
7 Sept	0.01	1	0.00	0	0.00	0	0.00	0.03
14	0.02	1	0.00	0	0.05	1	0.02	0.07
21	0.00	0	0.00	0	0.01	1	0.00	0.03
28	0.00	0	0.00	0	0.01	1	0.00	0.03
7 Oct.	0.00	0	0.00	0	0.05	1	0.02	0.03
7 (irrigated)	0.01	1	0.00	0	0.09	4	0.03	0.17

* out of ten

Table A5. Suberization and cork barriers of sampled lenticels in 1971

Sample date	PC		MJ		KE		Total	
	S*	C†	S	C	S	C	S	C
23 June	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
29	0.17	0.00	0.00	0.00	0.00	0.00	0.05	0.00
6 July	no sample		0.40	0.00	2.00	0.00	1.33	0.00
16	1.50	0.00	0.87	0.00	2.00	0.00	1.35	0.00
3 August	2.00	0.50	1.83	0.17	1.50	0.00	1.78	0.21
6	1.25	0.00	1.25	0.00	0.78	0.00	1.08	0.00
17	2.00	0.00	2.00	0.00	2.00	0.50	2.00	0.17
7 September	2.00	0.00	2.00	0.00	2.00	1.00	2.00	0.45
7 October (irrigated)	2.00	0.00	1.75	0.25	2.00	0.25	1.92	0.17

* S = Suberization (mean score)
† C = Cork barriers (proportion affected)

Table A6. Proliferation on sampled tubers in 1972

Sample date.....		29 June	4 July	7	10	13	18	27	8 Aug	18	29	19 Sept
cv and plot												
	Natural	0.45(6)	0.00(0)	0.00(0)	0.00(0)	0.03(1)	0.00(0)	0.00(0)	0.04(2)	0.00(0)	0.00(0)	0.00(0)
PC*	Dry	0.05(1)	-	-	-	-	0.00(0)	-	0.01(1)	-	0.00(0)	0.00(0)
	Wet	1.55(10)	-	-	-	-	0.07(5)	-	0.05(4)	-	0.00(0)	0.00(0)
	Natural	0.75(9)	0.28(9)	0.04(2)	0.00(0)	0.44(10)	0.00(0)	0.00(0)	0.40(7)	0.00(0)	0.00(0)	0.00(0)
MJ*	Dry	0.08(2)	-	-	-	-	0.00(0)	-	0.00(0)	-	0.00(0)	0.00(0)
	Wet	0.95(9)	-	-	-	-	0.20(9)	-	0.37(10)	-	0.01(1)	0.00(0)
	Natural	0.43(8)	0.28(9)	0.03(2)	0.13(5)	0.33(8)	0.00(0)	0.00(0)	0.35(8)	0.00(0)	0.00(0)	0.00(0)
KE*	Dry	0.13(5)	-	-	-	-	0.00(0)	-	0.00(0)	-	0.00(0)	0.00(0)
	Wet	1.48(10)	-	-	-	-	0.24(8)	-	0.64(8)	-	0.01(1)	0.03(1)
	Natural	0.54 (0.77)	0.19 (0.60)	0.02 (0.13)	0.04 (0.17)	0.27 (0.63)	0.00 (0.00)	0.00 (0.00)	0.26 (0.57)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
Total†	Dry	0.09(0.27)	-	-	-	-	0.00(0.00)	-	0.00(0.00)	-	0.00(0.00)	0.00(0.00)
	Wet	1.33(0.97)	-	-	-	-	0.17(0.73)	-	0.35(0.73)	-	0.01(0.06)	0.01(0.03)

* Mean score, followed by number (out of ten) in brackets. † Mean score, followed by proportion affected

Table A7. Suberization and cork barriers of sampled lenticels in 1972

Sample date...		29 June(1)		18 July(2)		8 August(3)		29 August(4)		19 September(5)	
cv and plot		S*	C†	S	C	S	C	S	C	S	C
	Natural	0.25	0.00	1.80	0.00	2.00	0.11	2.00	0.00	2.00	0.43
PC	Dry	0.00	0.00	1.86	0.00	2.00	0.00	2.00	0.17	2.00	0.63
	Wet	0.00	0.00	0.68	0.00	1.67	0.00	2.00	0.00	2.00	0.00
	Natural	0.00	0.00	1.88	0.00	0.67	0.00	2.00	0.50	2.00	0.38
MJ	Dry	0.00	0.00	1.30	0.00	1.33	0.00	2.00	0.00	2.00	0.43
	Wet	0.00	0.00	0.29	0.00	0.43	0.00	2.00	0.38	2.00	0.57
	Natural	0.14	0.00	1.50	0.00	1.00	0.00	2.00	0.25	2.00	0.38
KE	Dry	0.00	0.00	1.00	0.00	1.33	0.00	2.00	0.86	2.00	0.75
	Wet	0.00	0.00	0.00	0.00	1.33	0.00	2.00	0.57	2.00	0.75
	Natural	0.12	0.00	1.57	0.00	1.32	0.05	2.00	0.23	2.00	0.39
Total	Dry	0.00	0.00	1.39	0.00	1.56	0.00	2.00	0.35	2.00	0.61
	Wet	0.00	0.00	0.33	0.00	1.11	0.00	2.00	0.33	2.00	0.45

* S = Suberization (mean score) † C = Cork barriers (proportion affected)

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The effect of a few days of rain on the distribution of common scab (*Streptomyces scabies*) on young potato tubers

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(*Accepted* 23 *October* 1972)

SUMMARY

At Woburn, Beds. in 1967, potato tubers formed and grew in dry soil except after rain between 23 and 26 June. At lifting, Majestic tubers were severely and uniformly scabbed, except for distinct bands free from lesions. The position of the bands differed depending on the date when tubers started to form; they were further from the stolon attachment when tubers formed early and nearer it when tubers formed late.

Samples taken in June and July showed that the scab-free band and the late June rain were related. From 25 June to 1 July the soil was probably wet enough to prevent *Streptomyces scabies* infection, but only one or two tuber internodes remained free from infection; they were the internodes that began to expand about a week before the rain. Internodes that formed while the soil was wet became scabbed, showing they were still susceptible to *S. scabies* after the soil had dried again.

Recent work on lenticel formation has suggested a hypothesis to explain these results.

INTRODUCTION

Lapwood & Hering (1968, 1970) showed that the incidence and distribution of common scab lesions on tubers developing in a glasshouse in soil infested with *Streptomyces scabies* (Thaxt.) Waksman and Henrici depended on the duration of dry soil conditions and on the size of tubers at the time. Scab lesions developed on different parts of tubers when trickle irrigation was stopped during successive weekly periods.

This paper describes a natural occurrence of the converse situation, when a few rainy days during the period when tubers were most susceptible to *S. scabies* prevented infection of a band of the tuber surface.

MATERIALS AND METHODS

The experiment, on light land at Woburn, Beds. in 1967, was one of a series designed to relate scab infection to soil moisture. Four randomized blocks of three treatments were planted with the cultivar Majestic in plots with six rows each of thirty-five plants. The treatments aimed increasingly to delay the start of tuber formation; sprouted seed and unsprouted seed were both machine planted on 21 April, and unsprouted seed was hand planted into the formed ridges on 8 May.

Soil moisture tensions (−cm Hg) were measured with six tensiometers (Gallenkamp & Co. Ltd., London) set in ridges between potato plants, with the centre of the

porous pot 10 cm below the crest of the ridge. The initiation and development of tubers was studied by sampling four plants per plot on five occasions between June and August (Table 1). Eyes (nodes) were numbered from the oldest to the youngest along the spiral of phyllotaxy, and the internodes affected by scab were recorded from 28 June on the five largest tubers from each plant, usually on eighty tubers per treatment. At harvest on 25 August the modified Large & Honey (1955) key (see Lapwood & Dyson, 1966) was used to assess the incidence of scab on all tubers > 3 cm from ten plants per plot.

Table 1. *Development of tubers and distribution of common scab lesions from the three 'seed' treatments*

	June			July	
Sampling date ...	13	21	28	4	14
		Sprouted seed			
Tubers/plant	15	24	22	20	—
Total eyes formed/tuber† (mean of largest five)	5·7	7·6 ± 0·89*	10·1	11·1	12·5
Scab infection from stolon end limited at eye no.	—	—	5·4 ± 1·33*	5·9	5·3
No. scab-free internodes‡	—	—	—	—	1·2 ± 0·55*
		Unsprouted seed			
Tubers/plant	7	27	26	22	—
Total eyes formed/tuber† (mean of largest five)	3·8	5·5 ± 1·04	8·2	9·6	11·7
Scab infection from stolon end limited at eye no.	—	—	3·6 ± 0·78	3·8	3·8
No. scab-free internodes‡	—	—	—	—	1·3 ± 0·44
		Late-planted seed			
Tubers/plant	0	7	21	27	—
Total eyes formed/tuber† (mean of largest five)	—	—	5·5	7·3	9·6
Scab infection from stolon end limited at eye no.	—	—	1·9	1·0	1·4
No. scab-free internodes‡	—	—	—	—	1·5§

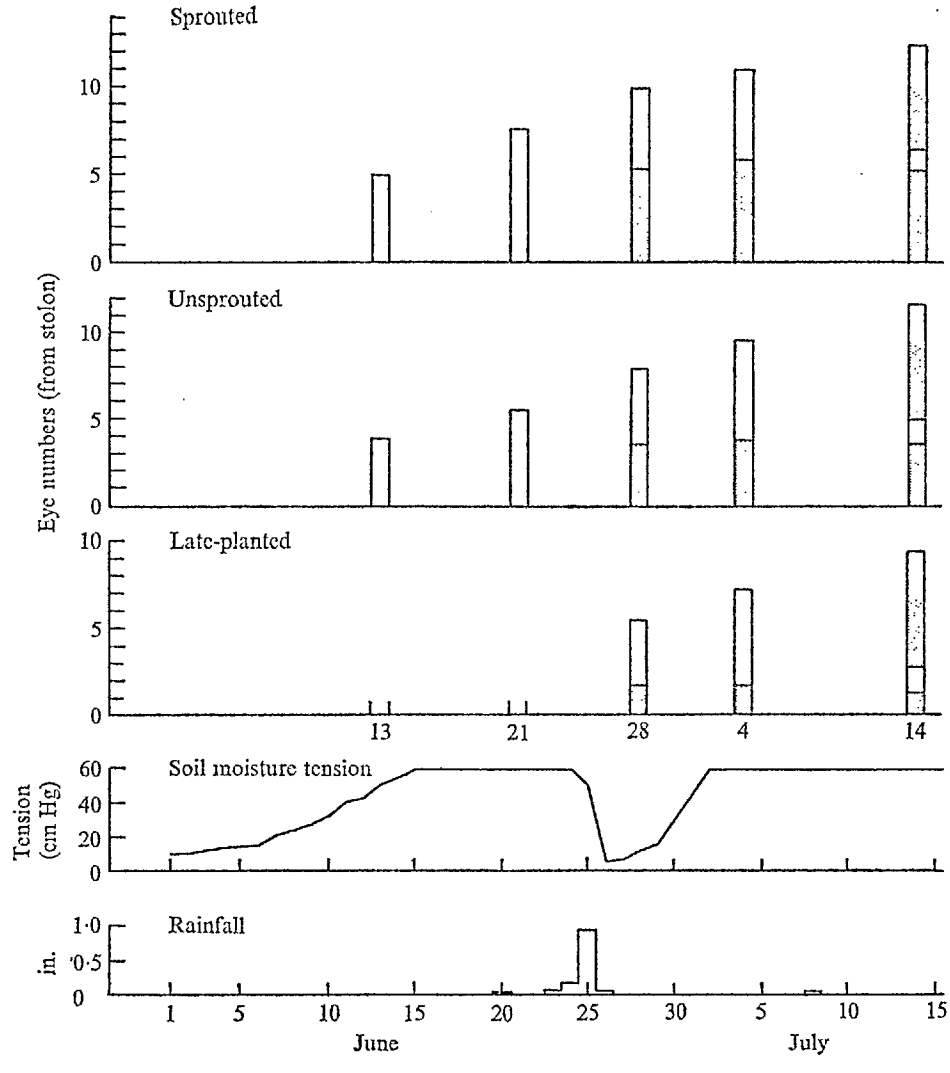
* S.E. single observation. † Includes apical bud.
‡ 40-tuber sample. § Only 20% tubers showed scab-free band.

RESULTS

On 25 August tubers from sprouted, unsprouted and late-planted seed treatments had respectively 67, 53 and 63 (±2·3)% of their surface area scabbed, and Plate fig. 1 shows that lesions occurred in two areas separated by a scab-free zone. The healthy zone was nearest the apex on tubers formed early from sprouted seed and nearest the stolon on tubers from late-planted seed.

Tuber formation in June and July occurred during dry weather, except for 1·3 in (33 mm) rain between 23 and 26 June, which increased soil moisture to field capacity for a few days (Text-fig. 1). A comparison of tuber samples taken before, during and after this rain (Plate fig. 2), showed that a scab-free zone was apparent by 4 July and that by 14 July there were many more scab lesions beyond this zone at the apex.

Text-fig. 1 shows how the growth of tubers (eyes produced) and the distribution of scab (Table 1), were related to the date, rainfall and soil moisture. On tubers developing from sprouted seed, surfaces between eyes 5 and 7 were free from scab lesions and were already formed by 21 June, before the rain. However, tissues from eyes 7 to



Text-fig. 1. Relation between tuber growth (number of eyes) and scab distribution from 28 June (shaded areas) at different dates for sprouted, unsprouted and late-planted seed treatments, and soil moisture and rainfall at Woburn 1967.

10 formed during the wet period up to 28 June and were scabbed on 14 July. Similarly on tubers developing later from unsprouted seed, tissues between eyes 4 and 5 remained healthy and those formed after eye 5 were scabbed.

DISCUSSION

Lapwood & Hering (1968) showed that when a scabby soil was allowed to dry for 5 days, four tuber internodes would usually be infected. By contrast, the Woburn experiment showed that when a soil was wet for about a week only one to two internodes escaped infection (Text-fig. 1 and Table 1). Further analysis of these and interpolated results suggests that changes in susceptibility as each internode ages may explain these differences.

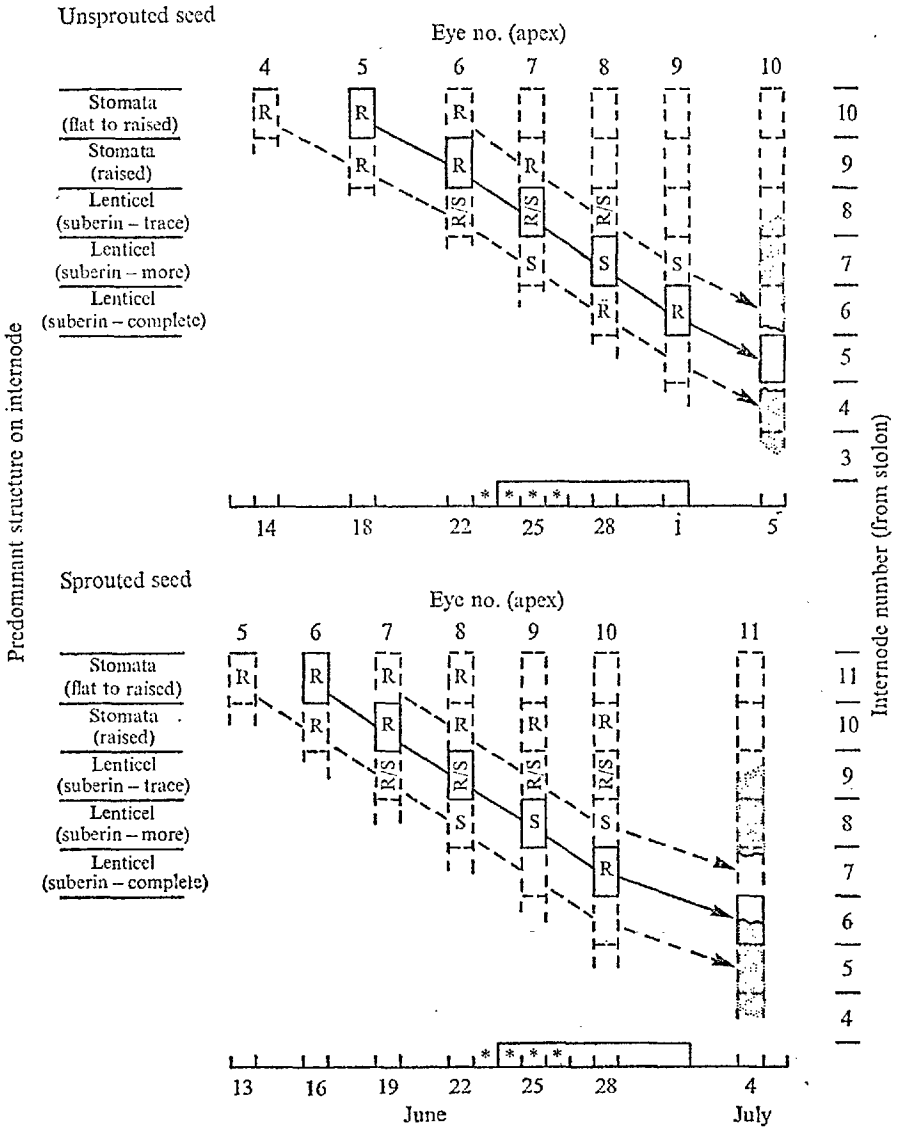
Table 2. *Relation between stages of tuber development, the scabbed and scab-free regions for the three seed treatments and the rain*

	Seed treatment				
	Sprouted		Unsprouted	Late-planted	
	(*a*) *Scab-free zone*				
Limits (eye no.)*	5·5–6·8		3·8–5·1	1·4–2·9	
	(*a*)	(*b*)		(*a*)	(*b*)
Scab-free internode†	6th or 7th		5th	2nd or 3rd	
Date internode started to form, June‡	16	19	18	16	19
Days before§					
(*a*) first rain	7	4	5	7	4
(*b*) main rain	9	6	7	9	6
	(*b*) *Second scab band*				
Starts: eye no.	7 (6·8)		5 (5·1)	3 (2·9)	
Internode no.	8		6	4	
Date internode started to form, June‡	22		23	23	
Days before§					
(*a*) first rain	1		0	0	
(*b*) main rain	3		2	2	

* From Table 1, eyes number from stolon along phyllotactic spiral.
† When data involves parts of internodes, the alternative whole internodes used.
‡ Date when relevant eye separated from apical bud by 0·5 mm, e.g. for 6th internode when eye 5 separated to make the apical bud the 6th eye position; calculated by interpolation from Table 1.
§ The first rain fell on 23 June; the main fall was on 25 June.

On tubers from unsprouted seed, the 5th internode (between eyes 4 and 5) was protected although it 'began to form' (was separated from the apical bud by 0·5 mm) on 18 June, 6 days before the soil became wet and unfavourable for infection (Table 2 and Text-fig. 2). The 4th internode, which formed about 10 days before the soil became wet, was usually scabbed later, suggesting that a newly formed internode is resistant to scab for at least 6 days, but is susceptible by the tenth day. The 6th internode which formed 2 days before the soil became wet and was scabbed by 5 July, must have been infected after the wet period, 10–13 days from its formation, while the 5th internode was about 14 days old when it re-entered this dry period, but did not become scabbed, suggesting that it was now resistant again. These data suggest that an internode is resistant for the first 6–8 days, then becomes susceptible for about 6 days and then again resistant. A similar pattern occurred on all seed treatments (Table 2).

These results differed from the findings of Lapwood & Hering (1968) who, from the relation between the rate of eye separation and rate of increase of infected internodes, assumed that the infected internodes included the one formed during the dry



Text-fig. 2. Relation between stages of lenticel formation (and the susceptibility (S) or resistance (R) of different tuber internodes), rain (dates*), period when soil moisture was unfavourable (dates boxed) and favourable for infection, and the distribution of scab (shaded) on internodes of tubers from unsprouted and sprouted seed.

period. Further examination of their Sutton Bonington data shows that in fact two apical internodes, including that formed during the 5 dry days did not become infected, which agrees with the Woburn data. For example, on tubers from unsprouted

seed exposed for 5 days in dry soil (Lapwood & Hering, 1968, Table 3), the scab-infected zone extended to internode 5 but not to internodes 6 or 7 although the 7th internode had been formed when the soil was dry.

The youngest tuber internodes bear stomata, which are converted to lenticels as the internode ages. Guard cells are generally unrecognizable on the third internode from the apex (Adams, 1972), and the data from Woburn and Sutton Bonington suggest that an internode in this position begins to be susceptible. We have recently found that the outer cell walls of non-proliferating lenticels of Majestic tubers are completely suberized on the fifth internode from the apex, which coincides with the end of the susceptible period. The Woburn results (Text-fig. 2) suggest that internodes bearing stomata were not infected in dry soil (R), but 'young' lenticels, where loss of guard cells exposed the inner tissues, were infected (S).

There is no convincing evidence that stomata can be infected by *S. scabies*. Fellows (1926) found stomata at the apex of scabbed tubers coloured brown, like newly infected lenticels, and assumed them to be infected, but could not detect mycelium in sections. Whether these tubers were from crops or artificially inoculated pots is not clear, but he could not reproduce the symptom on detached artificially inoculated tubers. Actinomycete mycelium is very fine and restricted initially to middle lamellae, so it is difficult to study the early stages of infection. Labruyère (1971) could not detect infection with a mieroscope sooner than with the unaided eye, when the affected structure was always a lenticel.

Older lenticels apparently resist infection, presumably because the packing cells are suberized (Fellows, 1926; Jones, 1931). Our results support this conclusion, although we studied suberization on non-proliferating lenticels. Whether at Woburn the young lenticels proliferated while the soil was wet, or whether proliferation delays suberization, is not known. However, if lenticel resistance depends on suberization, then lenticels on the fifth internode from the apex must already have been, or rapidly became, suberized when the soil dried, because they were not infected. In laboratory studies of proliferated lenticels we have observed suberization within 5 days, and it may happen as quickly in soil.

Lewis (1970) showed that periods when *S. scabies* could infect tissue in dry soil coincided with unusually small ratios of bacterial to actinomycete populations on the tuber surface. In wet soil the ratio was reversed, so he suggested that infection might be prevented by bacterial antagonism. We do not know whether apical tuber internodes had large bacterial populations between 24 June and 1 July; if so, the bacterial populations did not prevent *S. scabies* infecting these internodes when the soil dried, on some tubers within 3 days of the onset of dry conditions.

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(*Facing p. 283*)

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EXPLANATION OF PLATE

Fig. 1. Distribution of common scab on Majestic tubers at lifting on 25 August; left to right respectively, pairs of tubers from sprouted, unsprouted and late-planted treatments.

Fig. 2. Distribution of common scab on Majestic tubers from the sprouted seed treatment at different dates, respectively top to bottom 13, 21 and 28 June, 4 and 14 July; five tubers for each date (tuber apex always at the top).

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Mechanisms of Control of Common Scab by Irrigation

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INTRODUCTION.- Common scab blemishes potatoes superficially, affecting the sale of tubers rather than yield. The lesions or 'scabs', first seen as small (1 mm dia.) discrete spots, enlarge rapidly as the tuber swells. Labruyère (1971) recognized two types, 'superficial'and 'normal' and the disease is usually assessed on the proportion of tuber surface affected (Large and Honey, 1955; Lowings and Ridgman, 1959). Streptomyces scabies (Thaxt.) Waksman and Henrici is considered the main causal organism but other species may be involved (Corbaz, 1964). It penetrates through 'young' (recently formed) unsuberized tuber lenticels, perhaps stomata, but not intact skin (Fellows, 1926). The actinomycete mycelium is fine (0.5 - 1 µm dia.), initially confined to middle lamellae and so difficult to detect that the early stages of infection have not been convincingly recorded. The first macroscopic signs are brown spotted lenticels, and the histology of recognizable lesions has been described by Jones (1931) and Labruyère (1971). Penetration of the tuber tissues stimulates the formation of a wound barrier a few cells below the tuber surface and if the invasion is stopped a 'superficial' lesion is formed. If this barrier is breached, a second or third may be formed below, resulting in increasingly severe forms of 'normal' scab as the tuber grows; if the tuber stops swelling so does the lesion.

The disease is worst on light sandy or gravelly soils, on land recently limed or ploughed from long-term grass and in the U.K. when June is dry. S. scabies probably occurs in most arable soils and sporulates freely within the dead cells of lesions. Control of the

disease by seed disinfection has been tried (Emilsson and Gustafsson, 1954), but most control measures aim to decrease the soil population by using chemicals to kill the organism (Weinhold, Bowman and Bishop, 1964; McGregor and Wilson, 1964; van Emden and Labruyère, 1958 and McIntosh, 1973), sulphur to acidify soil (McCreary, 1967) or by soil amendments (Weinhold, Oswald, Bowman, Bishop and Wright, 1964). It has been long known that 'wet' soil prevents infection (Sanford, 1923; Dippenaar, 1933; Noll, 1939) and recently irrigation regimes have been designed in the Netherlands for seed (Labruyère, 1971) and in the U.K. for ware (Lapwood, Wellings and Hawkins, 1973) crops. In one Dutch experiment in 1967, scab on Bintje was decreased from 41% (unirrigated) to 0.2% tuber surface covered and in the U.K. in 1970 on Majestic from 32% to 3%.

This paper describes a practical irrigation regime and how it works, outlines some ways in which moisture changes affect the soil environment and finally discusses the possible mechanisms which prevent S. scabies infecting 'young' lenticels in 'wet' soils.

CONTROL BY IRRIGATION.- Irrigation regime.- If the soil is dry, irrigation must start at tuber initiation (when the stolon tips first start to swell) and in ware crops must continue for at least four to six weeks not allowing the soil to dry, or the 'water potential' (Griffin, 1969; Cook and Papendick, 1972) to fall to ~~suctions of~~ -0.4 bar (pF 2.6, (see Schofield, 1935) -0.4 atmospheres or -40 joules kgm^{-1} (see Slatyer and Taylor, 1960)).

An experiment at Rothamsted in 1967 with scab susceptible Majestic illustrated the main problems to devising a regime for controlling scab by irrigation. A planned 4 week irrigation regime coincided with the second to fifth weeks of a six week period predominately without rain.

Irrigation was begun when plants from seed planted unsprouted started to form tubers, and those from sprouted seed had tubers a week or more old, forming in the dry soil. Irrigation was applied to keep soil near to field capacity (-0.1 bar) or wetter than -0.4 bar (pF 2.6) or -0.7 bar (pF 2.9) for 4 weeks; other plots were unirrigated. At harvest, tubers grown from sprouted seed in plots kept close to field capacity had about 1% surface area affected, with scab lesions at the stolon attachment end of tubers, a middle region free from infection and a few tubers with scab at the apical end. Tubers from unsprouted seed had about 1% surface area affected but lesions were confined to the apical end. At -0.4 bar, tubers with about 2% scab showed less of a positional lesion effect and at -0.7 bar with 3% scab, lesions were scattered over the tuber surface as on tubers from unirrigated plots with 7-10% surface affected. To explain the disease incidence and distribution the way tubers grow has to be understood.

Tuber growth, soil water potential and scab incidence.- The beginning and length of the required irrigation period is related to the growth of tubers. These form as swellings at the tip of underground stems (stolons) and as they grow, nodes (eyes) are separated from the apical bud by expansion of internode tissue between them. When first formed, tuber internodes have stomata but as the tissue expands the stomata are transformed to lenticels (Fellows, 1926). Stomatal guard cells are gradually raised to sit at the top of a small 'pimple' by cell division and expansion beneath them, and eventually the stoma is ruptured to expose the inner cells, thus forming a 'young' lenticel. Most workers agree that this is the stage when infection is most likely (Fellows thought but did not prove that stomata were also susceptible). As lenticels 'mature' they become resistant to infection by S. scabies and therefore each developing

tuber internode passes through a period of susceptibility and then becomes resistant. Node (eye) separation is fastest (about two per week) during the first four weeks after tuber initiation, and then slows so that the mature (ware) tuber may have, depending on cultivar, twelve to fourteen separated nodes. Internodes also expand differentially, early ones expanding so much more than those formed later that most of the mature tuber surface is composed of internodes formed during the first month from tuber initiation.

Lapwood and Hering (1968 and 1970) showed that when soil was allowed to dry for 5, 10 or 15 days, on average four, five and six tuber internodes were infected. When soil was allowed to dry for 7 days at different dates during the first 8 weeks from tuber initiation, on average four to five internodes were scabbed after each occasion, but the later the date soil was allowed to dry, the nearer the tuber apex was the infection. Because of the differential expansion of tuber internodes, the proportion of tuber surface scabbed was greatest (15%) following drying during the third week and least (1.6%) following the eighth week (continuously irrigated control (1.7%) gave a measure of the efficiency of the trickle irrigation system).

To explain the results of the irrigation experiment previously described, irrigation for 4 weeks was insufficient time to protect tubers from unsprouted seed which showed four scabbed internodes extending towards the apex from node seven (numbering along the phyllotactic spiral from the stolon attachment). Scab at the attachment end of tubers from sprouted seed indicated that irrigation was not started soon enough, hence the need to start irrigation from tuber initiation and to continue for at least 6 weeks to ensure adequate protection in prolonged dry weather. The loss in the 'positional

effect' of lesion distribution showed the gradual loss of protection as soil dried and indicated that tubers were increasingly at risk from about -0.4 bar water potential or pF 2.6.

Internodes infected or protected.- When soil was allowed to dry for 5 days (Lapwood and Hering, 1968), on average four tuber internodes were scabbed but when wet for about a week, infection was prevented on only one or two internodes (Lapwood and Adams, 1973). In the experiment where soils were dried for 5, 10 or 15 days on average four, five and six tuber internodes were scabbed. Nodes were separating from the apical bud about one every 5 days and it was assumed, for example, in the 5 day treatment with four scabbed internodes that the internode which started to form (separated from the apical bud by at least 0.5 mm) during the dry days was infected, and that the stomatal/lenticel structures of three other internodes formed previously, were still susceptible to infection; this assumed that stomata were equally likely to be infected as 'young' lenticels.

In a 1967 experiment on light sandy scabby land (Lapwood and Adams, 1973), a few days of heavy rain interrupted prolonged dry weather and kept soil wetter than -0.4 bar (below pF 2.6) for at least 5 days protecting 1 to 1.5 internodes. However these internodes did not start to form during the wet period but at least a week before the rain when soil was dry. Since the experiment was done we have found that at any time early in tuber growth the internode visible nearest the apical bud (that most recently formed, defined as A minus (-) 1) has flat stomata, the second from the apex (A -2) predominantly raised stomata and the third (A -3) predominantly 'burst' or 'young' lenticels. In dry soil (and if not infected by S. scabies) suberin deposition on the outer walls of the outermost and exposed cells has started on the third internode from the apex (A -3) and is complete by the fifth (A -5). Although tubers were not checked in the experiment

just described, it is known that the uninfected internode was that which was third from the apex at the time of the rain and therefore probably had young unsuberized lenticels. This internode must have passed through the stomatal stages in dry soil, suggesting that stomata are not susceptible or unlikely to be infected by S. scabies. Further evidence to support this view came from checking data from Lapwood and Hering (1968), when it was found that the four internodes that became scabbed did not include the one formed during the five dry days but only extended to within two internodes of the apical bud at the time irrigation was restarted. If suberized lenticels resist infection and those on A -5 are suberized, and if nodes separate on average every 3 to 4 days, then it must take about as long for a lenticel to form and become resistant as for four nodes to separate, i.e. about 2 weeks. Subtracting the time spent in the stomatal stages (1 week) then the young lenticel would be susceptible to S. scabies for about 1 week. This would explain why, if soil remains wet enough to prevent infection for about a week, only that internode which can reach the resistant stage ('mature') in the time remains uninfected, and why the several internodes at different intermediate stages of lenticel maturation would be scabbed in soil allowed to dry for that week.

FACTORS AFFECTED BY CHANGES IN SOIL WATER POTENTIAL.- The relations between water and soil are considered in detail by Marshall (1959), water and the plant by Youngs (1965), and soil water and plant disease by Griffin (1969), Rotem and Palti (1969) and Cook and Papendick (1972). Only those possibly relevant to the infection of potato tuber lenticels will be considered here.

The soil.- In the simplest of terms, wetting and drying implies the filling and draining of pores in the soil. Wetting may fill all soil

pores rapidly, but draining takes much longer, with the larger pores emptying first (Childs, 1940). Although the modern concept of 'water potential' is perhaps more logical, the old soil moisture classification of Briggs (1897) favoured by Couch, Purdy and Henderson (1967), is more familiar. Gravitational water is that which may be removed from the soil as it dries from saturation (when all soil pores are filled with water) to field capacity (water held by soil against gravity) which approximates to the range (Marshall, 1959) -0.001 to -0.1 bar (pF 0 to pF 2); capillary water or the "readily available water", is that which may be removed as the soil dries from field capacity to permanent wilting point -0.1 to -15.0 bars (pF 2 to pF 4.2) and is the principal source of water used by plants; hygroscopic water and water vapour (Lebadeff, 1928) are beyond the range of interest here.

Common scab seems unimportant in wet soils with water potentials -0.4 bar and above (pF 2.6 and below) but increasingly important as soils dry in the range -0.4 to -1.0 bar (pF 2.6 to 3.0). A saturated soil will have few, if any, air-filled pores, oxygen diffusion will be severely restricted and the CO_2 content will rise from biological activity, but as a soil dries it will be increasingly aerated.

Rain is often associated with cool cloudy weather, for example during a wet period 8 to 20 June, 1971 at Rothamsted, when soils remained wetter than -0.1 bar (pF 2.0), soil temperatures at tuber depth only once exceeded 15°C and the average maximum and minimum temperatures were 14°C and 11°C. By contrast, during bright sunny weather from 4 to 11 June 1973, soil dried from -0.5 to -1.0 bar (pF 2.7 to 3.0) and soil temperatures exceeded 20°C for from 6 to 12 hours (av. 9.6) every day.

Changes in soil moisture which alter soil aeration also affect the

release of manganese which is greater in wetter soils. McGregor and Wilson (1964 and 1966) found that manganese sulphate added to a neutral soil with low water-soluble manganese content would control scab. They suggested that low scab incidence on acid soils could in part be attributed to the concentration of soluble manganese released by them.

The potato tuber.- The first observed effect of moisture stress on tuber growth is to slow the rate of swelling but the rate of node separation is also slowed as the stress increases. As the tuber swells it compacts the soil around it, and larger air filled pores are likely to be lost leading to the encirclement of the tuber by soil particles separated only by the smaller pores.

Temperature also affects tuber growth, for in the experiments where continuous trickle irrigation cooled the soil (Lapwood and Hering, 1968), a node separated about every 5 days during the first month from tuber initiation, whereas in unirrigated experiments at Rothamsted the rate was about two per week.

Changes in water potential also affect lenticel structure. In a dry soil lenticels become suberized by internode A -5 but in wet soil, following the rupture of the raised stoma and exposure of the inner cells, cell division continues and cells proliferate from the lenticel opening. When lenticels are suberized in dry soil the meristem may form a cork barrier, but if soil becomes wet the meristem may become active again cutting off parenchymatous cells which burst through the cork and proliferate from the lenticel opening. As a soil dries, proliferated cells collapse, a suberin layer is deposited and completed within 5 days. Metabolites are released into the soil, from developing lenticels but how far this process is affected by changes in soil water potential and their effect on the surrounding microflora (and S. scabies in particular) is not known.

However, Lawrence and Barker (1963) found that tubers produced aseptically were difficult to infect unless soaked in sterile water and that soaking increased the susceptibility of resistant Richter's Jubel; the dilution or elimination of 'soluble native products' was a possible explanation.

The pathogen.- Soil water potential affects aeration and Sanford (1926) considered low concentration of O_2 important in limiting scab in wet soil. Harmsen(quoted by Labruyère, 1971) found S. scabies grew in soil at field capacity provided it was aerated and that growth in sand was normal at 2.5% O_2, and could even be sustained at 0.625% O_2. In 50% CO_2 growth was about 70% of normal so the organism should easily tolerate a rise from 0.05 to 2% after irrigation. Williams, Shameemullah, Watson and Mayfield (1972) inoculated unidentified Streptomyces spp. from sandy soil under Pinus nigra, into sterile soil with different gas mixtures and found that decreasing O_2 in the absence of CO_2 had little effect on actinomycete growth, but increasing CO_2 above 10% (v/v) greatly decreased it. Growth was normal at CO_2:O_2 ratio 1:1 but drastically decreased by 3:1 and 10:1, and was greatest in humid but air filled pores (the pores in question would have emptied at pF 1.7, see Childs, 1940); radial growth was decreased in water-filled pores.

Labruyère (1971) found little difference between total actinomycete numbers in wet or dry soils but the number of tyrosinase producing isolates (including pathogenic S. scabies) was usually decreased in wet soil. The frequency of isolating tyrosinase positive actinomycetes from tuber lenticels was high in dry soils but low from wet (Lewis, 1970).

The speed of germination of spores of S. scabies is markedly affected by temperature (Shapovalov, 1915) and increased from over 48 hours at 10°C, 18 hours at 15°C, 11hours at 20°C, to 8 hours at 25°C. In Wisconsin tank experiments, Dippenaar(1933) found most scab lesions per tuber

at about 20°C (which was also most suitable for host development), although the disease occurred from 13° to 25°C. In culture the optimum temperature for growth of the S. scabies strains used varied from 28° to 36°C.

Microbial populations.- Williams et al (1972) studied numbers of actinomycetes, bacteria and fungi in soils at water potentials ranging from -0.001 to -400 bar (pF 0 to pF 5.6). All organisms were most common at -0.1 bar (pF 2) but decreased in the range -0.001 to -0.01 bar (pF 0 to 1.0) and drier than -10 bar (above pF 4.0). Between -0.2 and -0.5 bar (pF 2.3 and 2.7) the proportion of actinomycetes increased and that of bacteria decreased, and at -400 bar (pF 5.6) actinomycetes exceeded the total of bacteria and fungi. Streptomyces spp. mixed with sterile soil and kept at water potentials between -0.01 and -350 bar (pF 1.0 and pF 5.5) grew much better at about -0.1 bar (pF 2.0) than in wetter or drier soils.

In wet soil, a bacterial flora predominated over fungi and actinomycetes (Dippenaar, 1933) and bacterial numbers were greater than in dry soil (Labruyère, 1971) while numbers of tyrosinase positive actinomycetes were greater in dry than wet. Lewis (1970) isolated bacteria more frequently than tyrosinase positive actinomycetes from tuber lenticels in wet soils while in dry soil the ratios were reversed. Lewis (1962), by direct observation and culture, also studied populations of actinomycetes, bacteria and fungi in June and August, on the tuber surface of cultivars differing in resistance from Ulster Chieftain (very susceptible) to King Edward (intermediate) and Seneca (resistant). The total actinomycete population (including pathogenic S. scabies) on the periderm surface and in lenticels decreased with increasing varietal resistance, and between June and August. The probability of

isolating actinomycetes from a sample was less if bacteria were present. This, and the positive correlation between resistance and high frequencies of bacteria relative to actinomycetes indicated interactions between these organisms, but there was no evidence of antibiotic effects.

DISCUSSION.- It is difficult to study common scab disease and its relation to field soils because there is no quick way to distinguish pathogenic and non-pathogenic forms of S. scabies (Vruggink and Maat, 1968; Weinhold, 1970). Recently however, Knösel (reported by Labruyère, 1971) has found that pathogenic forms have greater pectin-acid transeliminase activity than non-pathogenic forms. Pathogenic isolates can be added to sterilised soil but in pot or glasshouse experiments it is difficult to control the water potential to ensure infection and yet maintain healthy plant growth (McIntosh, 1970) and in infection studies the tuber has to be manipulated while still attached to the plant without interfering with further development. Furthermore, these controlled conditions give little information about natural microbial interactions in field soil or at tuber surfaces. Thus, although the mechanisms of control by irrigation, in relation to presence or absence of infection or a particular distribution of lesions on tubers, are now more clearly understood, the actual mechanisms preventing lenticel infection in wet soil remain speculative.

Suberization may account for the resistance of mature lenticels in dry soil, while in wet the lenticels proliferate, but become rapidly suberized if the soil dries. It is, however, unknown if the pathogen fails to penetrate 'resistant' lenticels or is stopped before a recognizable symptom develops. Our results suggest that the internodes bearing flat or raised stomata, (A -1 and A -2) and the lenticels on

the fifth internode (A -5), are not infected. If true, this means that only young lenticels (recently lost guard cells) on internodes A -3 and A -4 are susceptible. Nodes separate every 3 or 4 days during the first month of tuber growth, which suggests young lenticels remain susceptible for a week

The water potentials limiting infection have not been measured precisely but the work of Labruyère (1971), Langton (1972) and Lapwood, Wellings and Hawkins (1973) suggests the disease is likely to occur in soils drying from -0.4 bar (above pF 2.6) and unlikely in wetter soils. In practical terms: on the 'sandlands' of Nottinghamshire, England, with 38 mm available water in the top 30 cm of soil, some scab infection was likely at soil moisture deficits greater than 20 mm or when over 50% available water was lost; on a Cambridge soil, Langton considered the critical range occurred when between 47 and 68% of available water was lost. Labruyère (1971) considered irrigation should be applied before 20 mm of available water was lost, although the figure varied with soil type. On Rothamsted and Woburn (Bedfordshire), farms following rain, critical levels (-0.4 bar) could be reached on average in 4 days and many lenticels could be infected if soil was allowed to dry 8 days to water potentials of -0.8 bar (pF 2.9). Baars (1968) indicated that during late May or early June, when tubers start to form, evaporation averages 3 mm per day in cloudy cool weather, 3.5 mm in 'normal' and 4.5 mm per day in sunny warm weather, hence Labruyère advocated irrigation at least every 5 days to prevent infection in dry sunny weather. It is interesting that Williams et al (1972) found that as soil dried from about -0.5 bar (pF 2.7) the proportion of actinomycetes isolated increased and that of bacteria decreased.

The lack of scab infection in wet soil has been attributed to

'antagonism'. Of many usages of the term we favour that of Park (1960) where antagonism includes "all those associations in which at least one of the interacting species is harmed". The three mechanisms of antagonism are antibiosis, "in which species A produces a chemical substance that is inimical to species B without species A deriving any direct benefit", exploitation "in which species A inflicts harm by the direct use of species B for its own benefit" and competition "which is found in the indirect rivalry of two species for some feature of the environment that is in short supply".

Sanford (1926) gave evidence of antibiosis where bacteria sometimes inhibited growth of S. scabies in culture but not by increasing the acidity, and Daines (1937) found the actinomycete susceptible to the antibiotic activity of Trichoderma. Orellana (1947) mentions a number of actinomycetes and bacteria which inhibit growth. The effectiveness of green manuring in controlling scab has also been attributed to antibiosis and with soya bean manuring Bacillus subtilis was considered the main antagonist (Weinhold and Bowman, 1965). We have found the germination of S. scabies spores to be inhibited by this bacterium and by metabolites from its culture medium. Millard and Taylor (1927) suggested that when green manuring failed to control scab it was because certain saprophytic Streptomyces spp. were absent from the soil, and when successful, green manuring encouraged growth of these species which then suppressed S. scabies. Labruyère (1971) searched for 'antagonists' among soil micro-organisms and found other actinomycetes to be the most active against S. scabies. Wet soils seem not to affect the total actinomycete population (Labruyère) but it is not known how 'antagonistic' species would be affected. Possibly they would prove most effective in decreasing the number of lenticels infected by S. scabies under favourable

infection (dry) conditions rather than preventing infection in wet soil.

There seems no evidence for exploitation, but competition between bacteria and S. scabies could be important. S. scabies is tolerant of low O_2 and high CO_2 concentrations and of a range of temperatures and will grow in sterilised soil even when wet. We do not know if lenticels can be infected in wet soil lacking other micro-organisms, although Barker and Page (1954) found that inoculated sterile tubers growing in glass tubes, produced most infections on areas in contact with the wall of the glass tube, where water persisted. In wet field soils with limited aeration, numbers of bacteria increase and the population of tyrosinase positive actinomycetes, including S. scabies, falls. In these conditions the demand for O_2 will be large, from bacterial activity, the rapidly swelling tuber and the meristematic activity of the lenticels. The bacteria will include facultative anaerobes which presumably will continue to multiply, so aerobic actinomycetes are likely to suffer most. Wet soils allow the movement of bacteria in water films and Lewis surmised that these could reach the lenticels in large numbers before the slower growing mycelium of the actinomycete, and Labruyère that the leakage of metabolites might also give the bacteria a further advantage. In dry soil, bacteria are unlikely to move rapidly or in large numbers through soil pore necks less than 1.5 µm., which would be drained at a water potential of-1 bar (pF 3.0) (Griffin, 1969) and so Labruyère suggested that they would be at a disadvantage, and that the actinomycete would have a greater chance of reaching the lenticel first. He also speculated that the organisms arriving first would probably maintain a dominant position because the supply of metabolites from the lenticels would favour their rapid multiplication, and in consuming them, chemotrophic attraction to other organisms would be decreased. Lewis found more

actinomycetes on the tuber surface of susceptible than resistant cultivars and in dry than wet soil and concluded that the chances of infection of the susceptible cultivar or tubers in dry soil are greater because of the presence of a larger number of propagules of the pathogen.

We have not studied microbial populations in soil or on tuber surfaces but our experiments suggest that internodes with flat or raised stomata forming in dry soil, with presumably a dominant actinomycete microflora, are not infected if at the 'bursting stage' (loss of guard cells) the soil is suddenly wetted from rain or irrigation. Conversely internodes bearing stomata in soil wetter than -0.4 bar (less than pF 2.6), with presumably bacterial dominated populations, may be infected if, by the time they reach bursting stage, the soil has become drier than -0.4 bar (pF2.6). Stomata become raised 100-120 µm. above the surrounding tuber surface and on 'bursting' expose a large surface area (relative to the stomatal pore), presumably of largely uncolonised cells. In the colonisation of this raised surface, the microflora on the tuber may perhaps be less important, and slower to change than that in the surrounding soil. The fact that S. scabies can still infect susceptible lenticels in drying soil, despite earlier periods when wet. soil prevented it, suggests that if antibiosis or competition do prevent infection in wet soil, these effects are soon lost. In wet soil 'protected' lenticels proliferate and, maybe, even the 'first colonists' (whatever they are and by whatever means they have become established) are pushed out and have no further role in preventing infection because if soil dries these lenticels will become suberized and resistant to S. scabies. In a drying soil, conditions become increasingly favourable for infection but even so there must be a fairly critical condition when infection can begin, for there is usually a sharp division between scabbed and scab-free tissues.

The evidence presented suggests that with potato common scab there could be a rather unique host-pathogen-antagonist situation because each lenticel may be challenged, protected or infected as it develops and this procedure continues until lenticel formation stops.

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