TAXONOMY AND PATHOGENICITY OF RUSTS FROM *ALLIUM* SPECIES IN THE U.K.

by

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SYNOPSIS

Comparison of seven isolates of rust from leek (Allium porrum), three from chives (Allium schoenoprasum) , one from A. scorodoprasum and one from A. babingtonii on the basis of telial and uredinial morphology showed there to be three morphologically distinct species. Application of these criteria to herbarium material confirmed these findings and showed the rust on leeks on leeks in the U.K. to be the same species found on European leeks , garlic and certain wild Allium spp. from the Mediterranean. It is suggested that the following names be adopted, viz. Puccinia allii (DC.) Rud. for the rust on leeks, Puccinia mixta Fuck. for the rust on chives and Uromyces ambiguus (DC.) Lev. for the rust on A. babingtonii.

Infection studies on isolates of each rust species supported the morphological evidence, and showed the three species to have different, extensive but overlapping host ranges within the genus Allium.

In the ampeloprasum complex, A. kurrat accessions were highly susceptible to leek rust whereas some accessions of A. ampeloprasum and A. babingtonii had high levels of quantitative resistance. There was no evidence of 'hypersensitive-type' resistance in the complex.

Tests within one leek cultivar (Musselburgh) showed older plants to be more resistant than seedlings in at least two quantitative components. However leaf tissue appeared to become more susceptible to infection with age, except in the leaf tips, which did not change in susceptibility over time.

Inoculation of 16 leek cultivars with leek rust isolates from different geographical areas, and subsequent analysis during the disease cycle of several components of resistance (viz; latent period, pustule density and pustule length) showed that some cultivars performed consistently better against all isolates. However, in most cases there was a considerable and complex cultivar-isolate-component interaction. There was no evidence of physiologic specialisation in the isolates, but low levels of specialisation could have been hidden by the high level of variation in the experiments.

Comparison of field cultivars of garlic with equivalent virus-free material using an isolate of leek rust gave inconclusive results, and further study of the rust-garlic-virus interaction is recommended.

Major trends in the infection/resistance studies included a high level of environmentally-dependent variation and a lack of 'hypersensitive-type' resistance, even in host species quite distantly related to the normal host.

TO

MUM AND DAD

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CHAPTER ONE

GENERAL INTRODUCTION & MATERIALS AND METHODS

1.1. INTRODUCTION

General Introduction

Rust has been regularly reported on leeks (Allium porrum L.) in the U.K. since 1919 (MAFF, various reports on diseases of cultivated plants in England and Wales, 1917-1973), but the damage done to the crop has varied from region to region and from year to year. It has also been reported in Scotland (Foister, 1961; Brokenshire, pers. comm.) on leeks and chives, especially in the Lothian region. It has been mentioned as a problem on leeks and garlic in France, (Bonnet, 1976; Grill, 1985)and present in Holland (van der Meer, 1984), and appears to be world-wide in distribution (Laundon & Waterston, 1965). However, disease outbreaks have become more severe and frequent in recent years in the United Kingdom (Dixon, 1976; Dobson, 1986; Norman, 1987). This has caused concern since leek production is increasing to supply an expanding demand. Leek production (harvested tonnes)in the U.K. has doubled in the past two decades, (figure 1.1), with the acreage of leeks in England and Wales increasing by 15.7% between 1984 and 1985 (Anon. 1986). Effort is now being put into producing leeks all year round. The current production season lasts from September to May, and new cultivars introduced in 1987 should be suitable for August harvesting. The present aim is to improve long-term cool

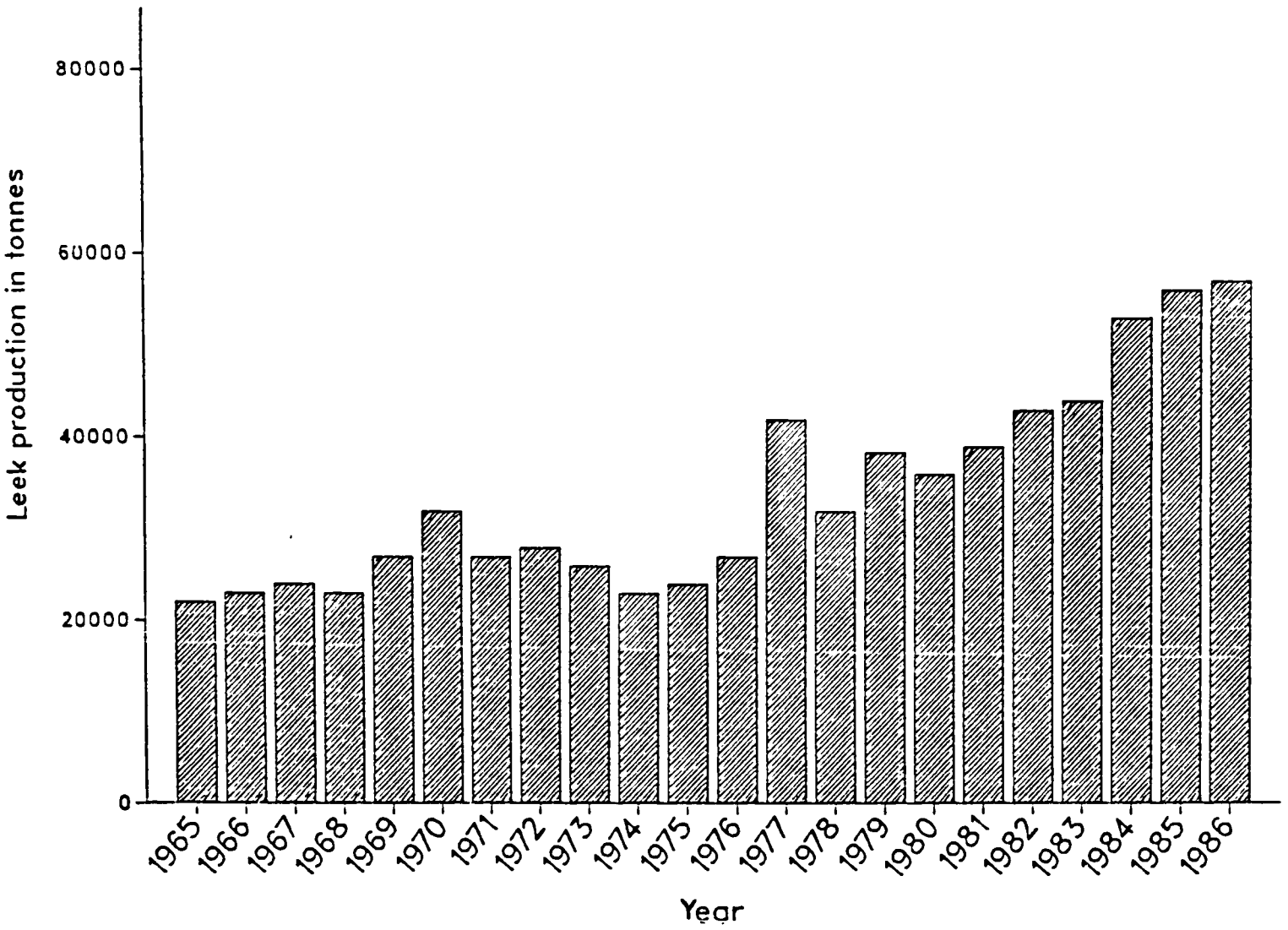


Figure 1.1
Leek production (harvested tonnes) in the United Kingdom from 1965 to 1986. Note the dramatic rise in production since 1975, after a decade of small fluctuations.

storage methods to close the June/July gap, by storing leeks harvested in April and May, (Norman, 1987).

The symptoms of the disease are bright orange circular-elongate pustules on the leaf surface which occur between the veins. These pustules, 1 mm to 3 mm across and up to several centimetres long, are the uredinial stage of the rust, and are often surrounded by a pale green halo. Before eruption, the sites of successful infection are visible as pale green flecks, though not all flecks on the leaf are due to the rust.

The Problem

Leek rust can cause several problems in the crop. Firstly, it can reduce marketable yield. One of the main causes of the increase in demand for leeks is the success of supermarket sales, particularly of the pre-pack type. Such outlets require blemish-free leeks (Dixon, 1976; Lovelidge, 1985). The statutory Class I category requires leeks to be rust-free; when leeks are in short supply, the presence of rust can downgrade a batch to Class III. (Anon, 1982). Leeks with rust must therefore either be trimmed more severely or sold in a lower category. Secondly, rust renders the plants more susceptible to frost damage (Jones, pers. comm.) - a serious problem, since most leeks are left in the field in the winter. Thirdly, heavily infected plants may be more susceptible to secondary infections. Fourthly, there can be direct yield losses. Bakhit *et al.* (1963) reported that rust reduced garlic yields by 33% to 50%, and Dobson (1986) stated that severe attacks on leeks reduced both growth and quality. Control of

the disease requires regular crop monitoring followed by fungicidal treatment, usually 'Bayleton' (+ Agral wetter), 'Trimanzone', 'Corbel' or 'Mistral' at two to three week intervals. Such treatments can provide effective control but are expensive, and Dobson (1986) reports that if rust is well established at the time of the first spray, 'good control may be difficult to achieve'.

The Disease

Relatively little is known about the epidemiology of the disease, or the variation in the pathogen in the field. The disease was first described on leeks in 1810 by Sowerby, and since then rust has been reported on many related species in the genus Allium. However, the taxonomy has been in a state of flux since then, especially in the U.K. One reason for this was that the telial stage was not described on leeks in the U.K. until 1984, when it was described by both Uma (1984) and Norwood (1985). Prior to this Doherty (1981) concluded that 'pending more detailed taxonomic studies' the rusts on all the cultivated species of Allium should be referred to as a single species, Puccinia allii (D C.) Rud., whereas Henderson and Bennell (1979) considered the rust on leeks to be distinct, calling it Puccinia porri (Sow.)Wint. Uma (1984) and Uma and Taylor (1986) in the light of the evidence provided by the new-found telial stage, named the rust Puccinia allii, whilst suggesting that Puccinia porri might be a separate species which could also attack leeks. Therefore, the problems of taxonomy and host species have still not been fully resolved.

Little is known of the epidemiology of the rust. Doherty (1981) concluded that the disease was carried from one season's crop to the next by a 'living bridge' of infected plants, since there are crops in the field all year round. Urediniospores could therefore be blown from either local or more distant foci of infection. Doherty (1981) found no evidence for the disease being maintained or spread by volunteer plants or through an alternate host. The first sign of infection in a spring-sown crop usually appears between June and August. The development and spread of the disease is enhanced by mild, wet weather (Dixon, 1976; Doherty, 1981; Dobson, 1986) and once established can remain throughout the winter , though the lower temperatures of early winter can slow the development of new infections (Dobson, 1986). Doherty (1981) found that urediniospores were present throughout the winter, and that sporulation only ceased below $4^{o}C$. Burchill and Everitt (1985) found that in the field, temperatures above $24^{o}C$ and below $10^{o}C$ were inhibitory to successful infection of leeks, and that prolonged heavy rainfall caused loss of viability in the field inoculum. Laboratory tests confirmed that prolonged immersion below the surface of water lowered viability significantly. Norwood (1985) stated that the banding of pustules on leek leaves was due to autoinfection from urediniospores collecting in the water trapped in the leaf sheaves. Burchill, Norwood and Everitt (1986) showed that leek rust spores required a relative humidity of 97% for at least four hours for successful germination and infection. Thus, leek rust requires a high level of humidity, preferably free water, but not immersion for successful infection. This, and the temperature requirement, may explain the apparent decrease in the

incidence of the disease in spring and early summer as temperatures rise and rainfall decreases.

The causes of the increase in the prevalence of the disease in the last decade or so have not been elucidated. Dixon (1976) suggested that mild winters in the mid-1970's enhanced the overwintering of the rust, but the relatively hard winters of the early 1980's do not seem to have reduced the disease levels in subsequent years. Thus Knight (pers. comm.) considers the major factor to be the intensification of production, which would be enhanced by the longer production season. Norman (1978) suggested that the new high-yielding cultivars may be more susceptible to the disease.

Biological Disease Control

Both Doherty (1981) and Uma (1984) made some progress toward biological disease control of leek rust. Doherty (1981) found that the bacterium Bacillus cereus could be applied as a spray to markedly reduce the level of disease on infected leaves in controlled environments compared with untreated controls. Uma (1984) investigated the effect of several hyperparasites of leek rust urediniospores, and reported that Verticillium lecanii and Ramichloridium schulzeri showed promise as biological control agents.

In the long term there are many benefits to be derived from breeding leeks, and perhaps other crop species in the genus Allium, resistant to rust, as an alternative to fungicide control of the disease, (Lester, 1986). Such a programme would require rather more informa-

tion of the host-pathogen interaction than is currently available in the leek-leek rust system.

Concepts of resistance and specialisation in fungal pathogens

The degree of specialisation between a fungal pathogen and its host varies considerably (Anikster, 1984). Some pathogen species are very specific, restricted to hosts within a single species, whereas others may have a host range within a single pathogen species, whereas others may have a host range within a single genus or a larger group. Epidemiological considerations may restrict the host range of a pathogen in the field compared with artificial inoculation tests in controlled environments. Rusts are usually considered to be in the more specific category; a rust species may exhibit levels of specificity within the host range, e.g. black stem rust, Puccinia graminis is restricted to members of the Gramineae. Certain forms of this rust are adapted to genera within the Gramineae, so Puccinia graminis is subdivided into formae speciales (f. sp.) on the basis of the host genus or species predominantly attacked, e.g. Puccinia graminis f. sp. tritici on wheat, P. graminis f. sp. secale on rye, and so on. These ranges are not absolute, and there is a restricted degree of cross-pathogenicity. 'Host-range' is not therefore used as a criterion for fungal taxonomy; fungal species must be defined by, for example, morphological characters (Shoemaker, 1981). Further specialization in the host-pathogen relationship can occur below the level of forma specialis, when a pathogen population contains 'physiologic races' with each race growing on a limited number of host cultivars. There is a high level of coevolution

between the genetic systems of the host and pathogen at this level. Studies have shown that both host resistance and pathogen virulence are under direct genetical control, often by a small number of genes (Manners, 1982). This was the basis of the gene-for-gene theory proposed by Flor (1942 & 1971). He summarised the evidence from his studies on flax rust (Melampsora lini) on flax (Linum spp.) as follows; 'for each gene that conditions reaction in the host there is a corresponding gene in the parasite that conditions pathogenicity' (Flor, 1971). Work has shown that virulence may be either dominant or recessive, and that the gene-for-gene theory holds in many host-pathogen systems besides rusts, e.g. smuts, powdery mildew, other fungal diseases and some diseases caused by other organisms, (Manners, 1982).

Such gene-for-gene relationships have been used by breeders looking for sources of easily utilisable resistance in crops, by selecting single genes for resistance (usually for an immune or hypersensitive reaction) for breeding into existing cultivars. However the main problem found with this sort of resistance is that the pathogen has only to incorporate a single gene for virulence, by hybridisation or mutation, to overcome the host resistance. The ability of rusts to incorporate new virulence genes varies. It is likely to be highest in those rusts with a sexual stage in their life-cycle, e.g. Puccinia graminis, where recombination occurs during meiosis. Recombination by mitosis can occur, when urediniospores of two different rust clones infect the same host plant and form dikaryons (Day, 1974). Exchange of genes can occur by reassociation and recombination of nuclei,

chromosomes and genes. This is the only form of recombination for hemicyclic rusts such as Puccinia striiformis (Day, 1974; Little & Manners 1969 (a) & 1969 (b)), and more importantly here, leek rust. Finally new virulence genes have been shown to arise through mutation (Flor, 1960).

Thus, 'single-gene' resistance may not prove to be durable in the field. Large monocultures of a crop depending on a single gene for a high level of resistance provide strong selection pressure for a race of the pathogen able to overcome that resistance. This has frequently occured in agriculture, especially in the Gramineae, producing the so-called 'boom and bust cycle' (Priestley, 1978). This has led to the desire for longer lasting or more 'durable' resistance (Johnson, 1984). Many workers feel that this may be obtained from 'race-non-specific' resistance (van der Plank, 1963). Hooker (1967) has reviewed the arguments and theory regarding 'multiline varieties' where several lines, each depending on one or a few genes for resistance, are mechanically mixed to provide more durable overall resistance. Also, resistance dependent on many genes should theoreticlly be more stable. However, the subject of resistance and durability is poorly understood - there is not necessarily a relationship between the type or level of resistance, its specificity and its durability. There are examples of quantitative resistance (e.g. slow-rusting, Wilcoxson, (1981)) which are race-specific and race-non-specific (Jones & Clifford, 1983) and durable and non-durable (Johnson, 1984). Furthermore, certain race-specific resistance has proved durable (Johnson, 1984).

Host Taxonomy

Before a programme of breeding for resistance could begin in the Allium - rust complex, the 'host-range' of the rust species within the genus Allium needs to be studied to examine the range of responses in leeks, onions and closely related species. The relationships between the wild and cultivated forms in the genus Allium have been summarised in figure 1.2, (after McCollum, 1976) and are based on the classification of Traub (1968). These classifications regard the leek as a part of a 'species complex' of A. ampeloprasum L., discussed in more detail in Chapter 4. Within the genus, the A. ampeloprasum complex is part of the section Allium, which also contains garlic (A. sativum L.) and its probable wild ancestor A. longicuspis Rgl., and the wild 'sand-leek' A. scorodoprasum found throughout Europe, including the British Isles. Most of the other cultivated Alliums are in the section Cepa and include the onion (A. cepa L.), the Welsh or Japanese bunching onion (A. fistulosum L.), chives (A. schoenoprasum L.) and rakkyo (A. chinense Rgl.). There is some possibility of interspecific breeding within sections, but none between. A major barrier to breeding is the obligate apomixis of garlic (Koul et al. 1979). Novel methods may be available to overcome these barriers, e.g. somatic hybrid formation by protoplast fusion. Interbreeding, albeit with variable levels of fertility in the hybrids, has been achieved between A. cepa x A. fistulosum, x A. galanthum, x A. drobovii and x A. pskemense using traditional methods (Saini & Davis, 1967 & 1969; Van der Meer, 1974). No crossing has yet been reported between A. schoenoprasum and A. cepa. New techniques such as in ovulo embryo culture are now being investigated as a means of improving

Figure 1.2
Relatioships between wild and cultivated species in the genus Allium, (after McCollum (1976), based on Traub, (1968)).

SECTION CEPA

Wild		Cultivated	
Allium sp.	(2x) →	A. cepa (2x)	ONION, SHALLOT
A. oschanii	(2x)		
A. pskemense	(2x) →	Sterile hybrids	Sterile hybrid → amphiploids
A. galanthum	(2x)		
A. vavilovii	(2x)		
A. altaicum	(2x) →	A. fistulosum (2x)	WELSH ONION or JAPANESE BUNCHING ONION
A. schoenoprasum	(2x) →	A. schoenoprasum (domesticated) (2x, 3x, 4x)	CHIVE
A. chinense	(2x) →	A. chinense (domesticated) (2x, 3x, 4x)	RAKKYO
	?(3x, 4x)		

SECTION ALLIUM

A. longicuspis	(2x) →	A. sativum (2x)	GARLIC
A. ampeloprasum (2x, 3x, 4x, 5x, 6x)	→	A. ampeloprasum	var. ampeloprasum (6x) GREAT HEADED GARLIC
			var. babingtonii BABINGTON'S LEEK
			var. porrum (4x) LEEK
			var. kurrat (4x) KURRAT

hybridisation between A. cepa x A. fistulosum, x A. vavilovii, x A. altaicum, A. schoenoprasum and between A.fistulosum x A.schoenoprasum, (Gonzalez & Ford-Lloyd, 1987). Interbreeding within the ampeloprasum complex has been carried out between A. porrum and A. kurrat (van der Meer, 1984; see also Chapter 4).

Thus while the potential for interbreeding and consequent improvement within crop species of the genus Allium is being expanded, there is still a significant gap in our knowledge of the leek-leek rust interaction. The general aim of this project was to elucidate certain areas of the interaction, as a basis for further work to improve the level of disease control.

Aims

The aims of the project were to:-

a) Investigate the taxonomy of the rusts on Allium species in the United Kingdom, to elucidate their relationships and classification, to end the confusion in this area;

b) Investigate the performance of a range of isolates from different Allium species on a wide range of (predominantly cultivated) Allium spp. to analyse host range and sources of resistance;

c) Investigate the possible sources of resistance within the ampeloprasum complex, which could be readily utilised in leek breeding work;

d) Investigate the nature of the disease within the leek plant, i.e. the effect of plant, leaf and tissue age on the disease;

e) Examine the performance of field isolates of leek rust on a selection of leek cultivars to look for cultivar resistance and indications of specialisation in the pathogen, and

f) Investigate the effect of the virus content of field cultivars of garlic on the garlic-leek rust interaction.

1.2. GENERAL MATERIALS AND METHODS

1.2.1. Rust Isolates

Isolates of rust were obtained from the sources listed in table 1.1. The NIAB and WSCOT samples were supplied as dry urediniospores. The remainder were collected from the field as pieces of infected leaf, and the urediniospores were harvested using the method in 1.2.6.

1.2.2. Host Species List

A list of the species used in this project is given in table 1.2.

1.2.3. Raising of Healthy Plants

a) Seeds

Seeds were sown in a John Innes No. 2 type compost, (see appendix 1) in three-inch plastic pots, and maintained at 20°C until germination, usually about four weeks later. When the seedlings had reached the two-leaf stage, they were repotted into individual pots and maintained in a heated glasshouse with a minimum temperature of 15°C, and supplementary lighting of soft white fluorescent tubes to provide a 16-hour daylength. The plants were fed weekly with a 1.25 mg l^{-1} solution of 'Vitafeed' (Steetly Minerals, Burscough, Lancs.) and repotted into larger pots as necessary.

Isolate	Host species	Origin
BIRM	Allium porrum	On shop-bought leeks. Maintained on A.porrum cv. Musselburgh
NIAB	Allium porrum	Various cultivars in field trials at NIAB, Cambridge
NVRS	Allium porrum	Various cultivars in field trials at NVRS, Wellesbourne, Warwick
LUDD	Allium porrum	Various cultivars in field trials at Luddington EHS, Stratford-on-Avon
STOCK	Allium porrum	Various cultivars in field trials at Stockbridge House EHS, Selby, Yorks.
WSCOT	Allium porrum	Field isolate from environs of West of Scotland College of Agriculture, Auchincruive, Ayrshire
CORN	Allium porrum	Field isolate from infected crops at Gulval, Penzance, Cornwall
CHIVE	Allium schoenoprasum	Various accessions in field plots at the University of Birmingham Botanic Gardens, Winterbourne, Birmingham
BABNT	Allium babingtonii	On wild plants near Penhale Sands, Newquay, Cornwall

Table 1.1

Details of host species and origin of the rust isolates used in this project.

Species			Type of source material
Section Allium			
Allium ampeloprasum	var	ampeloprasum	seed
	var	babingtonii	bulbs/bulbils
	var	kurrat	seed
	var	porrum	seed
Allium sativum			bulbs/cloves
Allium scorodoprasum			bulbils
Section Cepa			
Allium cepa			seed
Allium fistulosum			seed
Allium schoenoprasum			clones from plants
Allium vineale			bulbs/bulbils

Table 1.2

List of Allium species used during the project and the type of source material used for propagation

b) Vegetative Material

i) Allium sativum (garlic)

The bulbs were split into individual cloves, planted in 3.5 inch pots and maintained as above.

ii) Allium schoenoprasum (chives)

Mature plants from the field were split into rooted plantlets of 5-6 leaves, and repotted in 3.5 inch pots, and maintained as above.

iii) A. vineale, A. babingtonii, A. scorodoprasum

These are all perennials producing offset bulbils; A. vineale and A. babingtonii also produce bulbils in the seed head. In all cases, the bulbs and bulbils were vernalised at 5°C in the dark for 14 days before planting in 3.5 inch pots as above.

1.2.4. Spore Collection Methods

Large urediniospore samples were collected from whole plants by gently shaking the leaves over aluminium foil. Plant debris was removed with a fine paintbrush before storage. Smaller samples of spores were collected using a cyclone spore collector (figure 1.3), after Doherty (1981).

1.2.5. Maintainance of the pathogen in vivo

The BIRM isolate of the pathogen was maintained on leek cv. Musselburgh. The host plants were maintained in a glasshouse with supplementary lighting to give a 16-hour daylength, with a minimum temperature of 15°C. The plants were inoculated at 2 to 3 month

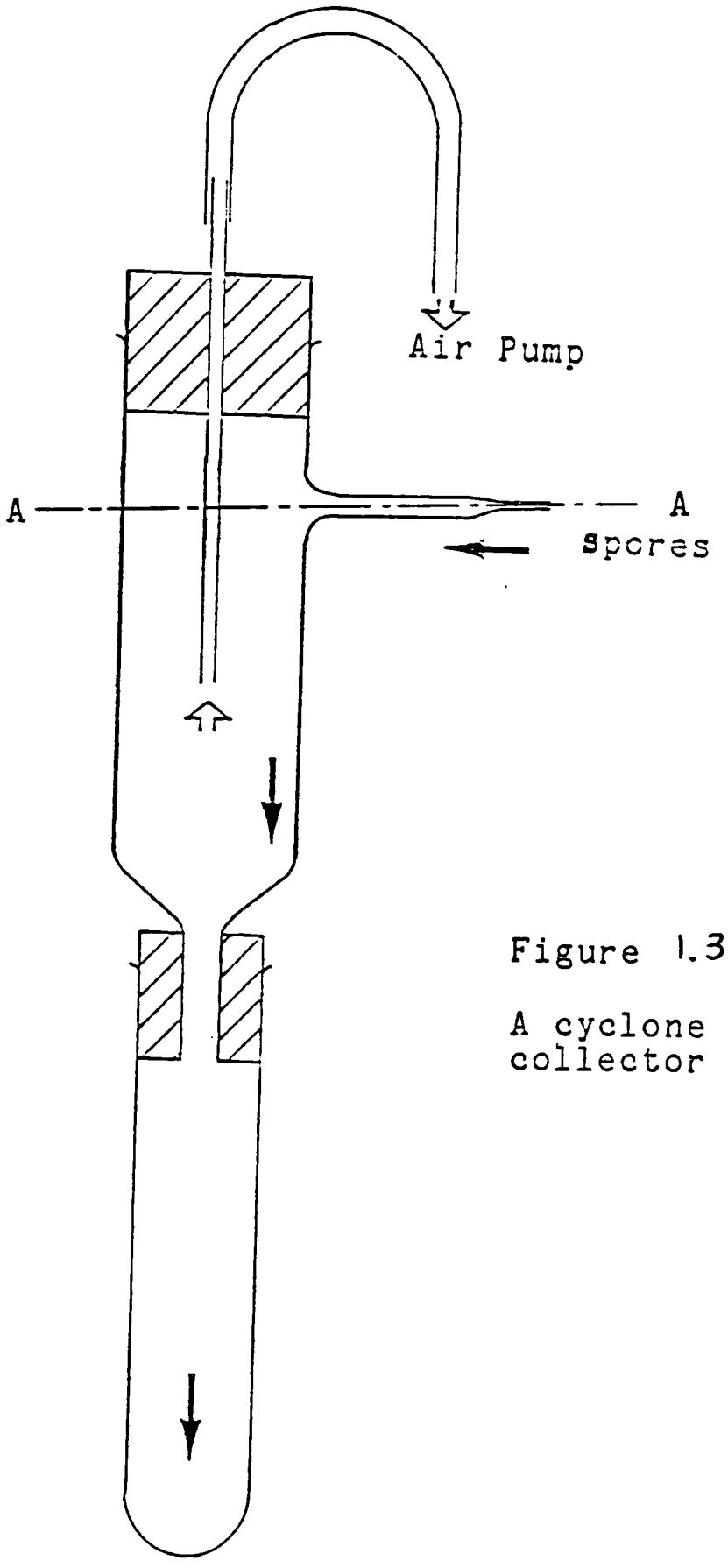
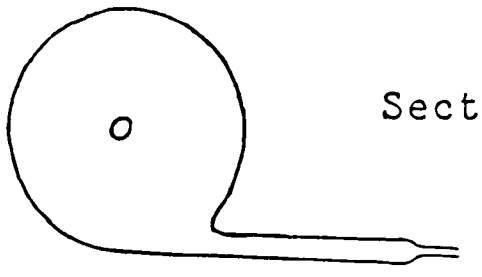


Figure 1.3

A cyclone spore collector



Section through A-A

intervals, (section 1.2.9.(a)) and spores were collected at one week intervals after pustule eruption.

1.2.6. Build-up of field isolates

Leaf pieces with erumpent uredinia ,collected from the field were maintained for 2 to 3 days in a 'dry-bed' chamber (figure 1.4) to facilitate production and collection of urediniospores. Build-up of the isolates to provide sufficient quantities of urediniospores for experiments was carried out by inoculating mature leek plants (cv. Musselburgh, cv. Albinstar and cv. Walton Mammoth) with the 'brush-on' method (1.2.9a). The inoculated plants were then maintained in a glasshouse in spore isolation chambers (fig. 1.5) to prevent contamination between isolates. The operation of these chambers is described in figure 1.5. The spores were collected from erumpent pustules using a cyclone spore collector.

1.2.7. In-vitro spore viability test for urediniospores

Spores were brushed using a small, soft paintbrush onto the surface of 0.5% water agar (Oxoid No.3, Oxoid Ltd., Basingstoke, Hants.) in 9 cm petri-dishes. The dishes were then incubated at 20 ^{o}C in the dark for 24 hours, before examination under a light microscope. A spore was considered to have germinated when the germ tube was at least the length of the spore. A figure for percentage germination was then counted from a count of at least 100 spores per dish.

Figure 1.4

Dry-bed method for collecting spores from infected leaf pieces from the field

The cut ends of the pieces were supported in saturated cotton wool to prevent the pieces from drying out. The pieces were surface sterilised with 95% ethanol prior to being placed in the chambers to reduce the growth of saprophytic and necrotrophic fungi.

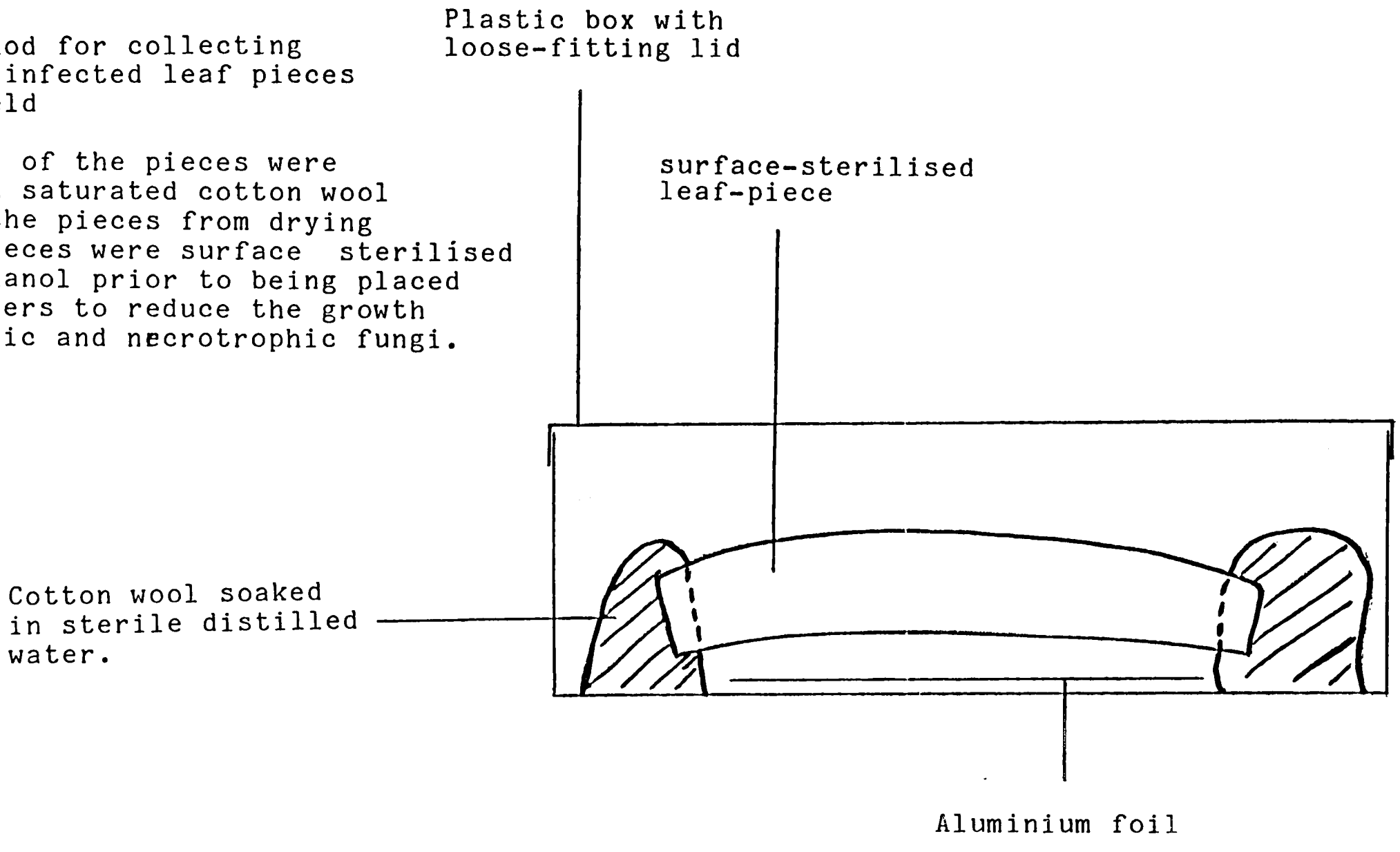


Figure 1.5

Spore isolation chamber

The isolation chambers consisted of a rectangular box frame of 2 cm square metal tubing, onto which 250-guage polythene sheeting could be attached to provide a spore proof growing area. The manifolds contained 5 μm diameter pore-size filters to prevent spore entry/exit, and a positive internal air-pressure was produced by reducing the diameter of the air exit vent. Each tray (65 x 18 cm) was capable of taking three 5-inch pots. All the plastic components were made of non-phytotoxic plastic. Water was supplied to the trays via a pipe through the polythene skin, which could be closed by an H-clip.

A - Fan (air in)
B - Fan (air out)
C - Manifold
D - Air duct (in)
E - Air duct (out)
F - Plant trays
G - Capillary matting
H - 2 cm tubular frame
J - Melamine base

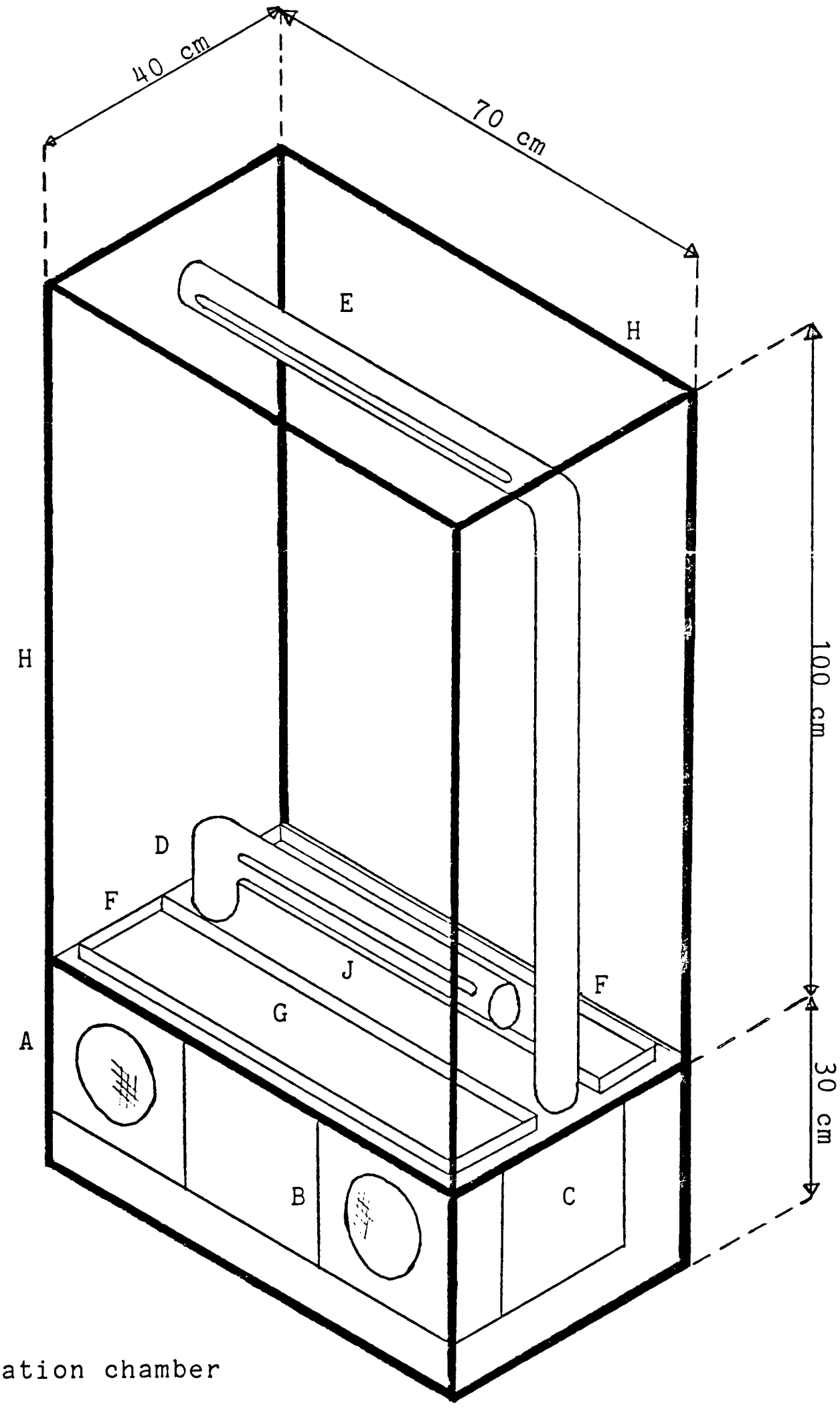


Figure 1.5

Spore isolation chamber

1.2.8. Urediniospore Storage

Urediniospores were stored in glass petri-dishes in a refrigerator at 5°C. This method was found to keep urediniospores sufficiently viable for reinoculation for up to 9 months. Small spore samples were stored in press-cap bijou bottles for up to 3 months in the same conditions.

1.2.9. Inoculation Techniques

In all inoculations, dead and senescing leaves and leaf tips were removed before inoculation to prevent infection with saprophytes.

a) Brush-on technique

Dry spores were collected from a petri dish on the head of a soft paintbrush and dusted liberally onto the adaxial surface of the leaf to be inoculated. The inoculated leaves were then mist-sprayed with a solution of Tween-80 (Koch-Light Laboratories Ltd.) in water (5×10^{-4} v/v) using a small aerosol hand spray. The inoculated plants were then covered in a polythene bag tied at the base of the plant or the rim of the pot for 48 hours to ensure a high humidity for spore germination and infection.

b) Spray Technique (for quantitative inoculation).

A known mass of spores was suspended in a solution of Tween-80 in water (as above) and allowed to stand for one hour to allow the spores to imbibe fully. The inoculum suspension was then sprayed evenly over the adaxial surface of the leaf until just before run-off, to give a

consistent dose per leaf. The plant was then enclosed in a polythene bag as in 1.2.9(a). The concentration of the spore suspensions were varied according to the dose required and the viability of the spore sample.

c) Dip technique

For routine inoculation of the CHIVE isolate onto chives, a modification of the dip technique (Browder, 1971) was used.

1.2.10 Disease assessment

a) Lesion Types

For qualitative assessment of the host plant reaction to challenge of the rust, a simplified codification of infection types was used, based on Zadoks' (1961) simplified glasshouse key:-

Code	Symptoms
i	No symptoms
0	Pale green/brown flecks, no pustules
1	As 0, but with few small pustules with brown halos
2	Pale green flecks, few small pustules with green halos
3	Few pale green flecks, pustules large with green halos
4	No flecks, pustules large with no halos

b) Pustule Quantity

For an assessment of pustule density on brush-on inoculated plants, a simplified coding was used, based on the ADAS rust key, figure 1.6.

Code	Description	% Density (on key)
0	No pustules	0
1	Few, single pustules	0.1
2	Few pustules	0.1 - 1.0
3	Dispersed pustules	1.0 - 2.0
4	Moderate covering of pustules	5.0 - 10.0
5	Heavy covering of pustules	25.0 - 50.0

c) Incubation period

Incubation period was defined as the time in days from inoculation to the appearance of the first flecks (lesion type 0), with a mean taken from replicate plants.

d) Latent Period

The time in days from inoculation to the eruption of the first pustules (lesion types 1 to 4), with a mean taken from replicate plants.

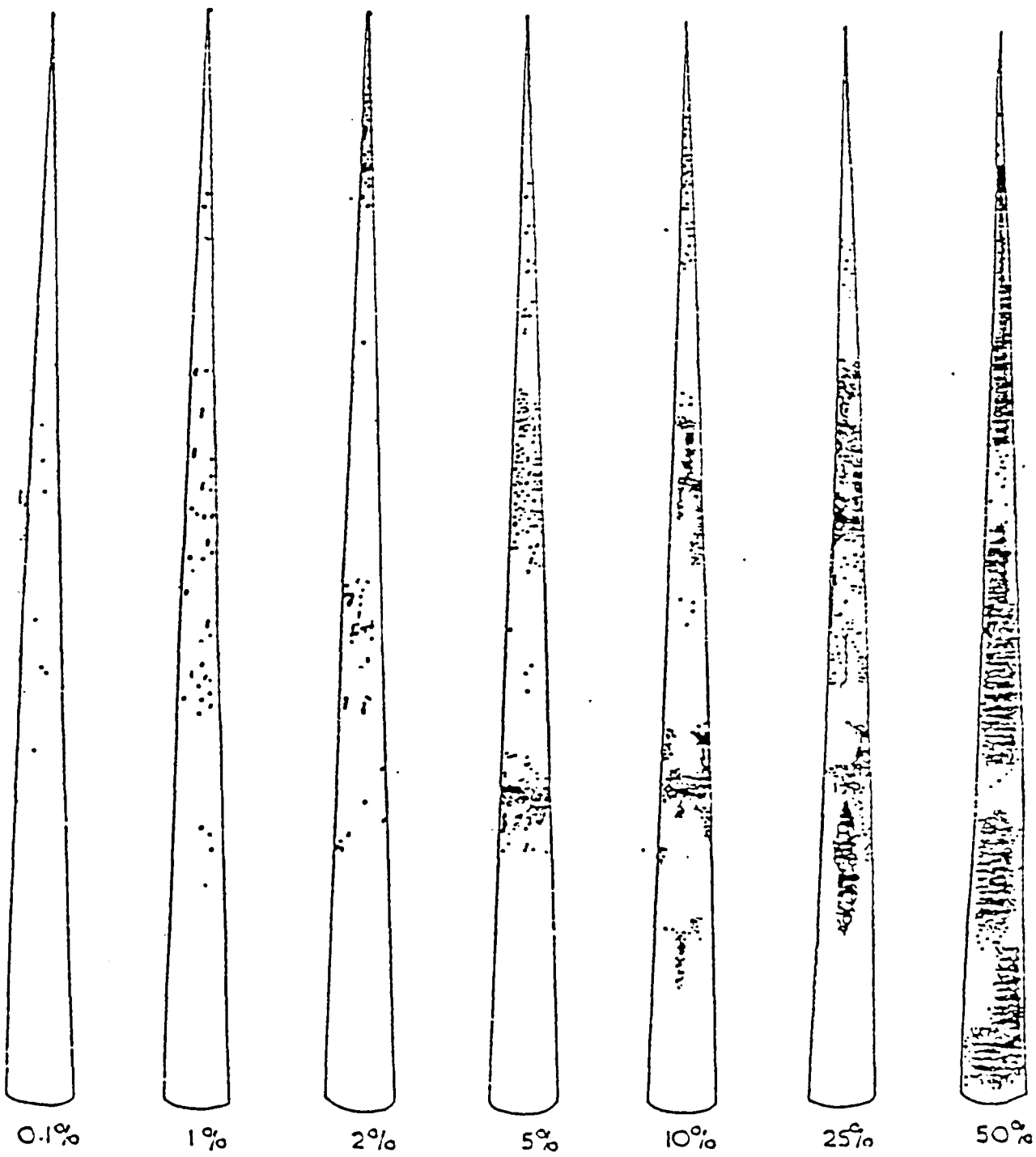


Figure 1.6
Key for assessing severity of rust infection on leeks in the field (From the Agricultural Development and Advisory Service).

1.2.11 Staining technique for examining fungal structures within leaf tissue.

A modification of the technique of Bruzzese and Hasan (1983) was developed, which was able to stain the colonies of rust within the leaves of leeks. Leaf pieces up to 5 cm long were immersed in the clearing-staining solution (Appendix 2) in stoppered vials or glass petri dishes in a fume cupboard for 48 - 72 hours (depending on the ability of the stain to work). The stained leaf pieces were then washed thoroughly in warm water, and then destained for 24 - 48 hours in a concentrated aqueous solution of chloral hydrate (2.5 g cm^{-3} chloral hydrate in distilled water), before being rinsed rapidly in distilled water and mounted on microscope slides in lactic acid.

1.2.12 Photography

Unless otherwise stated, all colour and black-and-white photographs and photomicrographs were taken on an Olympus OM2n 35 - mm camera. Fluorescence micrographs were taken on a Zeiss fluorescence microscope with a microscope mounted Zeiss M35 Camera.

1.2.13 Statistical Methods

Unless otherwise stated, analyses of variance followed the standard ANOVA pattern, and for correlations the product-moment correlation coefficient (r) was used (Parker, 1979). Where the variance was found to be proportional to the mean, a logarithmic transformation of the

raw data was carried out before the analysis. The arc-sin transformation was used before analysis on all data in the form of proportions and percentages (Parker, 1979).

CHAPTER TWO

SPORE STUDIES

2.1 INTRODUCTION

The taxonomy of the rusts on *Allium* species has a complicated history and has been in a state of flux since Sowerby (1810) described *Uredo porri* on leeks and De Candolle (1815) described *Uredo alliorum* on *A. multiflorum* D.C. (= *scorodoprasum* L.), *A. vineale* and *A. oleraceum*, and *Uredo ambigua* on an unknown *Allium* species. The detailed nomenclatural changes are described elsewhere (see chapter 8.1), but essentially the European situation was described by Gaumann (1959), summarising the work of Schneider (1912), Grove (1913), von Tavel (1932) and Goto (1933) in Japan. Gäumann described three species of rust using telial and teliospore characters, viz:

Puccinia allii (D.C.) Rud. -	with mostly two-celled teliospores in stromatic telia
Puccinia porri (Sow.) Wint.	with one and two-celled teliospores in non-paraphysate telia
Uromyces ambiguus (D.C.) Lev.	with one-celled teliospores in non-paraphysate telia

He also considered the species Puccinia mixta (Fuck.) to be a synonym of Puccinia porri. However lack of telial material on leeks in the U.K. had prevented classification using this system, so that Laundon and Waterston (1965) lumped all the species as P. allii and considered the other names as synonyms. Wilson & Henderson (1966) described U. ambiguus and P. allii as the two species in the U.K. with P. porri and P. mixta (on chives) as synonyms of P. allii. Doherty (1981) agreed with this system after a detailed literature survey. However Henderson & Bennell (1979) decided that the urediniospore morphology of the rust on leeks was sufficiently different to merit specific rank, and using the description of Savile (1961) named it P. porri. However in 1984 telia were found on leeks in the U.K. by Uma (1984) and Norwood (1985), enabling a comparative study with the continental system, and between the urediniospore and teliospore characteristics of this rust.

Goto (1934, 1935) compared quantitatively the two spore stages in his work on rust strains in Japan, and found no differences between the strains in uredinial characters but distinctive differences in telial morphology which parallelled those in Europe in P. allii and P. porri. However, considering the relative importance of the teliospore cell number in these descriptions, little is known of the relative viability of single and two celled spores where they occur in the same telium, though both Uma (1984) and Harrison (1987) described the germination of two-celled teliospores.

One major problem in using urediniospores is the lack of clear-cut morphological differences between 'species' compared with the variation within populations. Continuously variable characters such as spore length and width have been shown to vary between pustules, leaves or individual host plants, and with environmental conditions, (Littlefield, 1981). Thus classifications based on urediniospores are open to criticism. Characters such as germ pore number or morphology might be assumed to be more stable, and therefore 'good' taxonomic characters, (Wilson & Henderson, 1966; Cummins & Hiratsuka, 1983) but many authors have commented that the germ pores of the Allium-rusts are indistinct (Gäumann, 1959; Wilson & Henderson, 1966) and relatively few methods are available to enhance them (Urban, 1963; Savile 1961). Also relatively little is known about their formation or the germination process as it affects germ pores (Bennell & Henderson, 1978).

Numerical methods have been adapted for use in many fields of taxonomy and found particularly useful where the relationships between organisms are unclear (Michener & Sokal, 1957; Kendrick & Proctor, 1964; Kendrick & Weresub, 1966; Ibrahim & Threlfall, 1966; Lam & Chapman, 1985). Although the concepts described by the original proponents of numerical methods ideally required as many as 50 to 100 characters (Sokal & Sneath, 1963), it is possible to use multivariate analysis methods with far fewer characters as long as the limitations of the data are recognised (Dunn & Everitt, 1982).

The effect of temperature on the germination of urediniospores often varies between species (Stubbs et al. 1986) in terms of the range and and optimum temperatures, and may therefore be a useful physiological character as well as providing epidemiological information (Dunn & Everitt, 1982) although such information is not available from herbarium material.

Both scanning electron (SEM) microscopy and fluorescence microscopy provide additional ways of looking at fungal material. SEM provides an ideal method of studying surface features of spores and has been used extensively in many taxa (see review by Littlefield & Heath, 1979) but its accuracy as a measuring device is limited (Boyde, 1974). Uma (1984) examined uredinia and telia of P. allii on leeks using the SEM and described spines and germ pores on urediniospores, the structure of the telia and the surface sculpturing on teliospores (Uma, 1984; Uma & Taylor, 1986). Fluorescence microscopy has been found useful in germination studies on rusts; Rohringer et al. (1977) found that Calcofluor M2R White (CFW) caused intense fluorescence of urediniospores and germ tubes of Puccinia graminis f. sp. tritici and Melampsora lini, and several workers have concluded that CFW binds preferentially to actively growing regions. Therefore, the aims of this study were to do :-

a) A comparative morphological study of telia and uredinia of isolates from each of several different host species of Allium, comprising;

i) Light and SEM study of uredinial and telial material one representative isolate per host

ii) A quantitative study of selected urediniospore characters with statistical and multivariate analysis.

b) A comparison of a physiological urediniospore character - the effect of temperature on germination in vitro, using fresh isolates from different hosts.

c) A detailed examination of germ pore morphology and function (on one isolate)

d) A study of the comparative viability of single and two-celled teliospores

e) A comparative study of selected herbarium material from a European range (to compare with (a)).

2.2. MATERIALS AND METHODS

Materials

a) Urediniospore samples of isolates

The urediniospore samples of isolates were obtained from the sources in 1.2.1., with the addition of three herbarium specimens;

Isolate	Origin
KERR	On *Allium schoenoprasum*, from Aberdeenshire, Scotland, collected 2-7-1948 by A. Kerr
6504	On *A. schoenoprasum*, from Aberdeen, collected 30-6-1948 by E. Gray
H.70	On *A. scorodoprasum*, Kirkcudbrightshire, collected by D. M. Henderson, 1970

b) Teliospore samples of isolates

Isolate	Host plant	Origin
BIRM	*A. porrum*	Fresh material from cultivar trials with telia developing from uredinia

NVRS	A. porrum	Dried herbarium material of telia from field trials at the NVRS (same origin as urediniospore isolate). See Norwood (1985)
CHIVE	A. schoenoprasum	Dried herbarium material and fresh specimens from field plots at Winterbourne Botanic Gardens, University of Birmingham, and from in glasshouse trials from artificial inoculation with the CHIVE urediniospore isolate.
BABNT	A. babingtonii	Dried herbarium and fresh material from various Cornish sites and from artificial inoculation with the BABNT urediniospore isolate.

Methods

2.2.1. Urediniospore Studies

2.2.1.1. Non-quantitative

a) Macroscopic features of the uredinia

Fresh and herbarium specimens of the BIRM, CHIVE and BABNT isolates with uredinia were examined macroscopically, and with the aid of a

low-power binocular microscope, to describe surface features of the uredinia.

b) Spore Morphology

i) Direct light microscopy

Urediniospores were scraped gently from erumpent pustules, mounted in lactic acid under a coverslip, heated gently until boiling point, and then viewed under a Leitz direct-light microscope.

ii) Normarski Interference Microscopy

Spores were mounted as for viewing under direct light but were viewed instead on a microscope fitted with Normarski-Interference apparatus.

iii) Scanning Electron Microscopy

Dry, unfixed urediniospores were mounted on stubs using double-sided sticky tape, and gold-coated using a Polaron E5000 sputter coater. The coated spores were viewed using an ISI-100 A scanning electron microscope.

c) Germ Pore Morphology

i) Aniline-Blue Staining with Phase Contrast Microscopy

Urediniospores of the BIRM, STOCK, CHIVE and BABNT isolates were mixed with a drop of aniline blue stain in lactic acid, and heated until the stain began to 'smoke', and a coversip added whilst still warm. The coverslip was then pressed hard onto the slide until the spore contents had been evacuated and the spore walls were in one plane of

focus. The squashed spores were observed under a phase-contrast microscope.

ii) Scanning Electron Microscopy

Fresh, dry spores of the BIRM isolate were mounted on stubs using double-sided sticky tape, and a drop of $5x10^{-4}$ v/v Tween-80 in water was placed on the mounted spores at $20^{o}C$ for four hours. The stubs were then air-dried and placed in a desiccator for 24 hours before being prepared and viewed as for the dry spores in section b(ii).

iii) Fluorescence Microscopy

Two spore sample of the BIRM isolate were mounted in 0.5 mg ml^{-1} 'Fluorescent Brightener 28' (Calcofluor white new M2R; Sigma, Poole, Dorset) and viewed under a Zeiss ultraviolet-light microscope using an appropriate filter. The first sample consisted of fresh dry spores. The second was mounted in $5x10^{-4}$ v/v Tween-80 in water for four hours at $20^{o}C$ until the spores had begun to germinate, before adding the fluorescent brightener.

2.2.1.2. Urediniospore Studies (Quantitative)

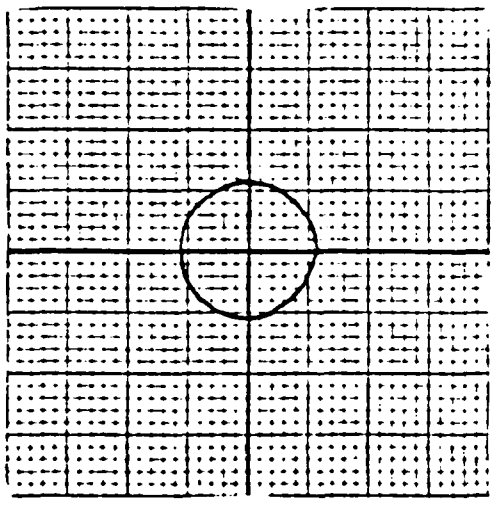
a) Urediniospore Length, Width, Spine density and Pedicel Scar Diameter.

Urediniospores were mounted in lactic acid under a coverslip, warmed gently in a bunsen flame until boiling point and then blotted until

the spores were immobile but not squashed. The length, width and pedicel scar diameter were measured by superimposing a grid scale centrally over a spore in the field of view using a projection tube attachment. The grid is illustrated in fig. 2.1.

Length was defined as the largest dimension bisecting the pedicel scar; width the largest dimension at right angles to the length axis; and the spine density was measured as the number of spines falling on and within the 100 μm^2 circle on the grid, see fig. 2.2.

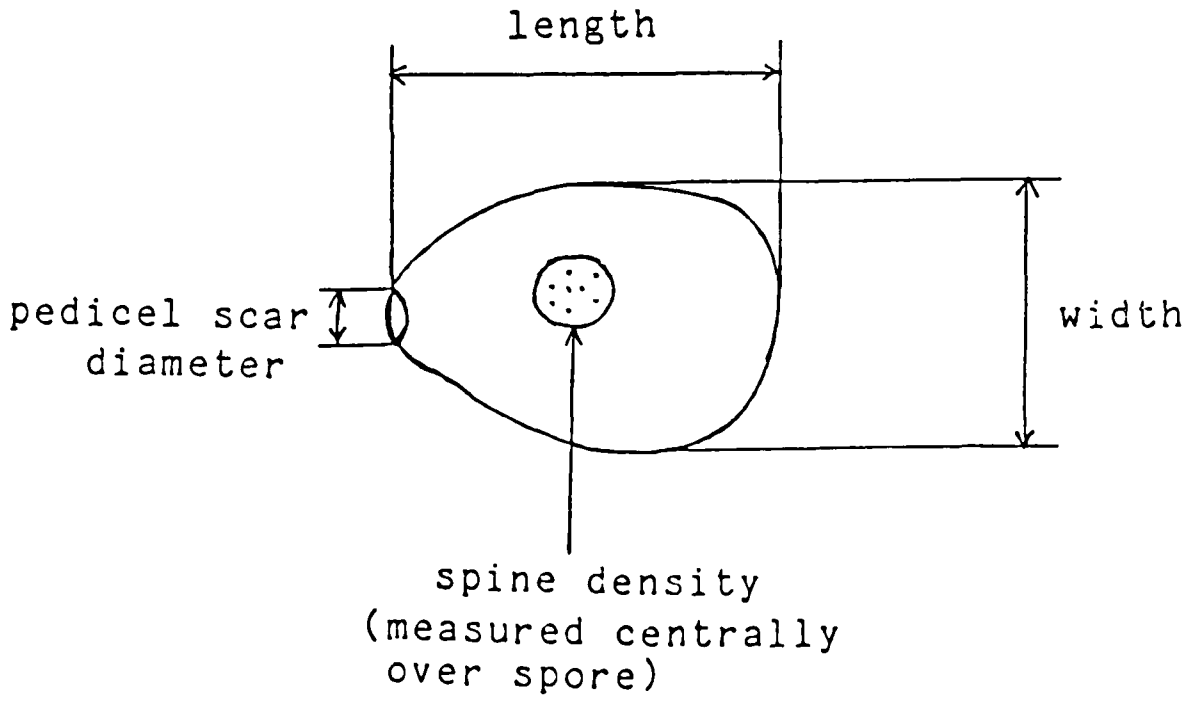
Figure 2.1 Grid for measuring length, width, spine density and pedicel scar diameter of urediniospores using a microscope with a projection tube attachment.



area within circle = 100 μm^2

not to scale

Figure 2.2 Dimensions measured on urediniospores using the grid in figure 2.1



Measurements on the isolates

A series of studies was first carried out using the BIRM isolate to determine the extent of variation within the technique (i.e. between slides), within a single isolate, and between fresh and herbarium material. The data from (i) and (ii) were analysed using a one-way nested analysis of variance, after Sokal & Rohlf (1981).

i) Variation between slides

Measurements were taken from 10 spores on each of two slides from each of 5 pustules (= 10 slides, 100 spores).

ii) Variation between pustules, leaves and plants

Spore samples from different leaves on different plants were subdivided as indicated below, with one slide per pustule.

10 spores > 10 pustules > 2 leaves > 2 plants

(= 4 leaves, 40 pustules, 400 spores)

iii) Comparison between fresh and herbarium material

Fresh spore samples of the BIRM isolate were compared with samples of the same isolate from herbarium specimens made two years previously. For each sample, 10 spores per slide, one slide per pustule from five pustules were measured, (= 5 slides, 50 spores).

iv) Comparison between isolates

For the measurements between the isolates, 10 spores from each of five pustules per isolate were examined (=one slide per pustule, 50 spores

per isolate), or where the spore material was in the form of a bulk sample, 10 spores on each of five slides were measured. The isolates examined are listed in the materials section (a).

b) Germ Pore Number

For each of the isolates in materials section (a) a single sample was prepared as in 2.2.1.1.c(i), and the number of germ pores per spore counted on 50 spores.

c) Multivariate Analysis

A multivariate analysis was performed on the quantitative characters of the twelve isolates using the data from a(iv) and (b) above. The isolate means of each character were analysed since the analysis has to be performed on data derived from the same taxonomic unit. In this case the smallest units possible were the isolates, since the germ pore character was measured on different individual spores from the other quantitative characters. These data were then analysed using the 'Clustan' programme (Program Library Unit, University of Edinburgh) on the Honeywell Multics computer at the University of Birmingham. The programme procedures were chosen to produce a principal component analysis and compute a similarity matrix to produce a dendrogram using a form of Ward's method (Ward, 1963). This produces a hierarchical grouping based on minimising the sum of squares for each character between the operative taxonomic units (in this case the isolates) in each cluster, i.e. clustered groupings based on minimum variance.

d) Effect of temperature on urediniospore germination (*in vitro*)

For each isolate (in section 1.2.1.) the standard *in vitro* viability test was carried out over a range of temperatures, three plates per temperature, and a percentage germination calculated from a count of 100 spores per plate after 24 hours (= 300 spores per temperature). The temperatures used were 5, 10, 15, 20, 22.5, 25 and 30°C.

2.2.2. Teliospore studies

a) Macroscopic Observations on the telia

Fresh and herbarium specimens (as in materials (b)) of host tissue with telia were examined macroscopically and with the aid of a low-powered binocular microscope, to examine the external appearance of the telia. Telia of the H.70, KERR and 6504 isolates were also examined.

b) Spore and Telium Morphology

i) Light Microscopy

Telia from both fresh and herbarium material (listed in materials (b)), and also the H.70, KERR and 6504 isolates) were dissected from the surrounding host tissue and either macerated with a scalpel or sectioned using a razor blade. The material was then mounted in lactic acid under a coverslip, heated gently to boiling point, and observed under a Leitz microscope.

ii) Scanning Electron Microscopy

Telia from herbarium material of the NVRS, CHIVE and BABNT isolates were sectioned and fractured by hand, and placed on a stub using double-sided sticky tape. The prepared stubs were then examined after gold coating as in 2.2.1.1.c(iii).

c) Germination studies

Teliospores were dissected from telia from fresh host specimens and plated onto 0.5% w/v agar plates (Oxoid No. 3 agar) incorporating 10 $\mu g\ ml^{-1}$ Rifampicin and 10 $\mu g\ ml^{-1}$ Streptomycin to reduce bacterial growth during the experiment. The plates were incubated at 5°C in the dark for 48 hours before transfer to 20°C. The plates were observed daily under the microscope for signs of germination.

2.2.3. Herbarium Material

Specimens of Allium spp. infected with rust of European and Mediterranean origin were examined from the herbaria of the International Mycological Institute, Kew; the Royal Botanic Garden, Edinburgh and Birmingham University. Both telia and uredinia were examined macroscopically and microscopically, and the appearance of the pustules and spores was described.

2.3. RESULTS

2.3.1. Urediniospore Studies

2.3.1.1. Non-quantitative studies

a) Macroscopic studies

BIRM isolate

The uredinia were usually large, rounded or more commonly 'eye' or 'lozenge' shaped. Single pustules were up to 10 mm long, but were often confluent into much larger pustules, and were up to 3 mm wide, and often with concentric rings of secondary pustules. The pustules were often raised with the epidermis coming away from the underlying tissue, giving a whitish appearance, usually with a pronounced tear along the axis of the leaf exposing areas of orange-brown urediniospores.

CHIVE isolate

The uredinia were generally rounded, or elongated along the leaf, and up to 10 mm long when confluent with adjacent pustules, but single pustules were more usually 1-2mm long. The epidermis was usually sharply raised above the surrounding tissue, with a single linear tear along the axis of the leaf, exposing the orange-brown urediniospores.

BABNT isolate

The uredinia were usually 1-4 mm long and 1mm wide when single, and

'lozenge' shaped. In naturally-infected specimens, the pustules were in long stripes, with adjacent stripes confluent into pustules 10 mm long. The epidermis covering the pustule was usually only narrowly slit, to reveal the bright orange urediniospores (see fig 2.3).

b) Spore morphology

i) Light Microscopy

Figure 2.4 shows urediniospores of the BIRM isolate viewed using light microscopy. There were no major morphological differences between the isolates when observed unstained under the light microscope, except for a higher spine density on the CHIVE and BABNT isolates compared with the BIRM isolate. In general the spores were subglobose to ellipsoidal or rarely globose in shape, with walls 1-2 µm thick, covered on the surface with delicate echinulations. The colour of the cytoplasm varied from pale yellow to bright orange, and coagulated after heating into a single central mass. The germ pores were only visible in profile, and appeared as areas of the wall locally thickened on the inner surface, sometimes with a lens-like cap on the outside. There were no consistent differences in germ-pore morphology between isolates. The pedicel scar was visible in profile as a locally flattened area devoid of spines.

ii) Normarski Interference Microscopy

This technique highlighted the walls, spines and germ pores in

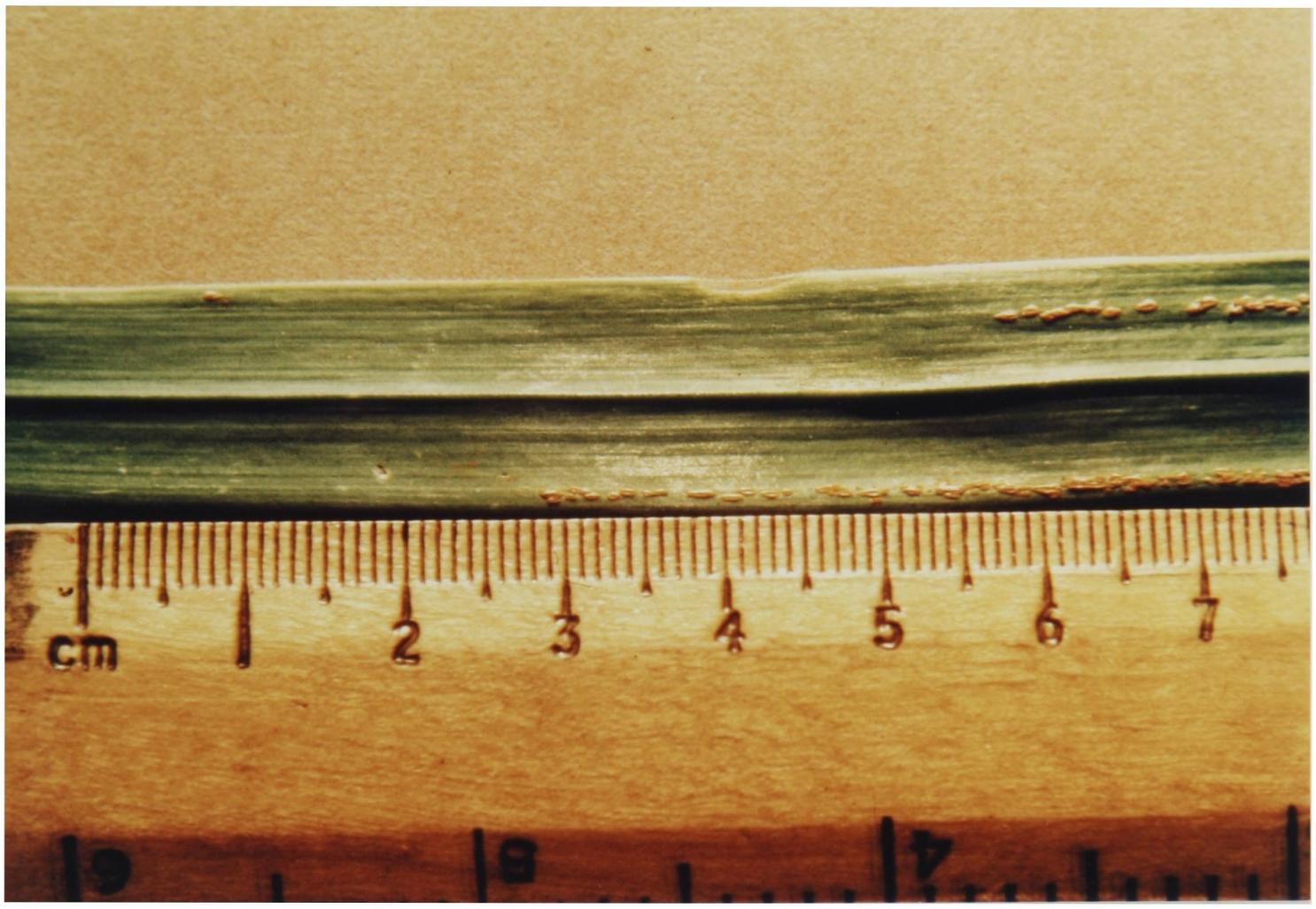


Figure 2.3
Abaxial leaf surface of A. babingtonii with natural infection of the BABNT rust isolate, from Penhale Sands, Cornwall. Note the uredinia in long stripes.

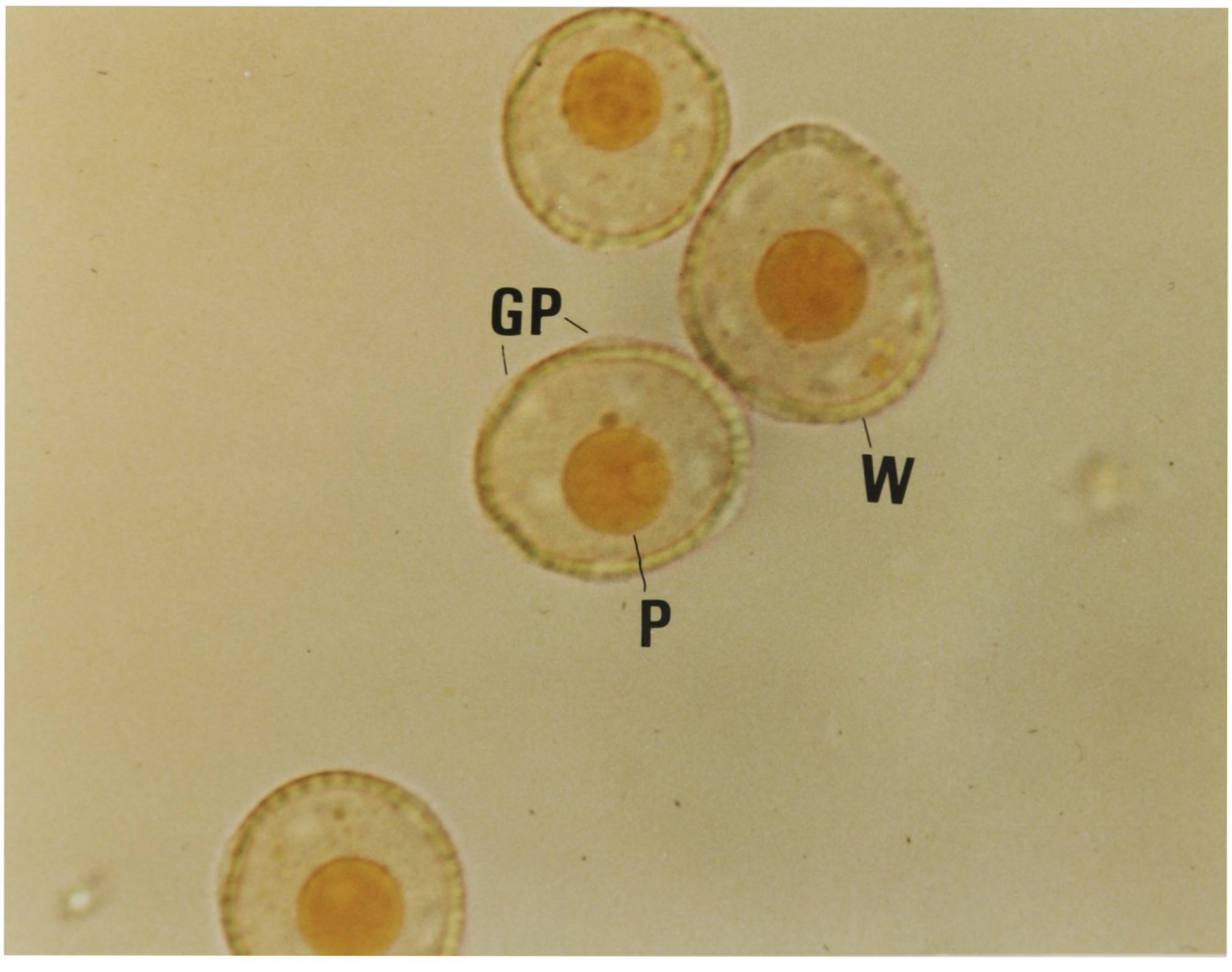
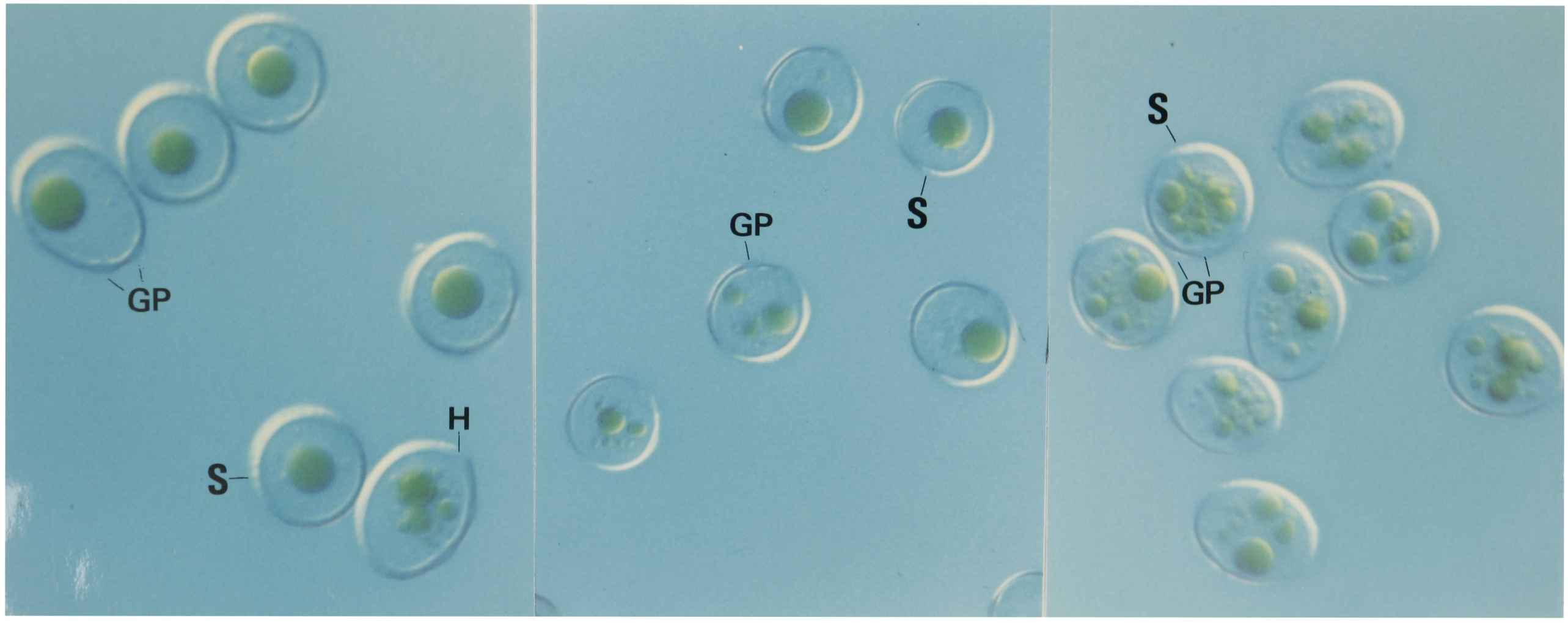


Figure 2.4
Transmitted light micrograph of urediniospores of the BIRM isolate, mounted in lactic acid, showing walls (W), the agglutinated protoplasm (P), and the germ pores (GP) in profile.

Fig 2.5

Fig 2.6

Fig 2.7



50 μm

50 μm

50 μm

Figures 2.5 to 2.7
Normarski interference-contrast micrographs of urediniospores of the BIRM, CHIVE and BABNT isolates, showing clearly capped germ pores (GP) in profile in all isolates, spines (S), walls and reduced wall thickness at position of hilum (H, fig. 2.5)

profile. The major differences between the isolates were the greater wall thickness and greater spore size of the spores of the BIRM isolate. However it must be noted that the spores of the CHIVE isolate illustrated in fig. 2.6 are not actually in profile, accentuating the size difference with the BIRM isolate spores (fig. 2.5) and the BABNT spores (fig. 2.7). The germ pores of all three isolates appear to have distinct 'caps'. There were no differences in spine density visible between the isolates.

iii) Scanning Electron Microscopy

Figure 2.8 shows an scanning electron micrograph of a partially collapsed urediniospore of the BIRM isolate. The surface is smooth and covered with scattered spines sited in small depressions on the surface. Figure 2.9 shows a urediniospore of the NVRS isolate. It is essentially similar to the BIRM isolate spore though the spines and depressions at their bases are more pronounced, encircled by raised annuli. At the base of the spore the pedicel scar is visible as a flat area devoid of spines, and in the centre of the scar there is a slight depression in the position of the septal pore. Figure 2.10 shows two urediniospores of the NVRS isolate, with more pronounced pedicel scars. Note that the spines and depressions in the upper spore are less pronounced than in the lower spore and the spore in figure 2.9. Figures 2.11 and 2.12 show spores from the CHIVE and BABNT isolates respectively. They are essentially similar to the leek isolates, though the surface of the CHIVE isolate is slightly

Figure 2.8
SEM of a urediniospore of the BIRM isolate, showing spines formed in small depressions.

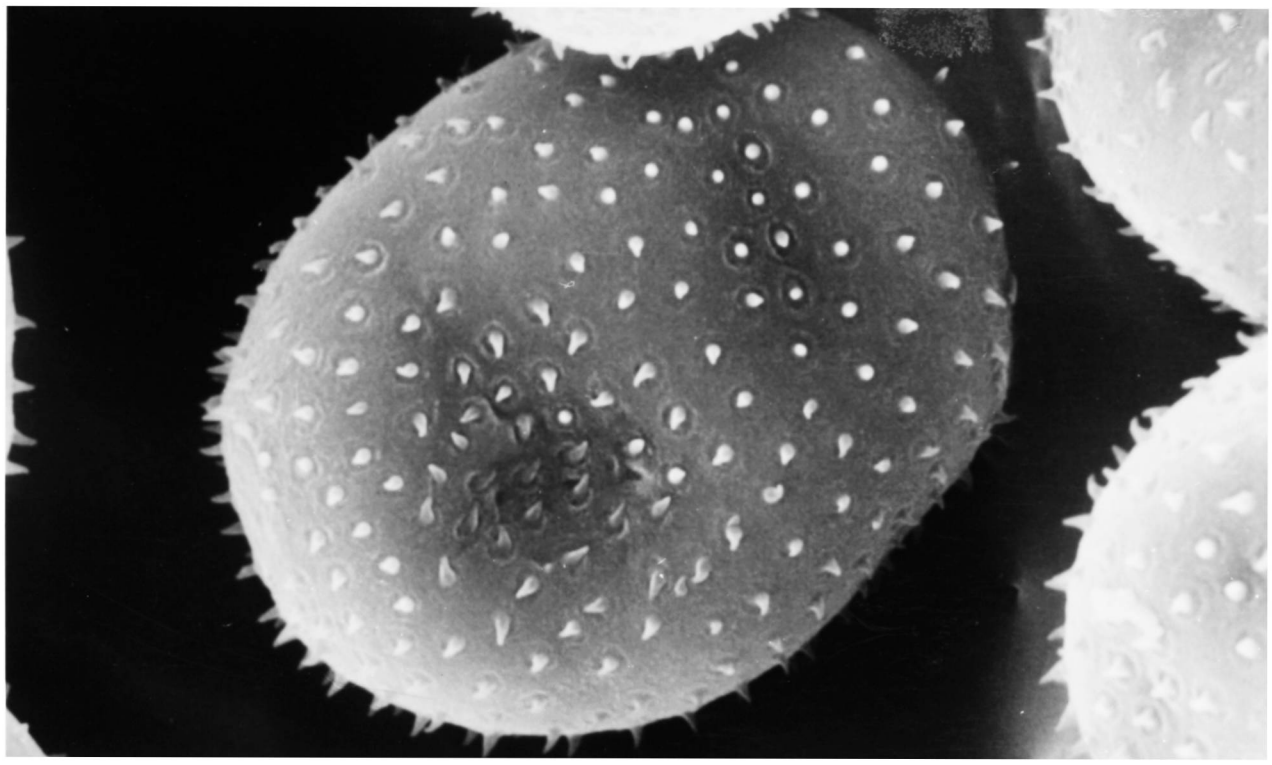
Bar = 10 μm

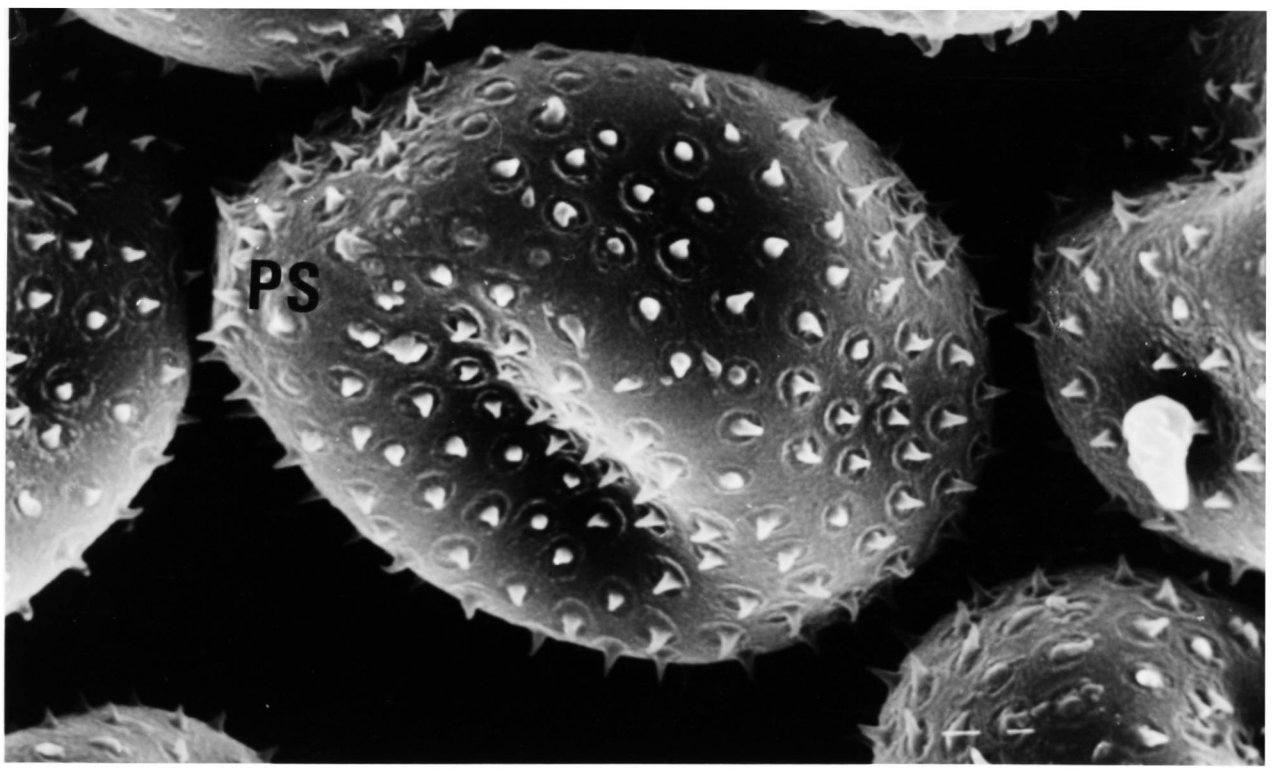
Figure 2.9
SEM of a urediniospore of the NVRS isolate, showing spines formed in small depressions with pronounced annuli, and the pedicel scar (PS) as a flattened area devoid of spines.

Bar = 10 μm

Figure 2.10
SEM of urediniospores of the NVRS isolate, with pronounced pedicel scars, both in profile (upper spore) and full on, with a septal pore (SP) visible in the centre of the scar on the lower spore.

Bar = 10 μm




PS

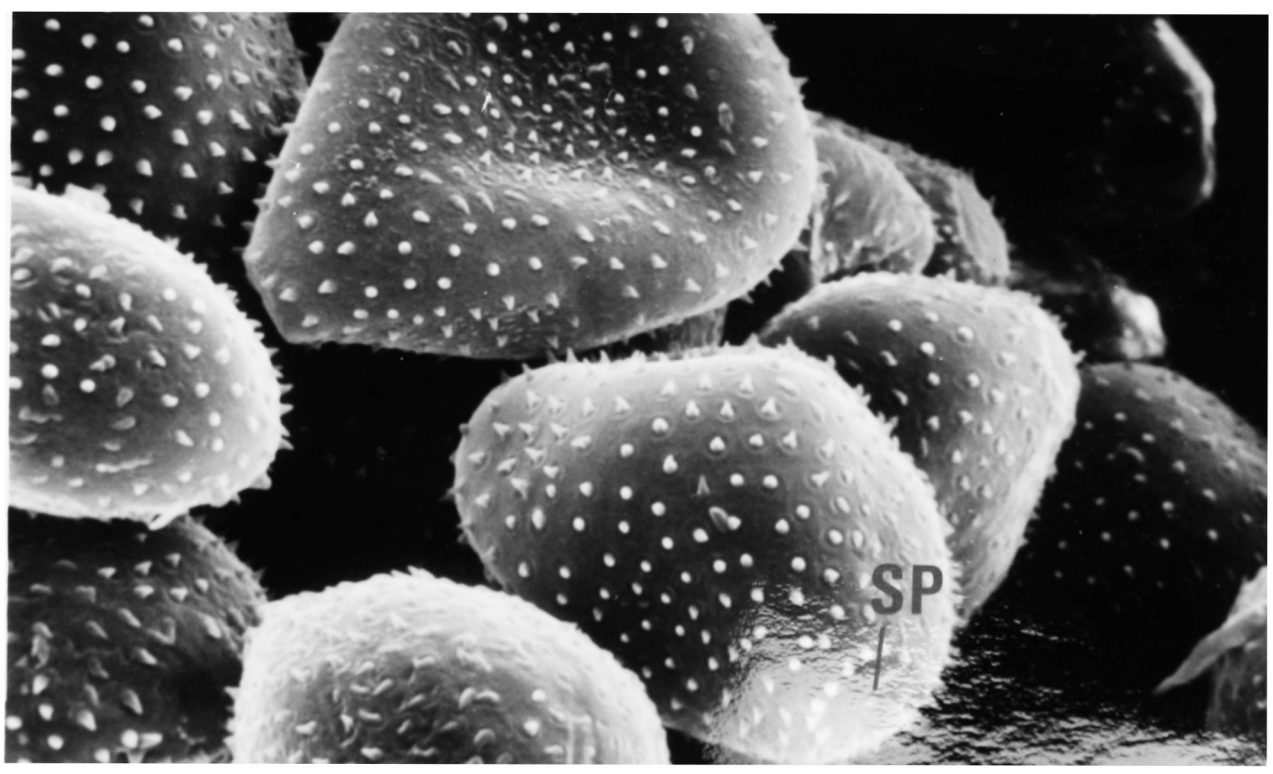

SP

Figure 2.11
SEM of a urediniospore of the CHIVE isolate, with spines in very shallow depressions and a pedicel scar in profile.

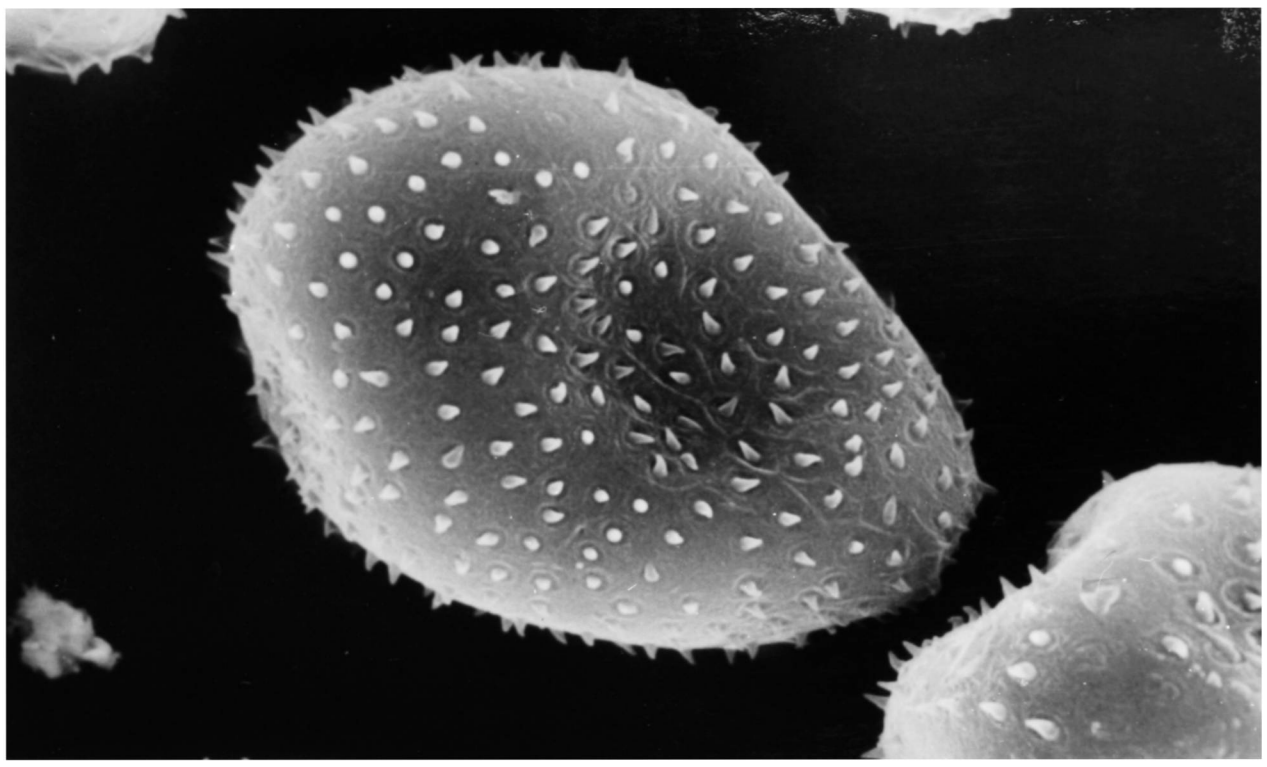
Bar = 10 μm

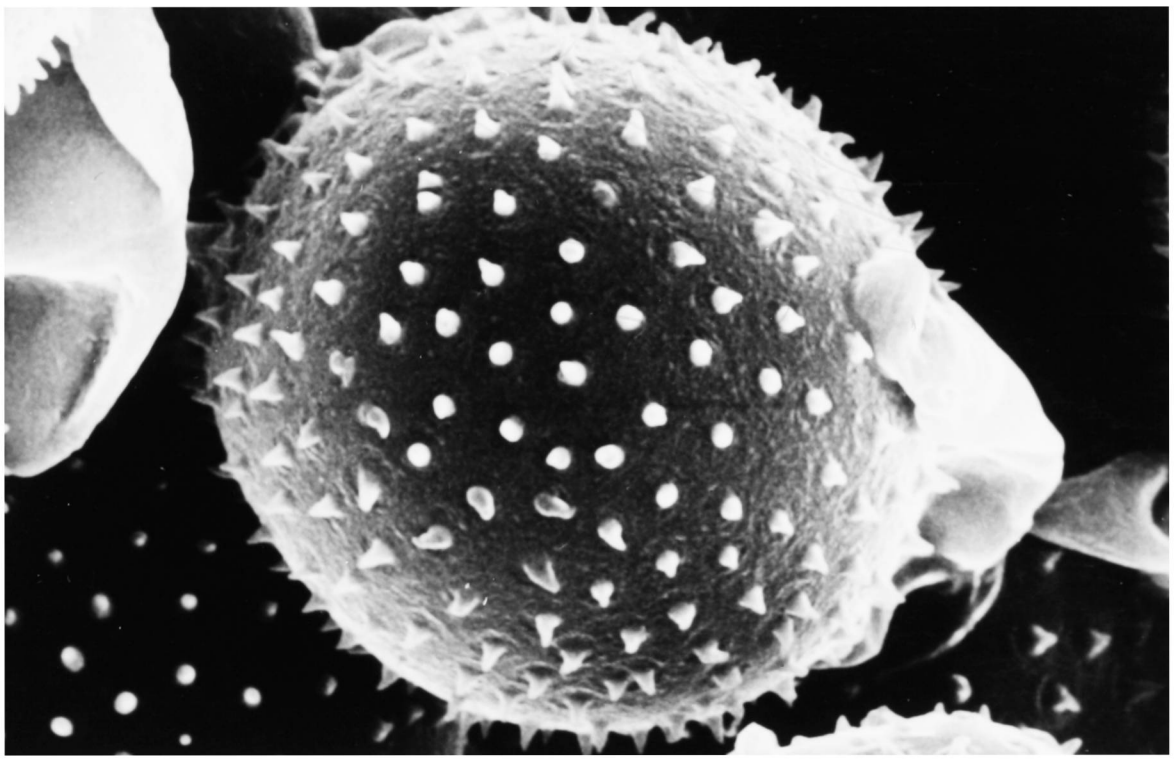
Figure 2.12
SEM of a urediniospore of the BABNT isolate, with very distinct regularly spaced spines in very shallow depressions and a slightly roughened appearance to the surface.

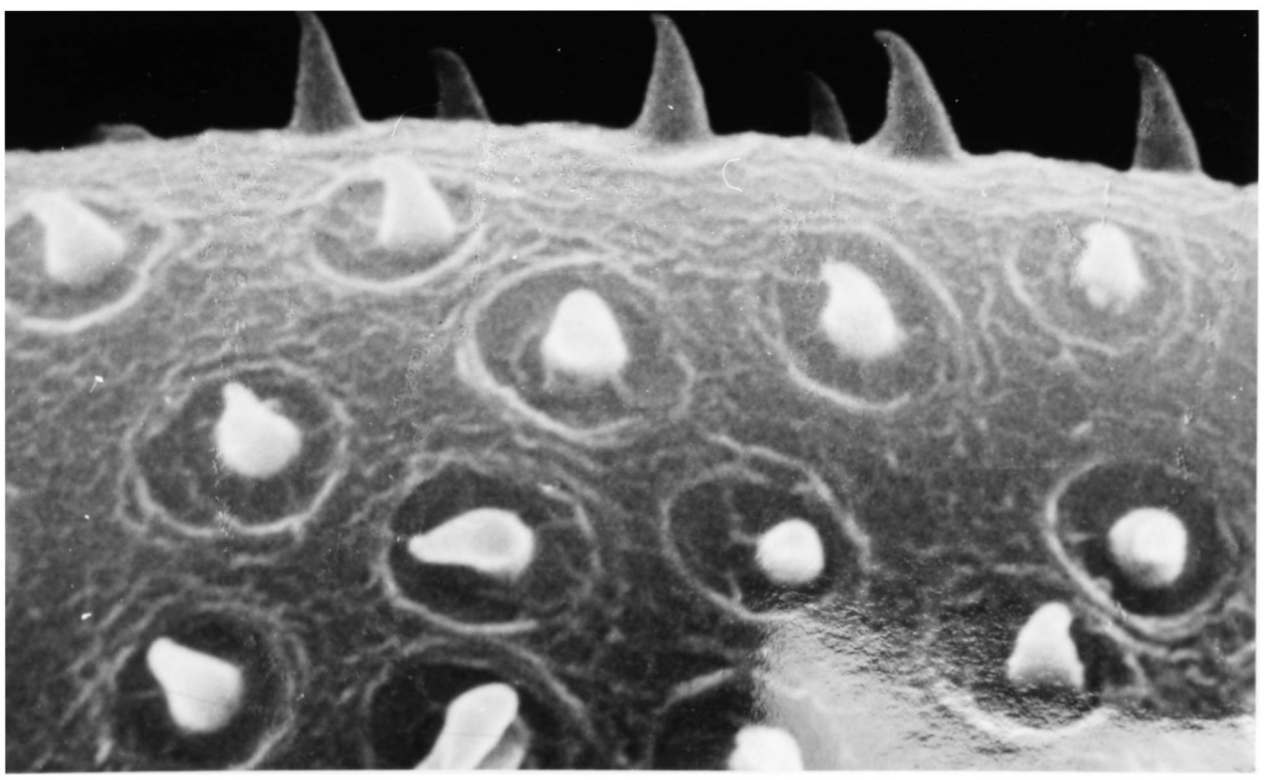
Bar = 10 μm

Figure 2.13
SEM of the surface of a urediniospore of the CHIVE isolate, showing spines with slightly curved tips, with distinct depressions surrounded by low annuli at the bases, with a wrinkly appearance to the surface between the depressions.

Bar = 1 μm







wrinkled. The spine spacing on the BABNT spore appears to be more regular than the others. Germ pores are not visible on any of these structures. Figure 2.13 shows a close up of the spines of the CHIVE isolate. The circular depressions around the spines are very clear, and there are very subtle surface markings outside the depressions. The spines are very uniform in shape, and are slightly curved at the apices.

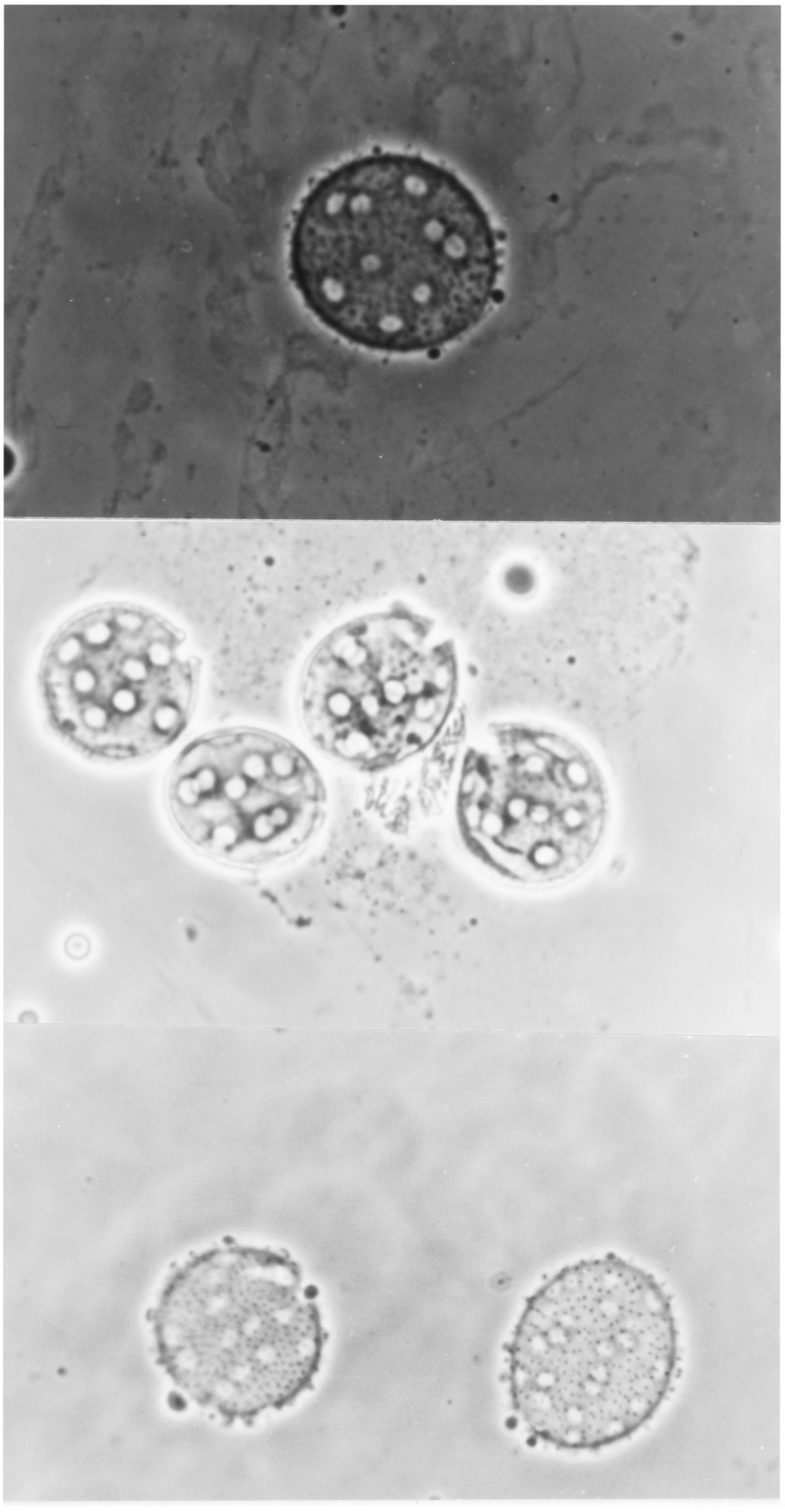
c) Germ Pore Morphology

i) Aniline Blue Staining with Phase Contrast Microscopy

Figures 2.14 to 2.16 show representative urediniospores from the STOCK, CHIVE and BABNT isolates, prepared to show the germ pores. In all the isolates the germ pores are clearly visible both full on and in profile. The spines are also clearly visible on the wall surface. The most noticeable difference between the isolates is the greater number of smaller germ pores on the BABNT isolate (fig. 2.16) compared with the STOCK and CHIVE isolates, (figs. 2.14 & 2.15). Germ pore number varies from 9 in the STOCK isolate (fig. 2.12), 10 and 11 in the CHIVE isolate (fig. 2.15), and 15 and 20 in the BABNT isolate (fig. 2.16).

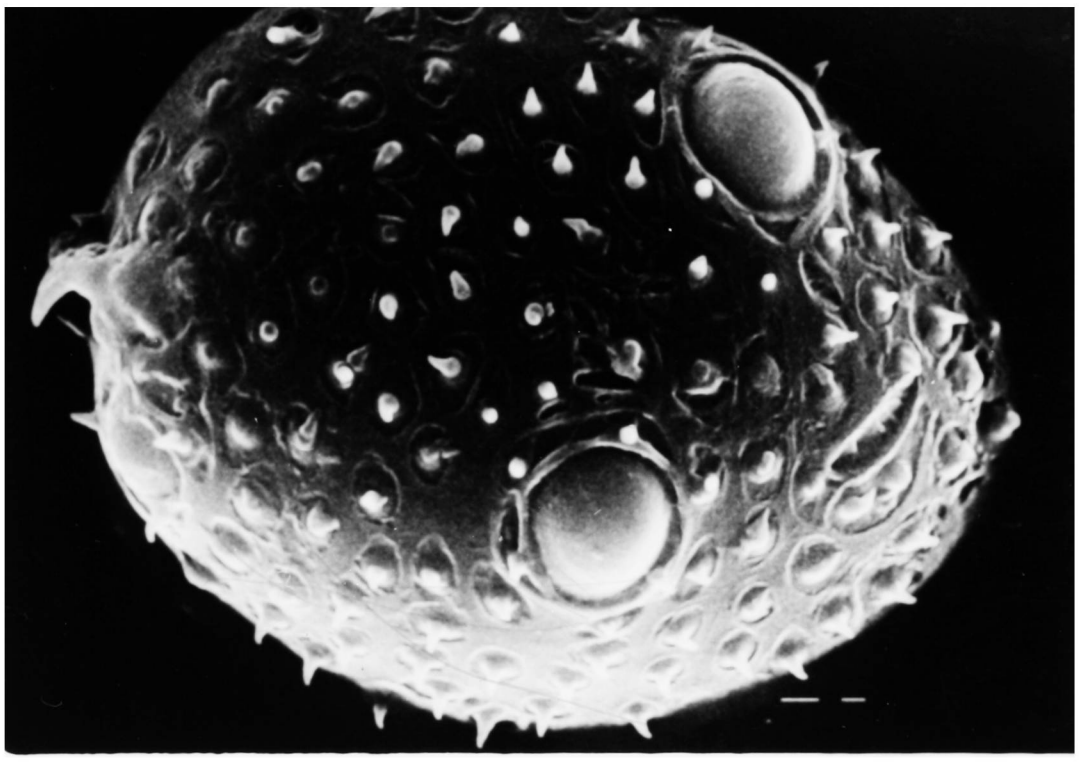
ii) Scanning Electron Microscopy

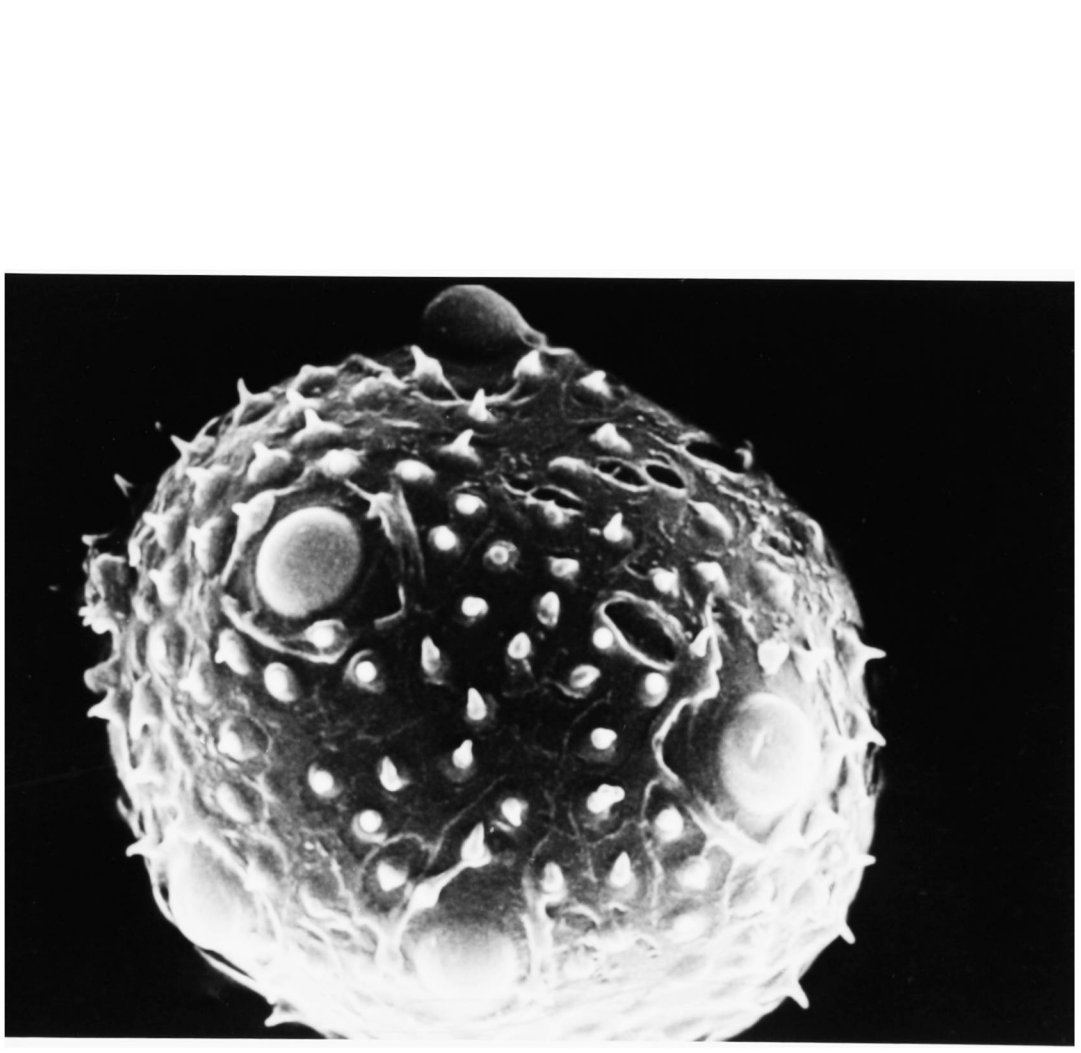
Figures 2.17 and 2.18 show urediniospores with multiple swelling of germ pores. In both cases the protuberance from the germ pore has



Bars = 50 μm

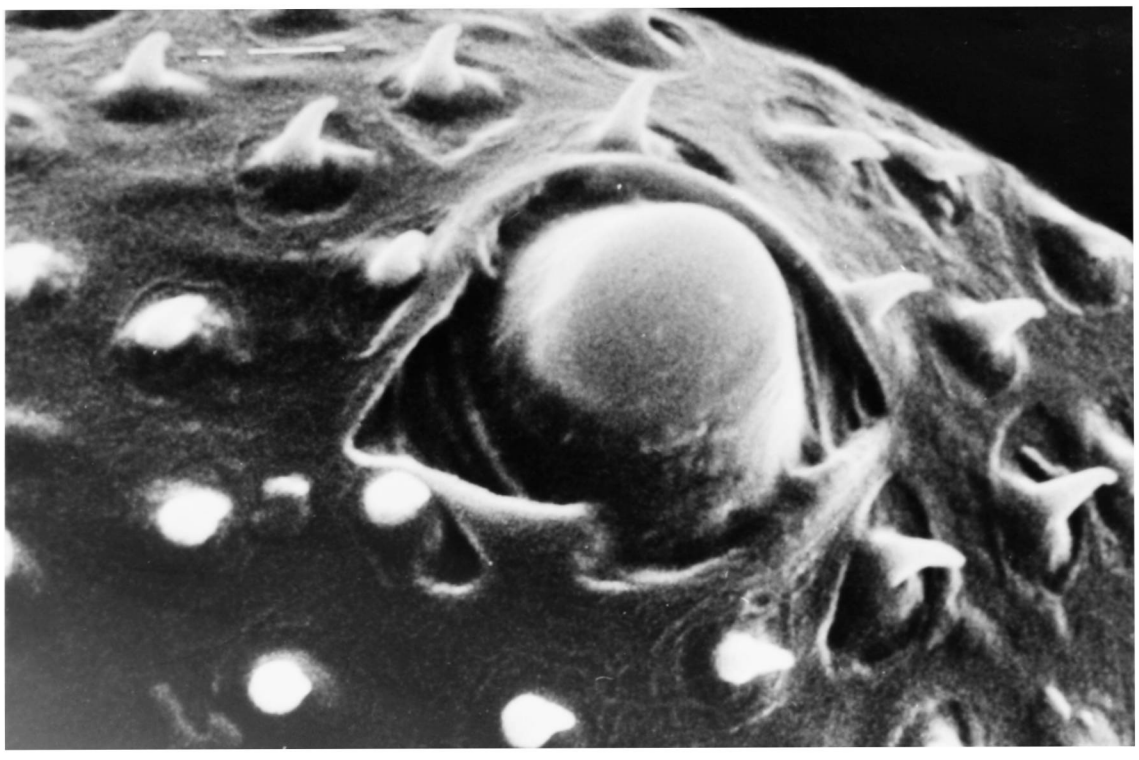
Figures 2.14 to 2.16
Phase contrast micrographs of urediniospores stained in aniline blue and squashed to show germ pores . Figure 2.14; spore of the STOCK leek rust isolate, with 9 pores visible, all full on. Figure 2.15; four spores of the CHIVE isolate, with between 10 and 11 germ pores visible, in profile and full on. Figure 2.16; two urediniospores of the BABNT isolate, showing 15 (left) and 20 (right) germ pores respectively.





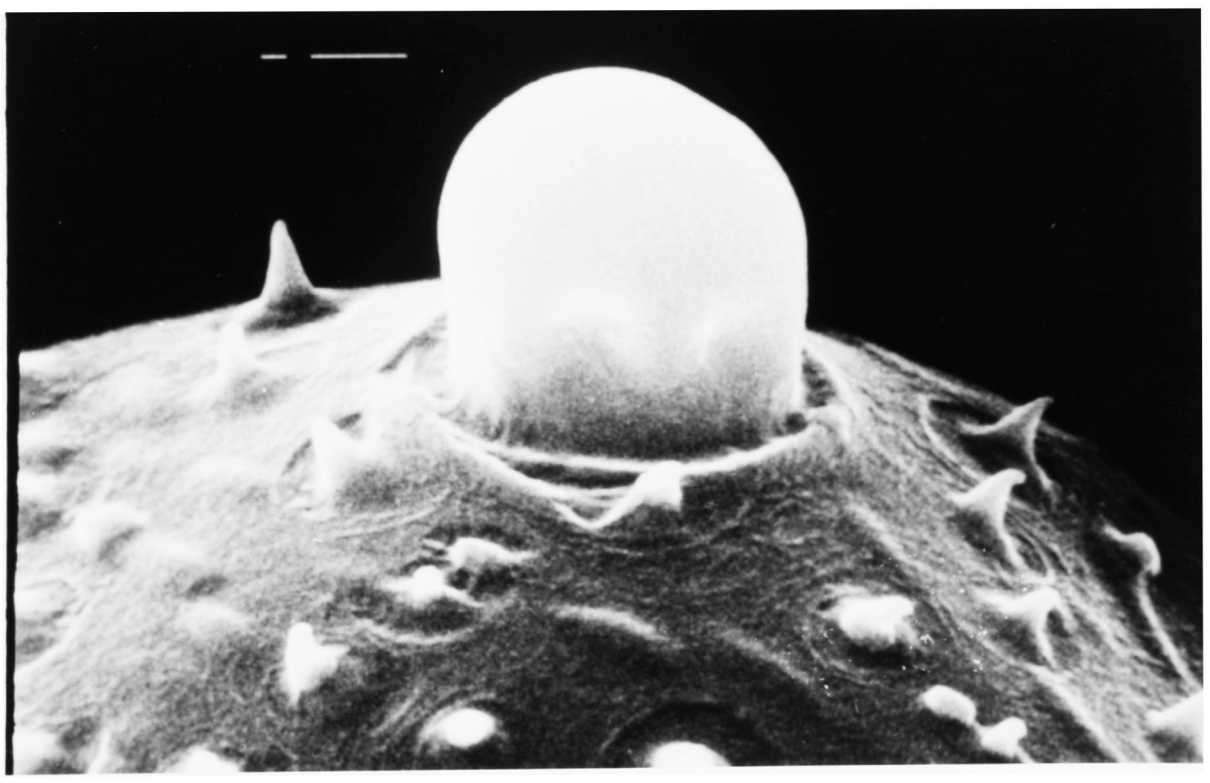
Bars = 10 μm

Figures 2.17 and 2.18
SEMs of urediniospores of the BIRM isolate, showing swollen germ pores pushing through the outer wall, and very pronounced depressions at the base of the spines, (after imbibing treatment).



bar = 1 μm

Figure 2.19
SEM of a urediniospore of the BIRM isolate showing germ tube emergence in greater detail, with the pronounced tear in the outer wall.



Bar = 1 μm

Figure 2.20
SEM of a later stage of germ tube emergence from a urediniospore of the BIRM isolate, showing the slightly swollen tip of the germ tube.

'peeled back' the outer wall and displaced the spines from the area of the wall directly above the pore. The protuberances in fig. 2.17 are larger relative to the size of the spore than those in fig. 2.18. In figure 2.18 the protuberances appear to be continuous with a layer beneath the outer wall (in which the spines are embedded). In both cases the depressions around the spines have become more pronounced than those in ungerminated spores, with the base of the spines sometimes forming a raised area in the centre of the depression. Figure 2.19 shows an early stage of germ tube emergence in more detail. The germ tube has ruptured the outer wall layer, and a continuous layer beneath is visible. Figure 2.20 shows a more advanced germ tube, with a distinctly swollen tip growing out from the spore, and again the outer wall has the appearance of being ruptured.

iii) Fluorescence micrography

In general the hilar region region fluoresced in both metabolically active and inactive spores. In freshly imbibed spores, fluorescence was seen on the interior of the spores, either from the protoplasm or an inner wall layer, but in the later stages of imbibition the fluorescence appeared more concentrated on the germ pore regions, and finally in the germ tubes and other, less well developed, external protuberances. The outer wall layer (i.e. in the thickness of the wall) did not fluoresce.

Figure 2.21 shows spores fixed from a fresh unincubated sample. Bright fluorescence is visible only at the hilar region as a conically-shaped fleck, with less bright fluorescence inside the outer wall layers, from an inner wall layer or protoplasm. Figure 2.22 shows spores after incubation with the fluorescence concentrated in the germ pore regions, which can be seen both full on and in profile. The paler fluorescence inside the spores varies from a very even homogenous appearance to a more heterogenous granular appearance. Figures 2.23a and 2.23b show the transmitted light and fluorescence micrographs respectively of a group of spores, one of which has germinated. The germinating spore has produced two protuberances, only one of which has expanded to form a tube. In the ungerminated spores the contents or inner walls fluoresce weakly, with an area of bright fluorescence which corresponds to the hilar region in the light micrograph. Comparison of the two photographs makes evident the lack of fluorescence in the main wall. Figure 2.24 shows two spores germinating. The germ tubes are fluorescing as well as the remains of 'activated' but non-functioning germ pores. The bright area in the upper spore is the hilar area. Figures 2.25a and 2.25b show a light micrograph and fluorescence respectively of a spore which has produced two protuberances, one of which appears to be extending to form a germ tube. The smaller protuberance has grown beyond the outer wall but has failed to develop further. It is again clear from figs. 2.25a and 2.25b that the main thickness of the wall does not fluoresce. Figure 2.26 shows a spore where several germ pores have produced protuberances but none has produced a germ tube. The protuberances are clearly continuous with the interior of the spore

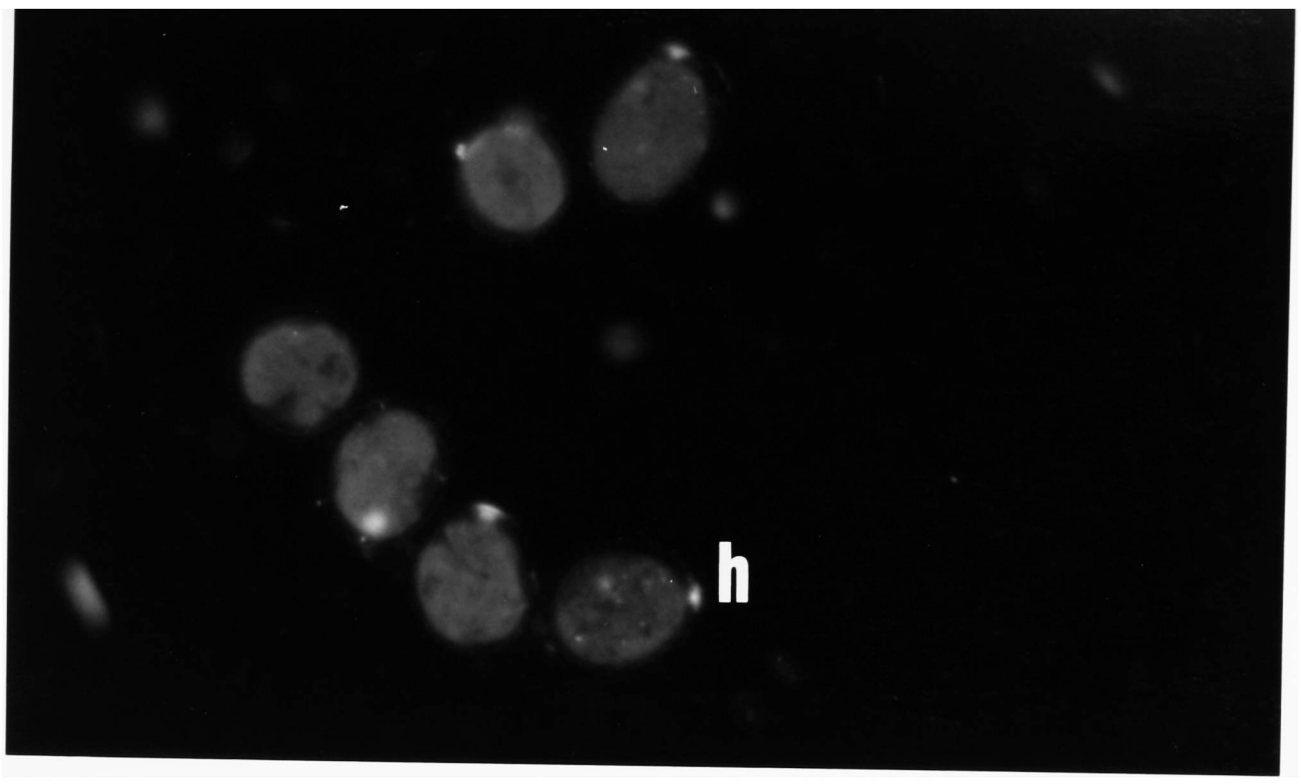


Figure 2.21
Fluorescence micrographs of unincubated spores of the BIRM isolate, showing fluorescence at the hilar region (H) and paler fluorescence which appears to be inside the wall.

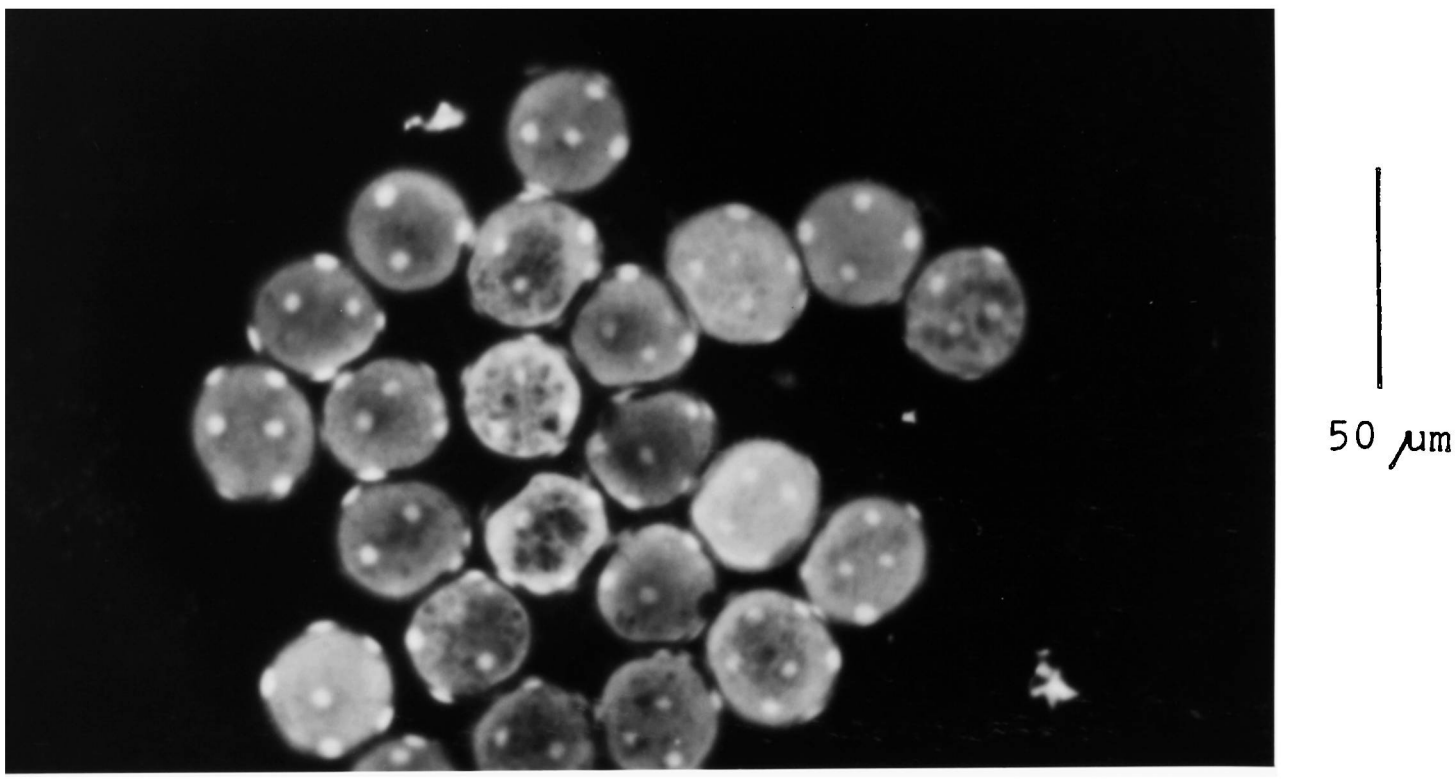
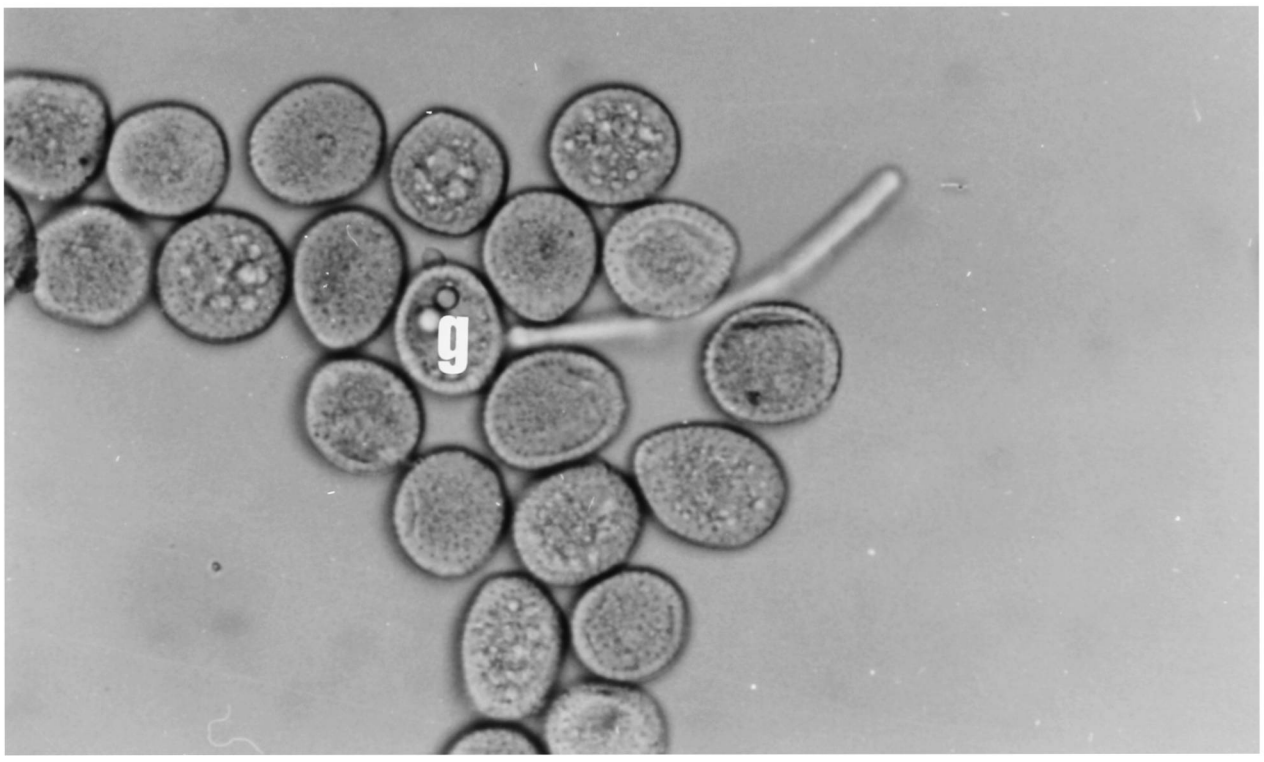
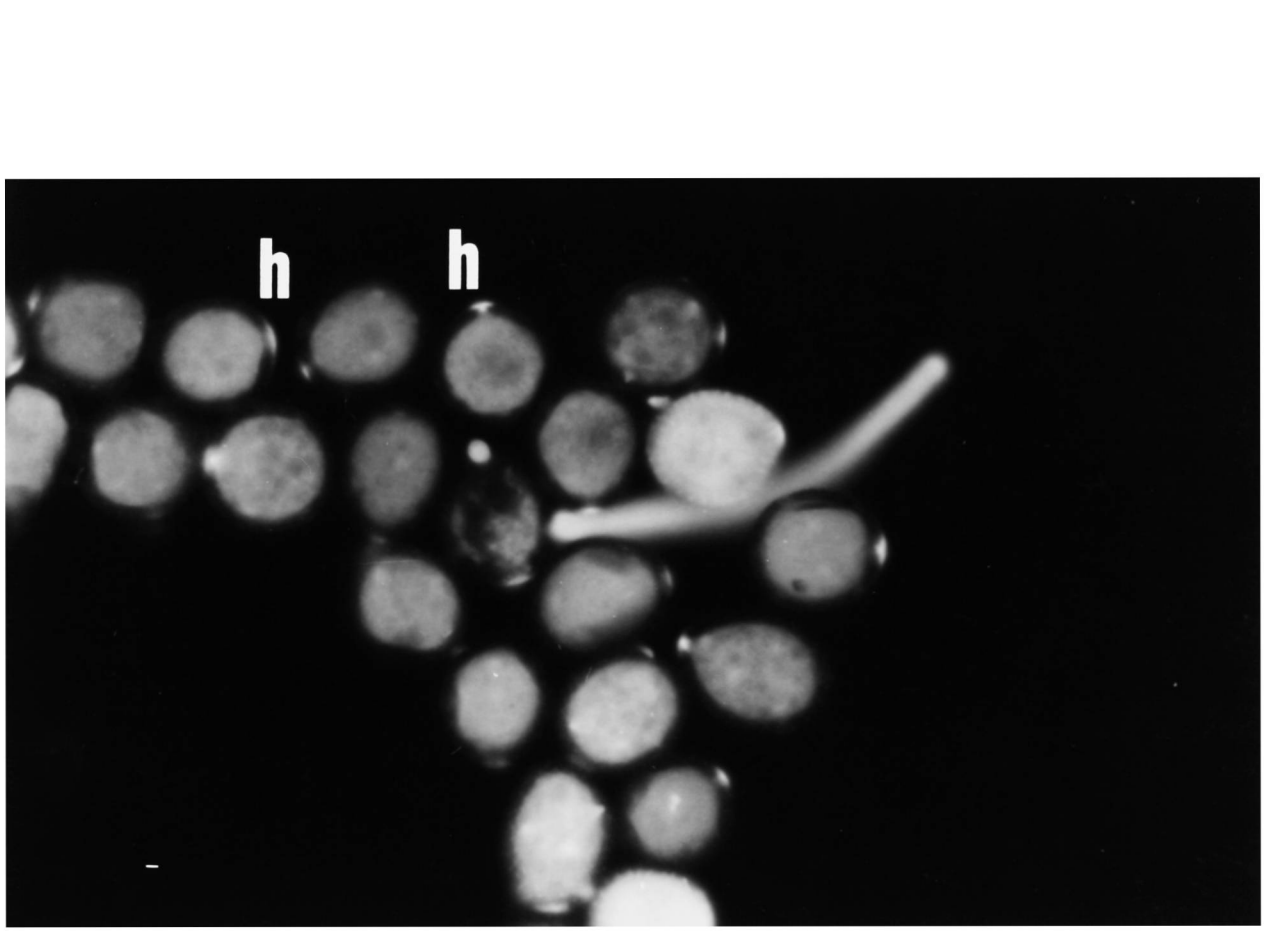


Figure 2.22
Flourescence micrograph of incubated but ungerminated spores of the BIRM isolate, showing intense fluorescence from the regions around the germ pores.





50 µm

Figure 2.23a and 2.23b
Light micrograph and fluorescence micrographs respectively of incubated spores of the BIRM isolate, with one spore germinating (G). Note the fluorescence near the hilar region (H) of the spores, and the lack of fluorescence in the thickness of the walls.

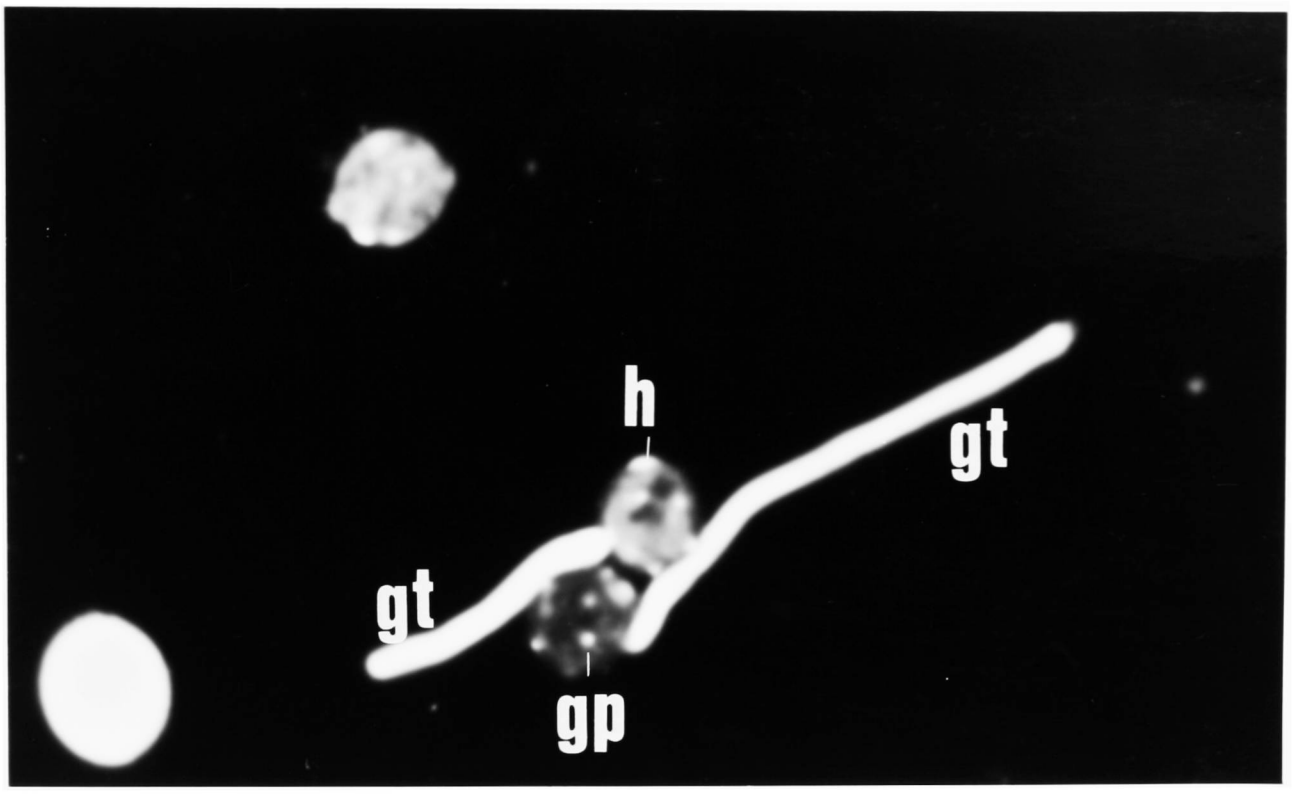


Figure 2.24
Fluorescence micrograph of two spores of the BIRM isolate germinating, showing intense flourescence of the germ tubes (GT) and 'points' of fluorescence at the site of germ pores (GP) which have not produced germ tubes, and at the hilar region (H).

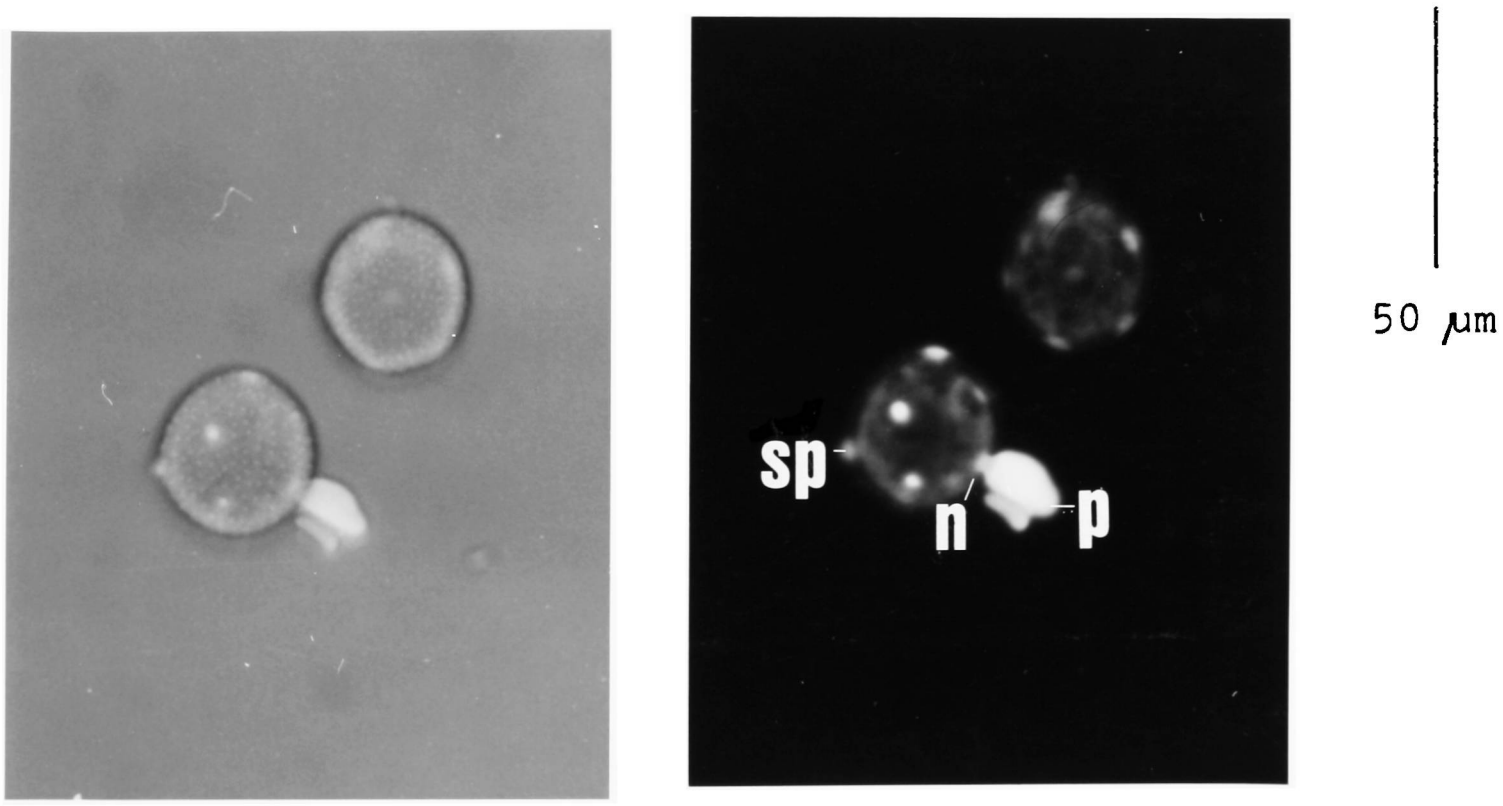


Figure 2.25a and 2.25b
Light and fluorescence micrographs respectively of two spores one which has germinated, showing brightly fluorescing protuberance (P) with a 'neck' (N) in the non-fluorescing wall, and smaller protuberance (SP) developing through the wall.

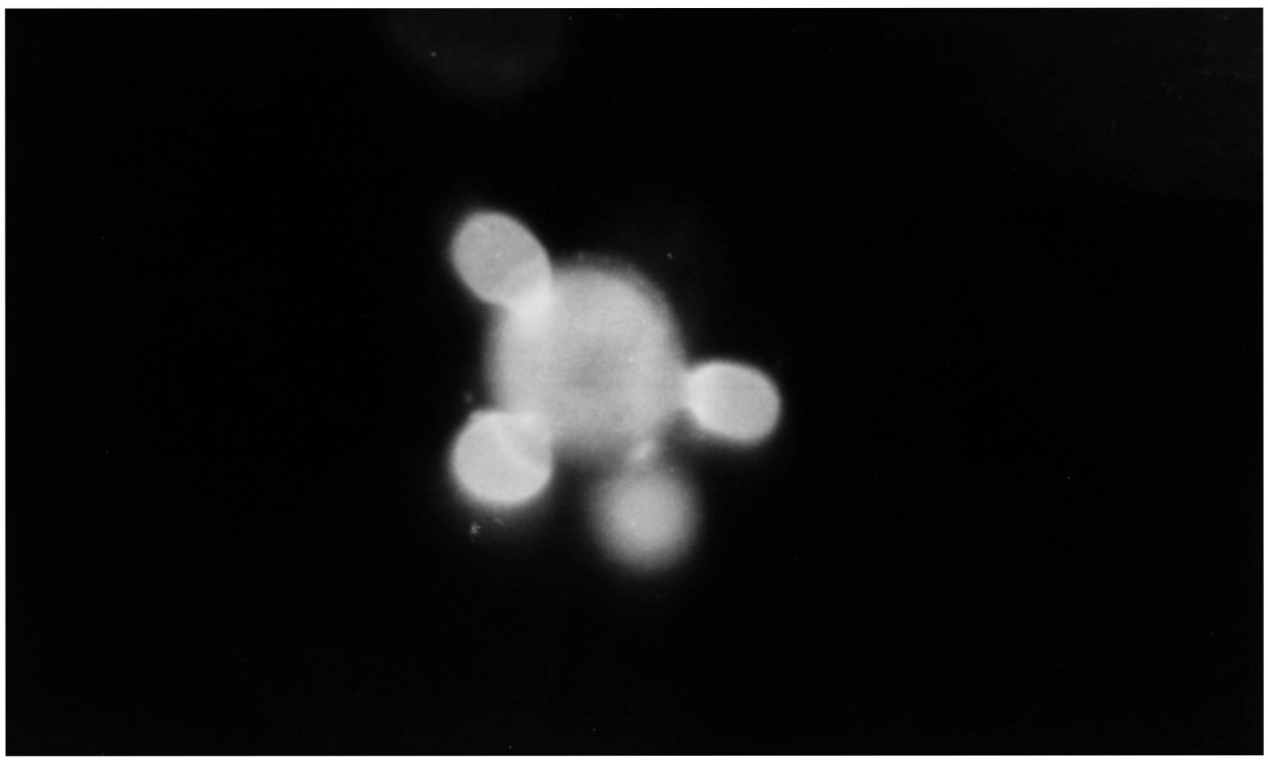


Figure 2.26
Fluorescence micrograph of a spore which has produced multiple protuberances, showing that the protuberance is continuous with the interior of the spore and passes through the wall.

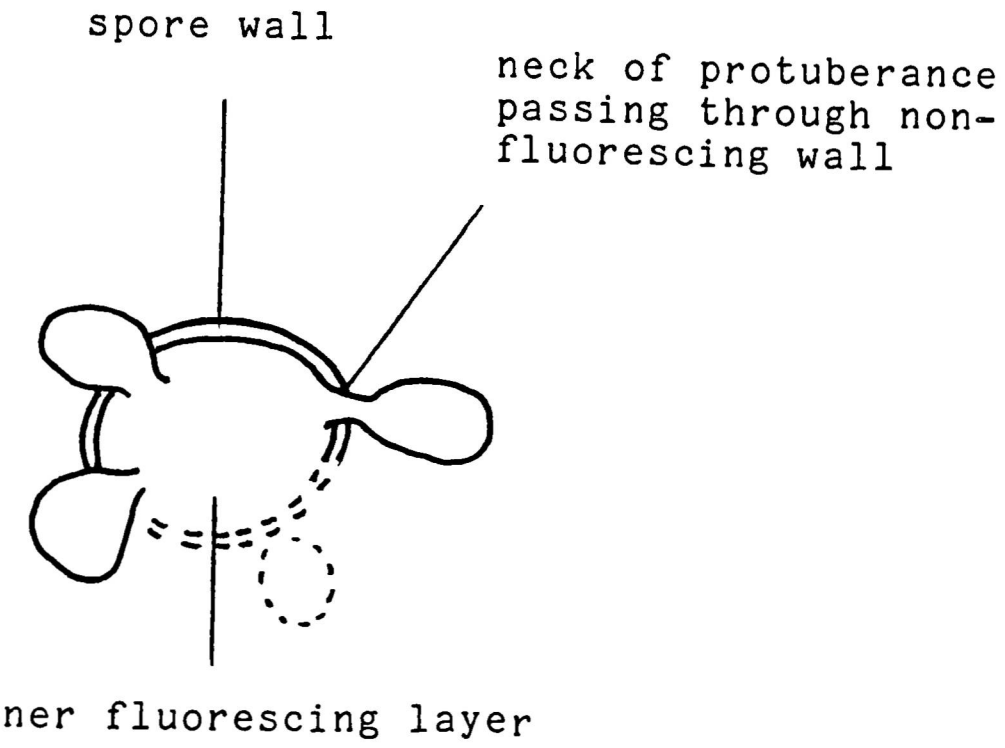


Figure 2.26(a)
Diagram to illustrate the main features visible in figure 2.26.

and pass through the outer wall. In some spores such protuberances could be seen to have collapsed where a germ tube had been formed later.

2.3.1.2. Quantitative Studies on Urediniospores

a) Urediniospore length, width, spine density and pedicel scar diameter

i) Variation in the technique (between slides within pustules)

In general, the spore characters did not vary significantly between slides from the same pustule, or between pustules. The exception was that spore width differed significantly between slides ($P < 0.001$). Table 2.1 shows the means for each character between slides and pustules with the nested analyses of variance for the characters in tables 2.2(a)-(d).

ii) Variation within the BIRM isolate between plants, leaves and pustules

There were no significant differences in any of the characters between spore samples from different plants or leaves within plants. However, spore length, width and spine density varied significantly between pustules ($P < 0.001$). Pedicel scar diameter did not differ significantly between spore samples from different pustules, leaves or

Character						
Length (μm)	Slides	33.00	32.40	32.70	33.75	32.45
		32.35	32.40	32.90	32.80	32.90
	Pustules	32.68	32.40	32.80	32.78	32.68
Width (μm)	Slides	26.95	26.70	27.40	28.35	28.25
		27.15	26.65	26.00	27.05	28.65
	Pustules	27.05	26.68	26.70	27.70	28.45
Spine Density *	Slides	16.60	16.20	17.20	16.10	15.70
		17.30	15.60	15.90	16.20	17.10
	Pustules	16.95	15.90	16.55	16.15	16.40
Pedicel Scar (μm)	Slides	4.50	4.60	4.45	4.50	4.50
		4.70	4.60	4.60	4.80	4.10
	Pustules	4.60	4.60	4.53	4.65	4.30

Table 2.1

Means of spore length, width, spine density and pedicel scar diameter between different pustules and slides within pustules, of urediniospores of the BIRM isolate (ten spores per slide, two slides per pustule).

* Number of spines in 100 μm grid area

Table 2.2

Summaries of nested analyses of variance of selected urediniospore characters, ten spores per slide, two slides per pustule, from the BIRM isolate.

a) Length

Item	d.f.	SS	MS	F	P
Pustules	4	8.218	2.0545	1.311	N.S.
Slides	5	7.837	1.5674	0.741	N.S.
Spores (error)	90	190.425	2.1158	-	-
total	99	206.480	-	-	-

b) Width

Item	d.f.	SS	MS	F	P
Pustules	4	45.890	11.4725	2.217	N.S.
Slides	5	25.870	5.1740	7.020	< 0.001***
Spores (error)	90	66.318	0.7369	-	-
total	99	138.078	-	-	-

c) Spine Density

Item	d.f.	SS	MS	F	P
Pustules	4	12.740	3.185	0.706	N.S.
Slides	5	22.550	4.510	1.274	N.S.
Spores (error)	90	318.500	3.539	-	-
total	99	3353.790	3.539	-	-

d) Pedicel Scar Diameter

Item	d.f.	SS	MS	F	P
Pustules	4	1.485	0.371	1.147	N.S.
Slides	5	1.618	0.324	1.350	N.S.
Spores (error)	90	21.575	0.2397	-	-
total	99	24.678	-	-	-

plants. The means for each character at the various levels are given in table 2.3 to 2.6 with the nested analyses of variance in 2.3(a) to 2.6(a).

iii) Comparison of fresh and herbarium material

There were no significant differences between spores from fresh and herbarium samples, in any of the characters (table 2.7).

iv) Comparison of different isolates

The data for all the characters were normally distributed, with the exception of pedicel scar diameter where the class number was reduced by the limits of accuracy of measuring the diameter.

Length

There were large and significant differences between the isolates ($P < 0.01$, see figure 2.27). The mean lengths of the CHIVE and KERR isolates were similar, and less than the leek isolates and 6504. There was a large amount of variation among the leek isolates as a whole, with the CORN and STOCK isolates differing significantly ($P < 0.05$).

Width

There were large differences between several of the leek isolates ($P < 0.01$); the BABNT isolate was only marginally smaller than the smallest leek isolate WSCOT and the KERR and 6504 isolates were within

Table 2.3

Means of spore length from different pustules, within and between leaves, within plants, infected with the BIRM isolate on A.porrum cv. Musselburgh

Partition	Mean spore length (μm)			
Pustules	32.95	32.40	32.10	32.65
	33.05	32.00	32.10	32.65
	33.10	30.95	34.05	34.05
	34.20	32.95	32.05	32.30
	32.00	32.20	33.20	34.15
	32.50	32.20	34.50	32.10
	32.80	32.70	35.60	33.15
	31.80	31.45	32.90	34.00
	32.90	31.50	33.95	33.25
	32.55	33.90	32.60	32.50
Leaves	32.79	32.23	33.31	33.08
Plants	32.51		33.19	
Total	32.85			

Table 2.3(a)

Summary of nested analysis of variance of spore lengths from pustules within and between leaves and leaves within plants (data in table 2.3)

Item	d.f.	SS	MS	F	P
Plants	1	14.42	14.42	1.584	N.S.
Leaves	2	18.21	9.10	1.154	N.S.
Pustules	36	283.85	7.89	2.737	< 0.001***
Spores (error)	360	1037.00	2.89	-	-

Table 2.4

Means of spore width from different pustules, within and between leaves, within plants, infected with the BIRM isolate on A.porrum cv. Musselburgh

Partition	Mean spore width (μm)			
Pustules	26.10	26.00	25.20	25.45
	26.10	26.30	25.40	26.95
	25.60	25.80	27.50	26.30
	26.00	26.10	25.50	25.50
	27.30	25.35	24.50	25.90
	25.30	25.90	26.70	25.40
	26.45	27.00	26.30	25.75
	25.70	25.80	26.05	27.70
	25.20	26.10	26.50	26.65
	25.95	26.95	27.75	26.00
Leaves	25.97	26.13	26.17	26.16
Plants	26.05		26.16	
Total	26.11			

Table 2.4(a)

Summary of nested analysis of variance of spore widths from pustules within and between leaves and leaves within plants (data in table 2.4)

Item	d.f.	SS	MS	F	P
Plants	1	1.27	1.27	1.98	N.S.
Leaves	2	1.28	0.64	0.14	N.S.
Pustules	36	166.69	4.63	4.20	<0.001***
Spores (error)	360	397.25	1.10	-	-

Table 2.5

Means of spine density for spores from different pustules, within and between leaves, within plants, infected with the BIRM isolate on Allium porrum cv. Musselburgh

Partition	. Mean spine density ($100\mu m^{-2}$)			
Pustules	14.4	15.6	15.4	17.3
	15.4	16.0	16.3	15.5
	15.8	16.4	16.0	16.8
	15.8	16.4	16.3	15.1
	16.2	16.5	16.3	15.7
	16.8	16.7	17.3	15.8
	15.3	16.4	16.0	14.3
	17.2	16.3	15.8	16.0
	15.5	17.1	17.5	16.0
	16.5	16.7	18.8	17.2
Leaves	15.9	16.4	16.6	16.0
Plants	16.1		16.3	
Total	16.2			

Table 2.5(a)

Summary of nested analysis of variance of spine density from spores within pustules within and between leaves and leaves within plants (data in table 2.5)

Item	d.f.	SS	MS	F	P
Plants	1	1.82	1.82	0.21	N.S.
Leaves	2	30.00	15.00	2.20	N.S.
Pustules	36	246.07	6.84	1.71	<0.001***
Spores (error)	360	1437.10	3.99	-	-

Table 2.6

Means of pedicel scar diameter of spores within pustules within and between leaves, within plants, infected with the BIRM isolate on Allium porrum cv. Musselburgh.

Partition	Mean Pedicel Scar Diameter (µm)			
Pustules	4.80	4.75	4.70	5.00
	4.80	4.70	4.75	4.80
	4.70	4.85	4.50	4.70
	4.80	4.85	4.70	4.80
	4.30	4.75	4.90	4.80
	4.80	4.90	4.70	4.70
	5.00	4.55	4.80	4.80
	4.70	4.80	4.80	4.60
	4.75	4.80	4.70	4.80
	4.70	4.80	4.75	4.80
Leaves	4.74	4.78	4.73	4.78
Plants	4.76		4.76	
Total	4.76			

Table 2.6(a)

Summary of nested analysis of variance of pedicel scar diameter of spores within pustules within and between leaves and leaves within plants (data in table 2.6)

Item	d.f.	SS	MS	F	P
Plants	1	0.00	0.00	-	-
Leaves	2	0.21	0.11	0.66	N.S.
Pustules	36	5.59	0.16	0.68	N.S.
Spores (error)	360	82.20	0.23	-	-

Table 2.7

Means of Urediniospore length, width, spine density (SD), and pedicel scar diameter (PSD) for fresh and herbarium spore samples of the BIRM isolate

Material	Length (µm)	Width (µm)	SD ($100\mu m^{-2}$)	PSD (µm)
Fresh	32.67	26.27	16.82	4.76
Herbarium	32.72	26.12	16.98	4.78

$p > 0.05$ in all cases

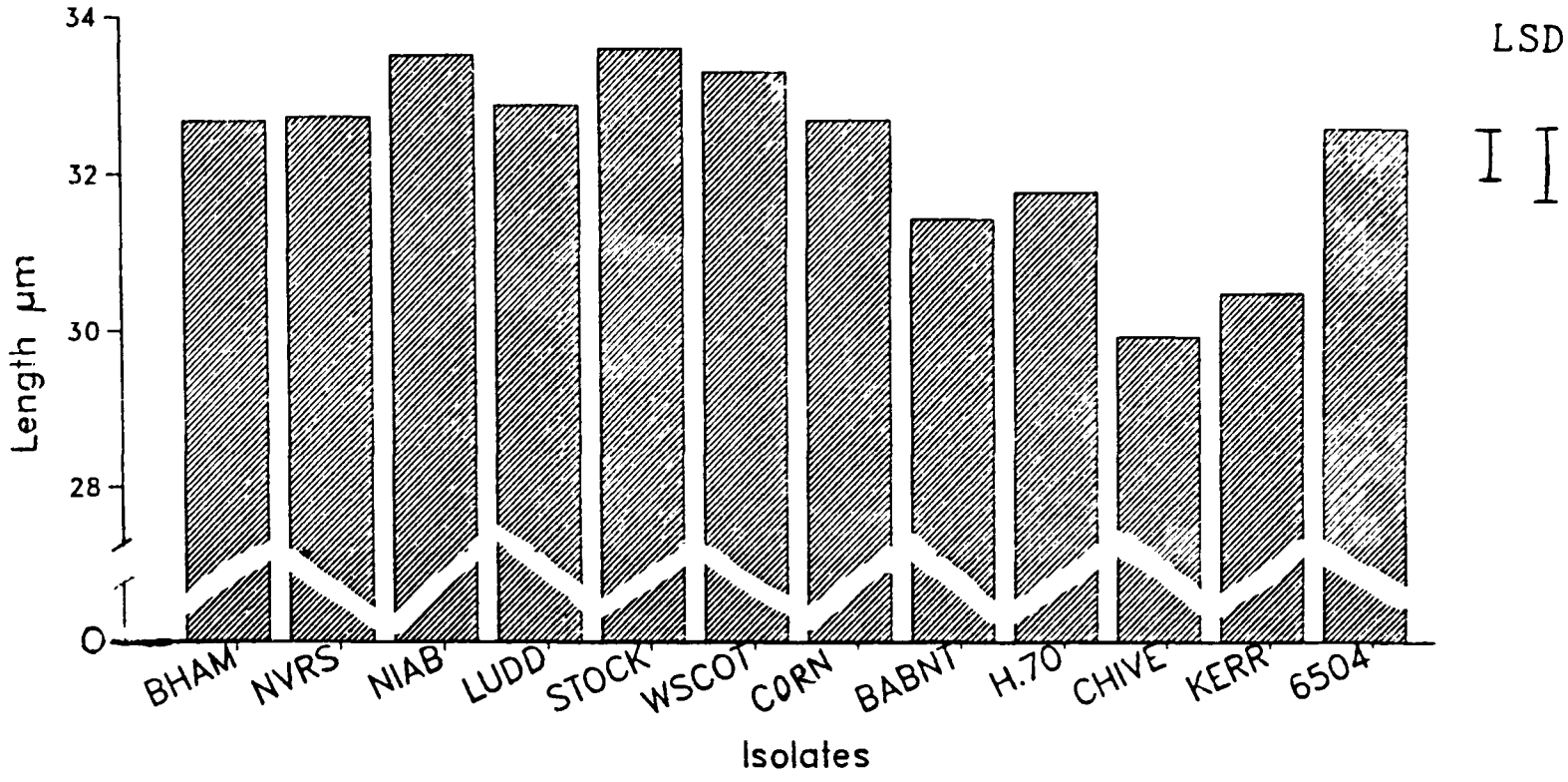


Figure 2.27
Means of urediniospore length for fresh and herbarium isolates from Allium spp.
(50 spores per isolate, mounted in lactic acid).
LSD = least significant differences at P = 0.05 and 0.01

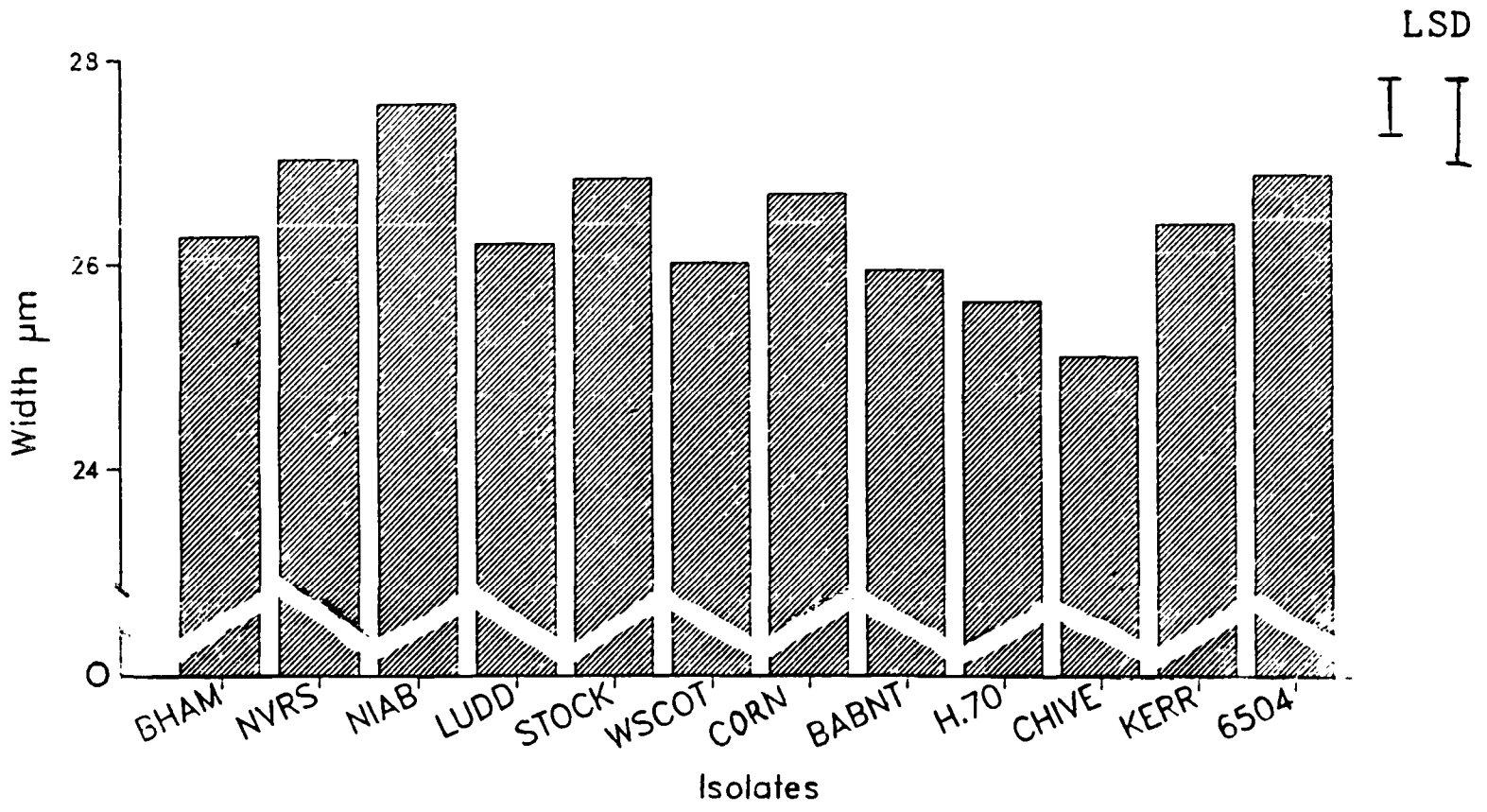


Figure 2.28
Means of urediniospore width for fresh and herbarium isolates from Allium spp.
(50 spores per isolate, mounted in lactic acid)
LSD = least significant differences at P =0.05 and 0.01.

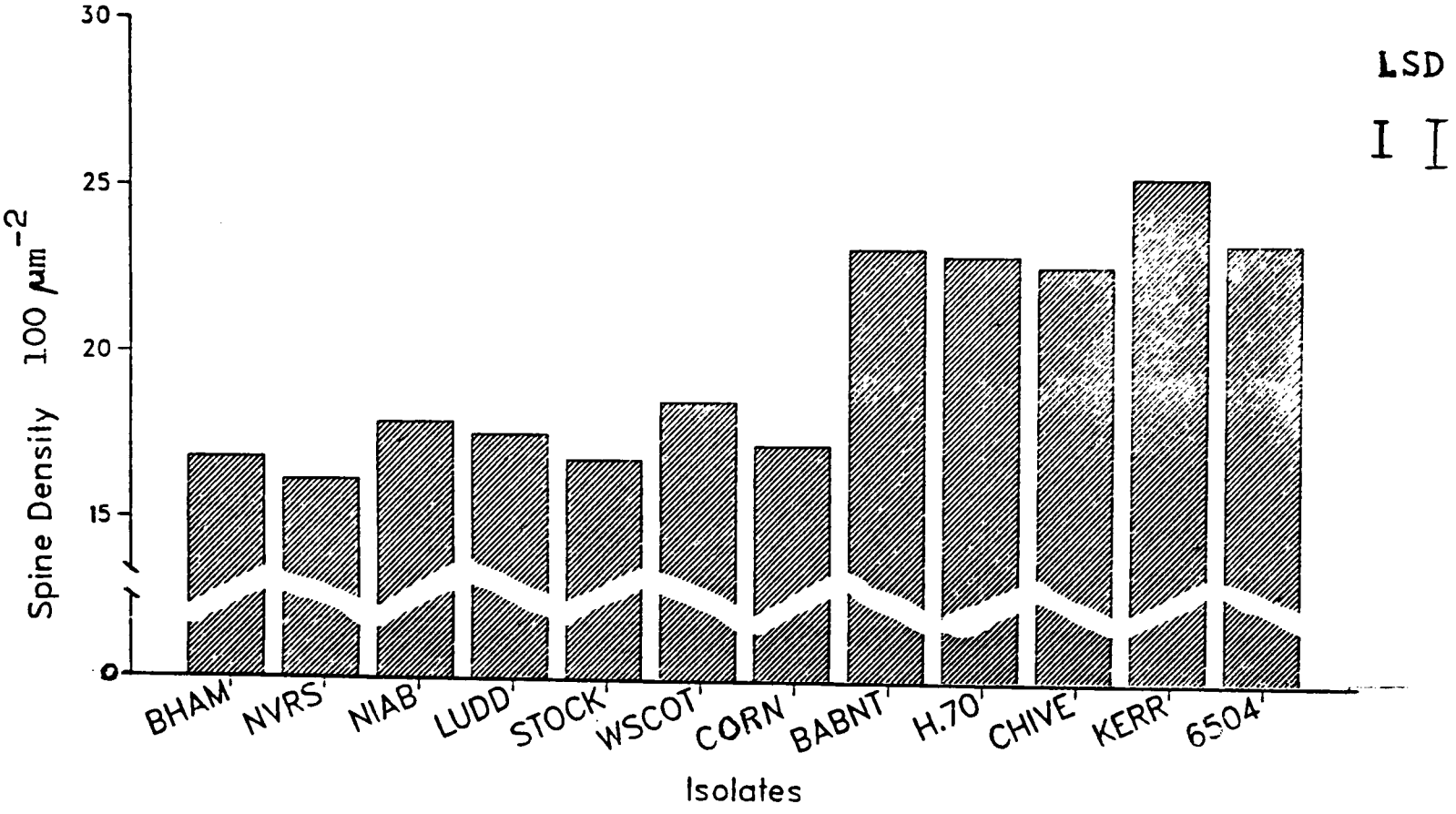


Figure 2.29
Means of spine density on urediniospores from fresh and herbarium isolates from Allium spp.
(50 spores per isolate, mounted in lactic acid).
LSD = least significant differences at P = 0.05 and 0.01.

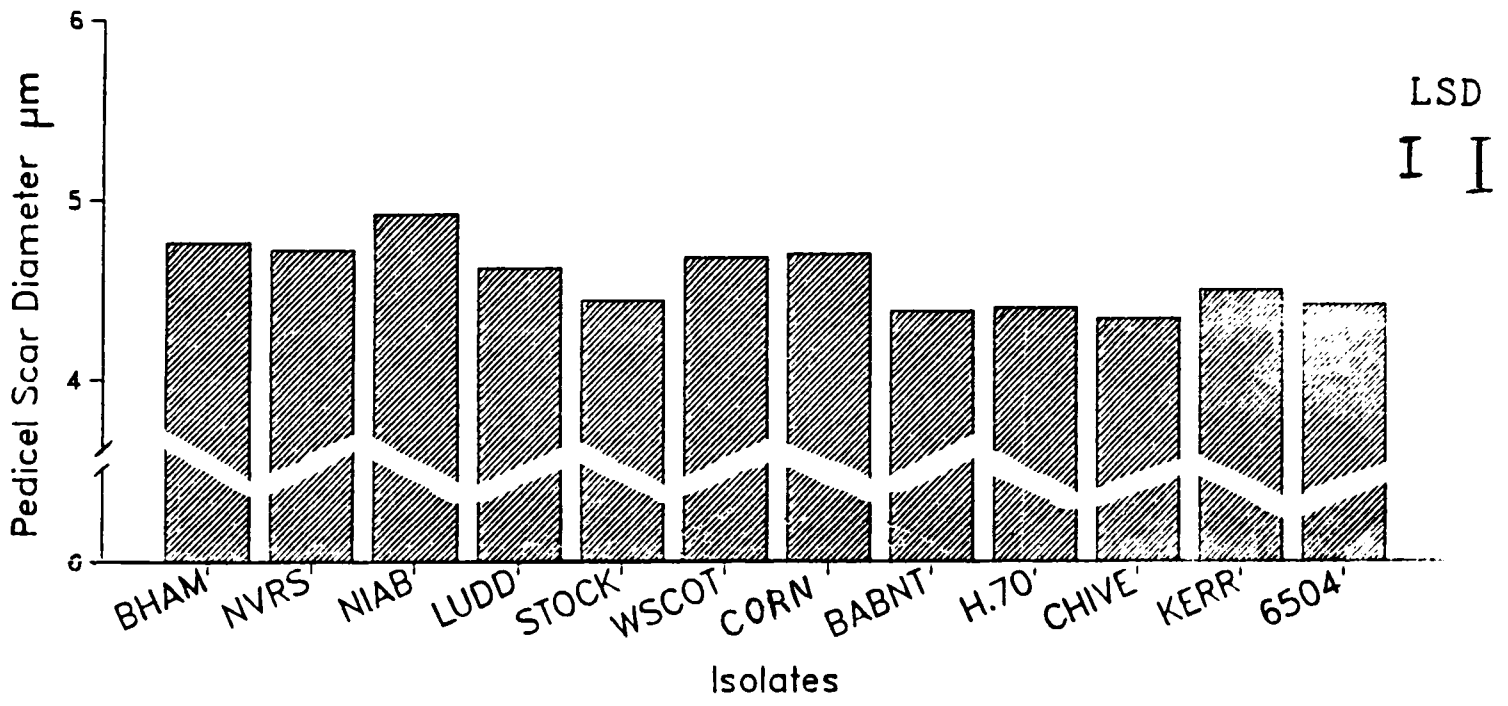


Figure 2.30
Means of urediniospore pedicel scar diameter for fresh and herbarium isolates from Allium spp.
(50 spores per isolate, mounted in lactic acid).
LSD = least significant differences at P = 0.05 and 0.01.

the limits of the leek isolates. Mean spore widths of the CHIVE isolate was also significantly less than the leek isolates (see figure 2.28).

Spine Density

The analysis of spine density separated the isolates into two distinct groups, leek and non-leek, which were very significantly different from each other. The non-leek isolates had considerably higher spine densities than the leek isolates. However there were small but significant differences between some of the leek isolates ($P < 0.01$) and the KERR isolate had a significantly higher spine density than the other non-leek isolates (see figure 2.29)

Pedicel Scar Diameter

In general the non-leek isolates had smaller pedicel scar diameters than the leek isolates (see figure 2.30). The STOCK leek isolate had a smaller mean pedicel scar diameter than the KERR isolate and was similar to the H.70 and 6504 isolates. There were also significant differences between some of the leek isolates (see figure 2.30).

Ratio of spore length to spore width

The KERR isolate had a significantly smaller length/width ratio than the other isolates ($P < 0.01$) but the ratios of the other isolates did not show any pattern which could be attributed to host (see figure 2.30a).

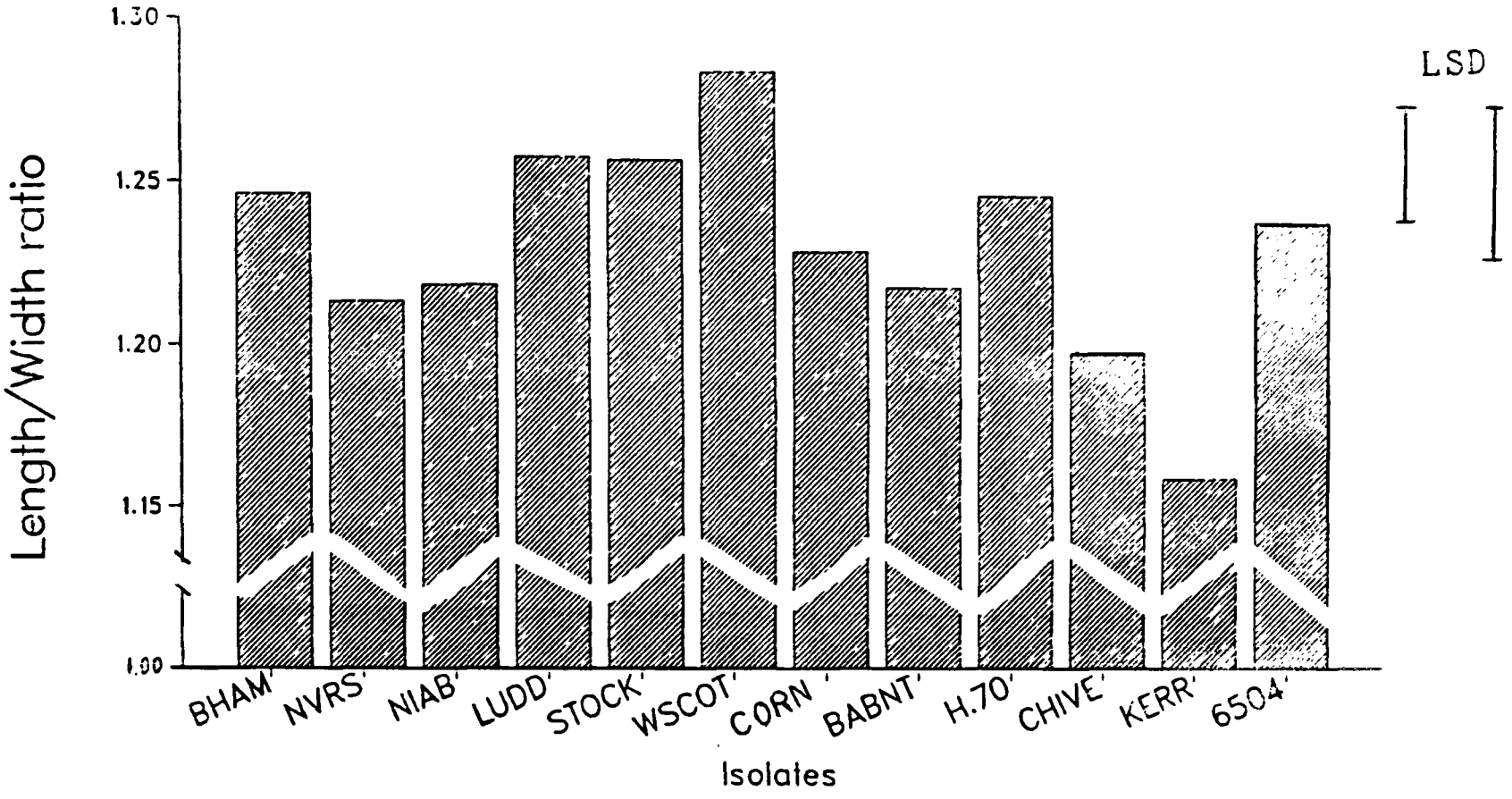


Figure 2.30a
Means of spore length - width ratio for fresh and herbarium isolates from Allium spp. Calculated from data from 50 spores per isolate mounted in lactic acid.
LSD = least significant differences at P = 0.05 and 0.01.

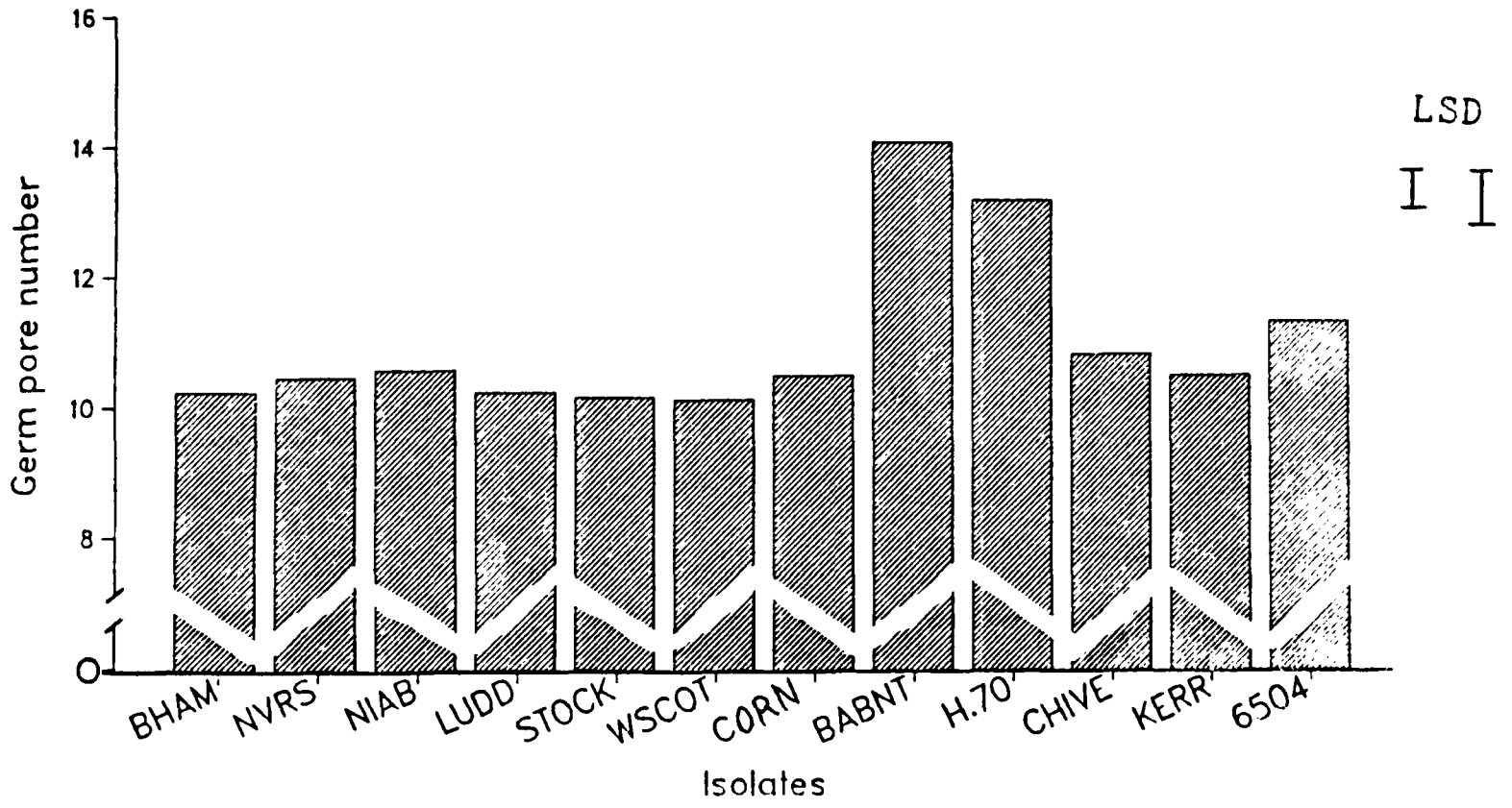


Figure 2.31
Means of germ pore number for fresh and herbarium isolates, from Allium spp. measured on 50 spores per isolate.
LSD = least significant differences at P = 0.05 and 0.01.

b) Germ pore number

Figures 2.14 to 2.16 show the spores prepared for counting germ pore number. The number of germ pores did not differ significantly between leek isolates, the CHIVE or KERR isolates; the 6504 isolate had a small but significantly larger number of germ pores than several of the leek isolates and the KERR isolate, ($P < 0.05$), but not the CHIVE isolate. The BABNT and H.70 isolates however had a very much greater number of germ pores per spore than all the other isolates ($P < 0.01$) and a small but significant difference between each other ($P < 0.05$), see figure 2.31. Although the distribution ranges of germ pore number between the BABNT and for example, the BIRM isolate overlapped, the upper limits were considerably higher for the BABNT isolate.

c) Multivariate Analysis

The percentage variance accounted for by the first principal component was high (table 2.8) and considerably greater than the second component. The Eigenvalues (table 2.8) indicate a similar result with only the first component greater than unity, and therefore the only component of significance according to Kaiser's rules. The correlations between the different variables are given in table 2.9. All the characters were correlated with each other, except germ pore number which was not correlated with any other character. Figure 2.32 shows the first principal component plotted against the second. The first component has broadly split the isolates into 'leek' and 'non-leek' groups. The second component, though of less significance than the first component (accounting for only 15% of the variation)

Table 2.8

Percentage and cumulative variance and Eigenvalues for the individual components of the principal component analysis of mean spore length, width, spine density, pedicel scar diameter and germ pore number of the twelve *Allium* rust isolates

Principal component	1	2	3	4	5
% Variance	68.38	14.24	8.77	6.02	2.59
Cumulative % Variance	68.38	82.62	91.39	97.41	100.00
Eigenvalues	3.42	0.71	0.44	0.30	0.13

Table 2.9

Correlation coefficients (r) between the variables in the multivariate analysis ^

	Width	Germ Pore number	Spine density	Pedicel Scar Diam.
Length	0.692*	-0.363	-0.787**	0.623*
Width		-0.393	-0.584*	0.707*
Germ pore no.			0.569	-0.533
Spine density				-0.727**

* Significant at $p<0.05$
** Significant at $p<0.01$

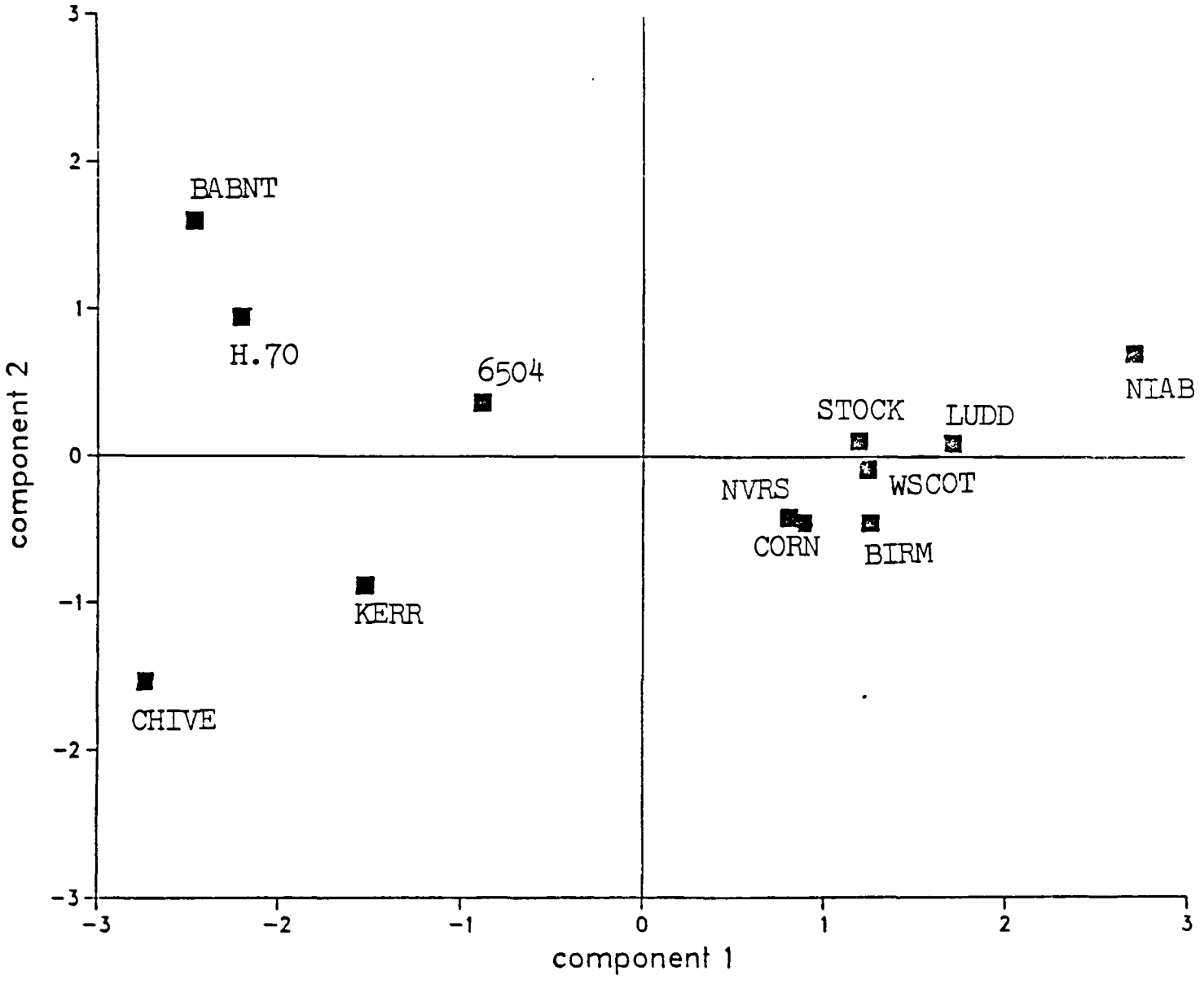


Figure 2.32
Plot of first and second principal components from the multivariate analysis of data from urediniospore isolates from Allium spp.

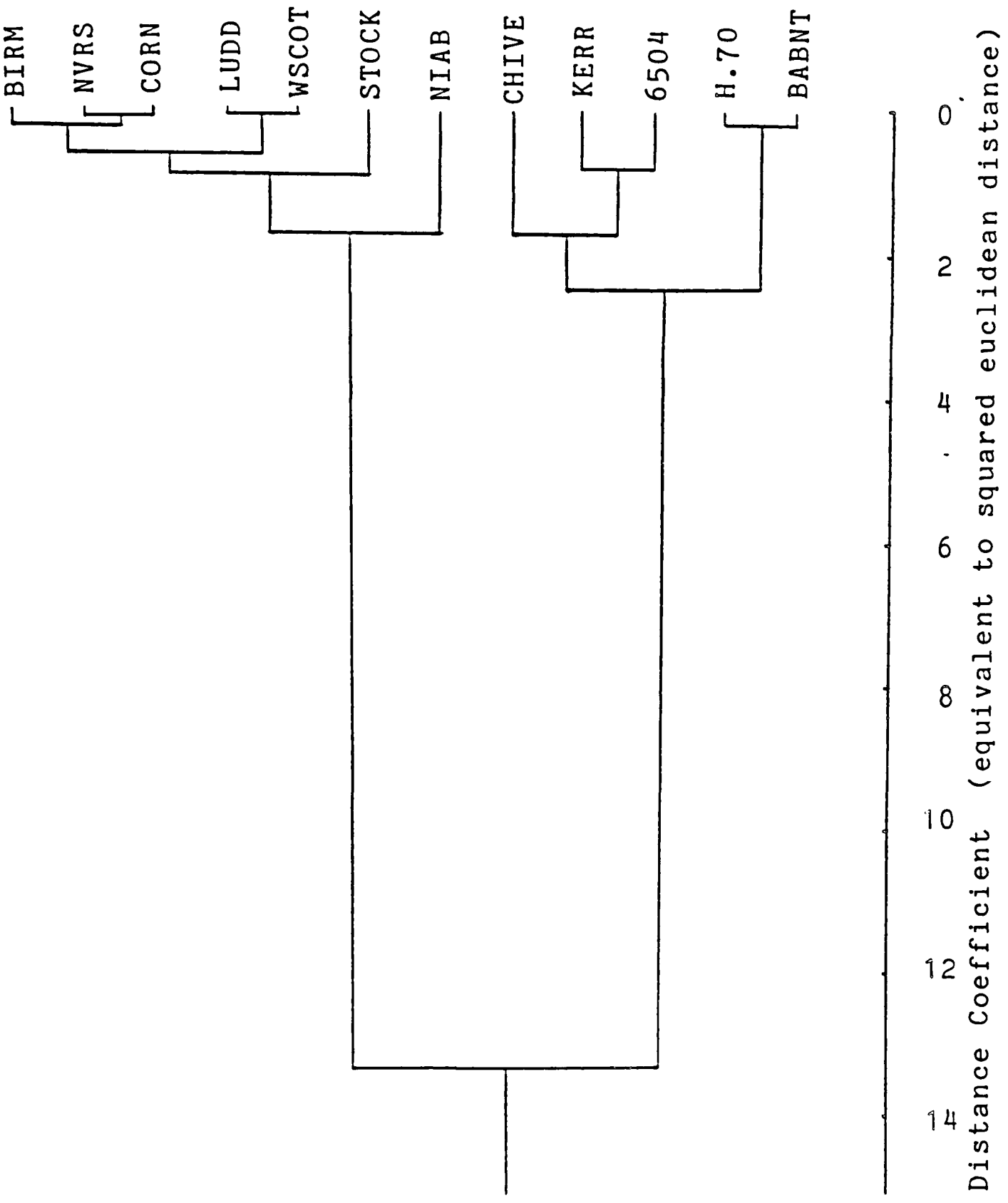


Figure 2.33
Dendrogram of the distance coefficients based on the cluster analysis of the data from the urediniospore isolates from Allium spp.

has split the BABNT and H.70 isolates from the CHIVE isolates, leaving the CHIVE, KERR and 6504 isolates dispersed.

The dendrogram of similarity coefficients based on the cluster analysis (figure 2.33) summarises all the data. The major feature is the very strong split into 'leek' and 'non-leek' groups, with a less strong split between the chive isolates and the strongly-linked BABNT and H.70 isolates. The two herbarium chive isolates are more closely linked to each other than to the fresh CHIVE isolate. The leek isolates are generally closely linked with each other, with the exception of the NIAB isolate.

d) Effect of Temperature on Urediniospore Germination in vitro

Figures 2.34 (a) to (h) show the in vitro percentage germination rates of the isolates over the temperature range from 0°C to 30°C. In general all the leek isolates showed relatively high levels of germination over a temperature range from 5°C to 22.5°C, with the BIRM and CORN isolates having an optimum of 20°C and the LUDD, WSCOT,STOCK and NVRS isolates an optimum of 15°C. The CHIVE isolate showed a distinct optimum at 15°C, with percentage germination falling off sharply above and below this temperature. The BABNT isolate had a less pronounced optimum at 10°C, but a very low germination rate at 25°C. (NIAB isolate not tested as viability too low).

Figures 2.34 (a)-(h)
Plots of percentage germination <u>in vitro</u> over a range of temperatures for the following urediniospore isolates; (a) BIRM (b) NVRS (c) LUDD (d) STOCK (e) CORN (f) WSCOT (g) CHIVE (h) BABNT. Bars give standard errors, except when S.E. =0

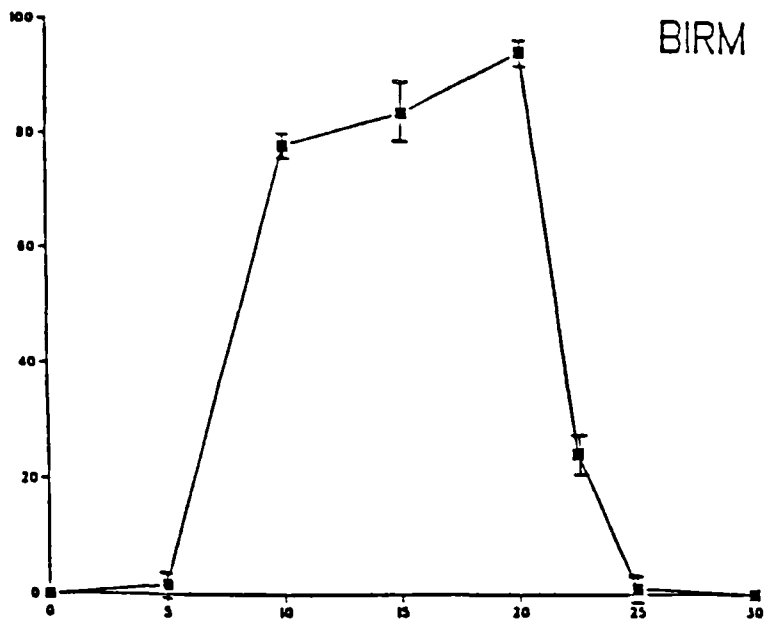


Fig 2.34(a)

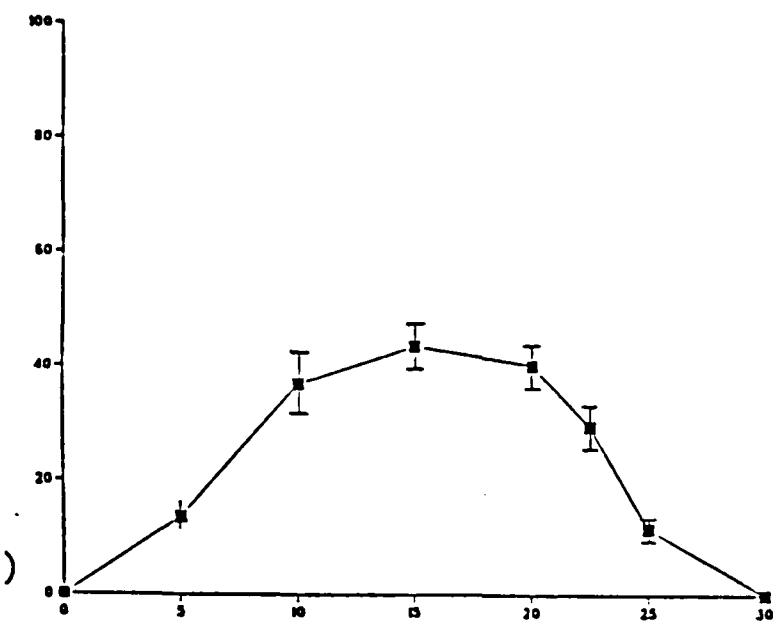


Fig. 2.34(b)

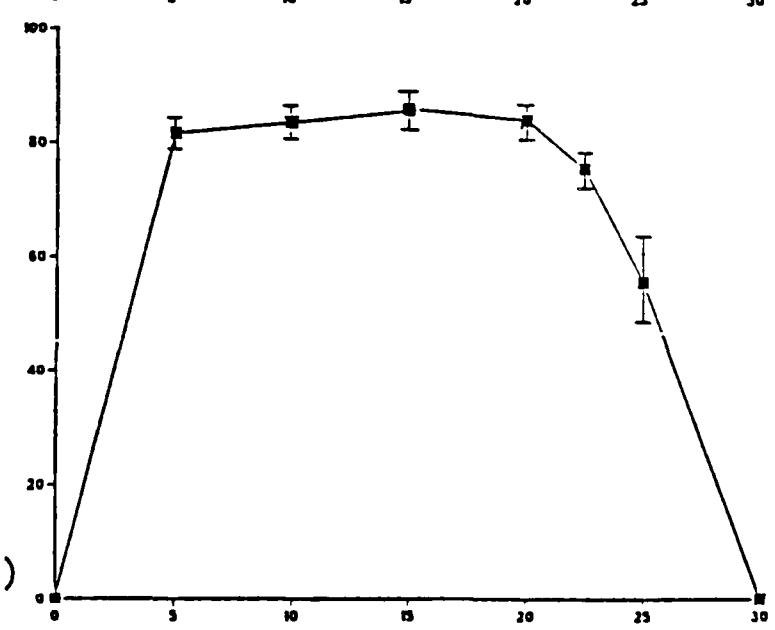


Fig. 2.34(c)

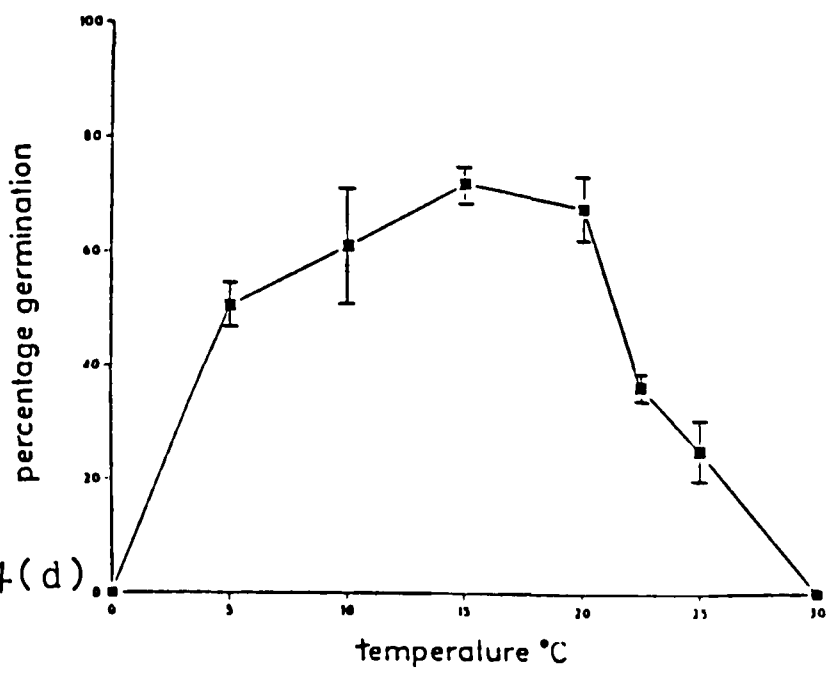


Fig. 2.34(d)

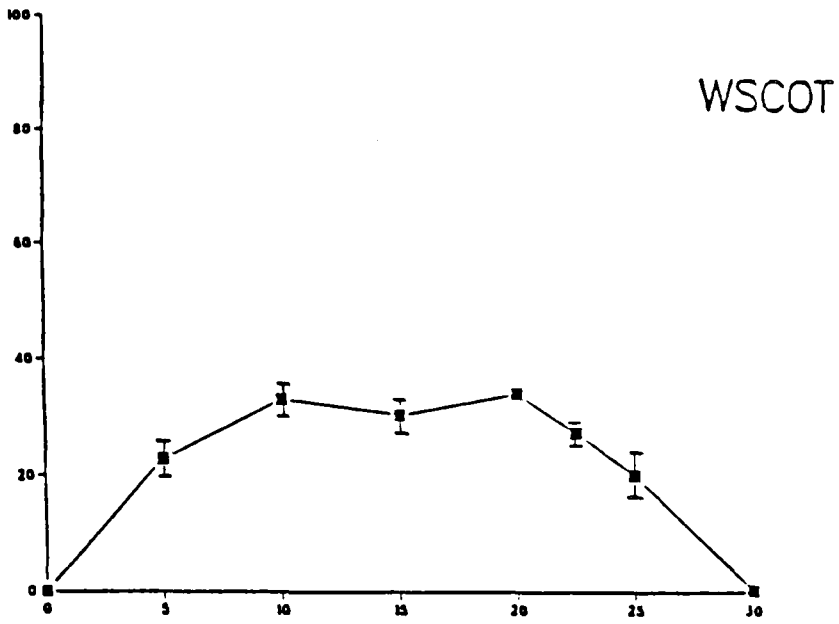


Fig. 2.34(e)

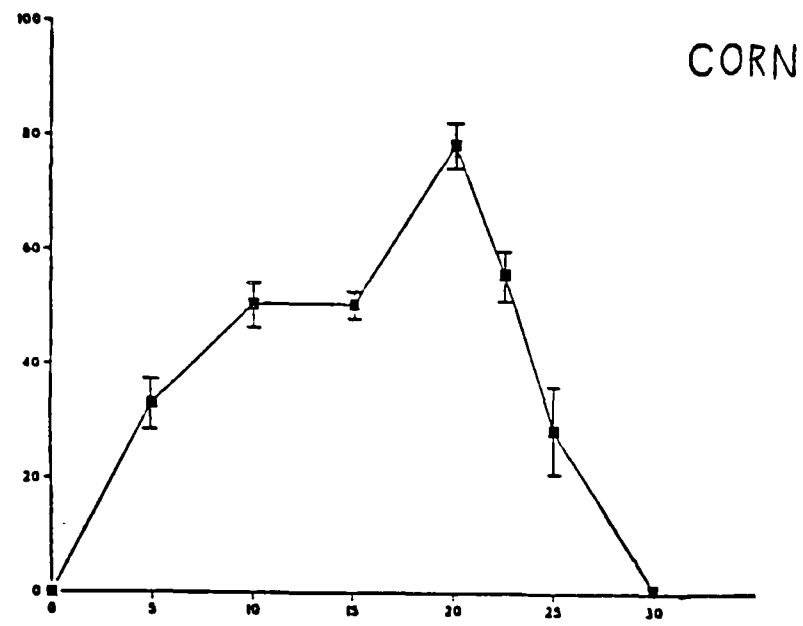


Fig. 2.34(f)

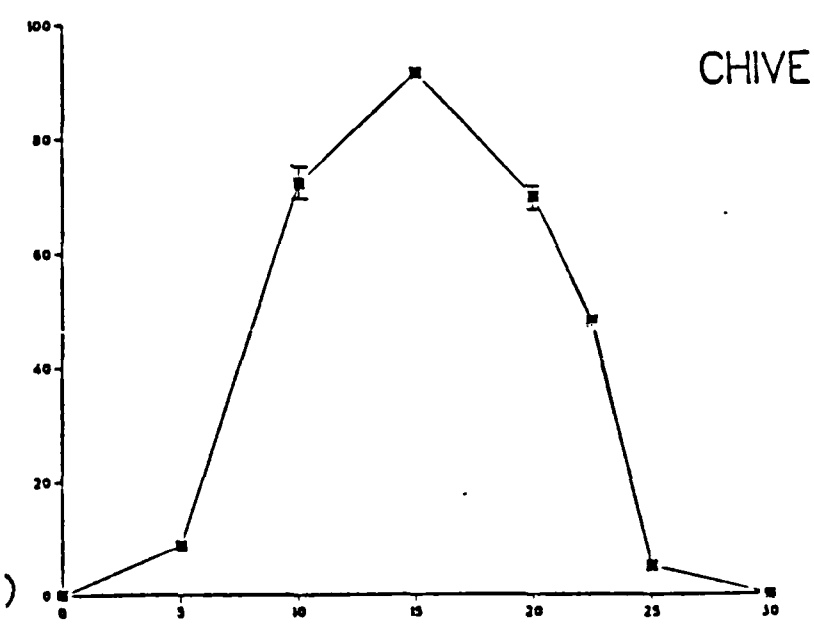


Fig. 2.34(g)

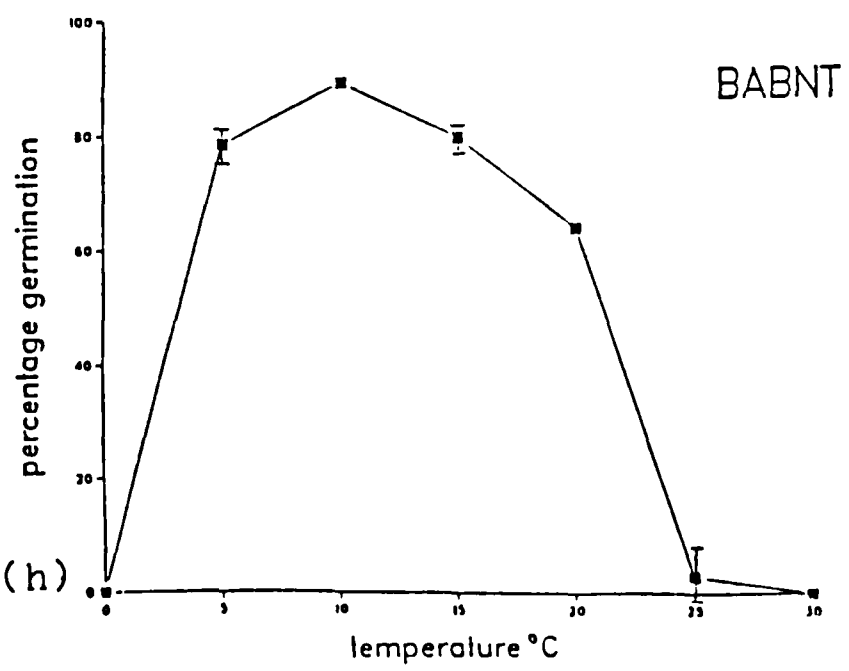


Fig. 2.34(h)

2.3.2. Teliospore Studies

a) Macroscopic Studies

BIRM isolate

Telia formed only rarely in material infected by this isolate. On a few occasions telia began to form around the circumference of uredinia, four to five weeks after inoculation. The telia did not mature before the leaves senesced. Figure 2.35 shows a telium forming around the circumference of a uredinium. It shows as a darkened area around the uredinium, with no separate structure visible.

NVRS isolate

Telia appeared either scattered or more rarely in groups when they tended to coalesce. The telia appeared as hard, thickened areas, black or more rarely dark grey in colour, slightly pulvinate and occasionally lozenge shaped, up to 4 mm long and 2 mm wide, along the axis of the leaf. The teliospores were rarely exposed, the epidermis usually remaining intact.

CHIVE isolate

Telia formed readily after uredinia, beginning as a dark border to the uredinia, and gradually increasing in size until replacing it.The telia were scattered along the length of the leaves and flower stalks, occasionally coalescing. The telia appeared as flattish, pale grey areas, round or oblong in shape, up to 5 mm long and 2 mm wide. The teliospores were rarely exposed either in the field or the glasshouse, remaining under the epidermis.

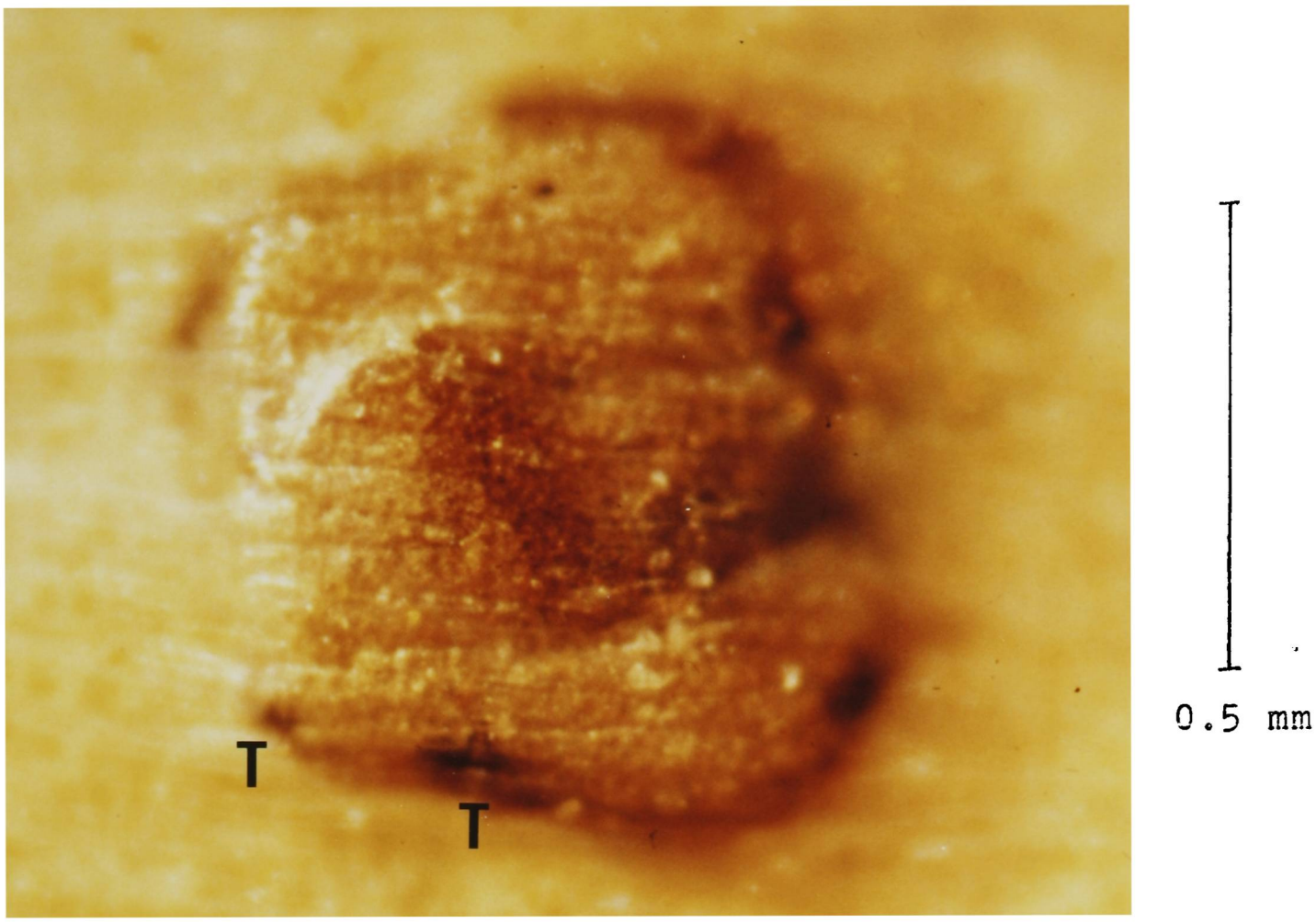


Figure 2.35
Uredinium of the BIRM isolate on leek cv. Rolan, with telium (T) forming at the circumference.

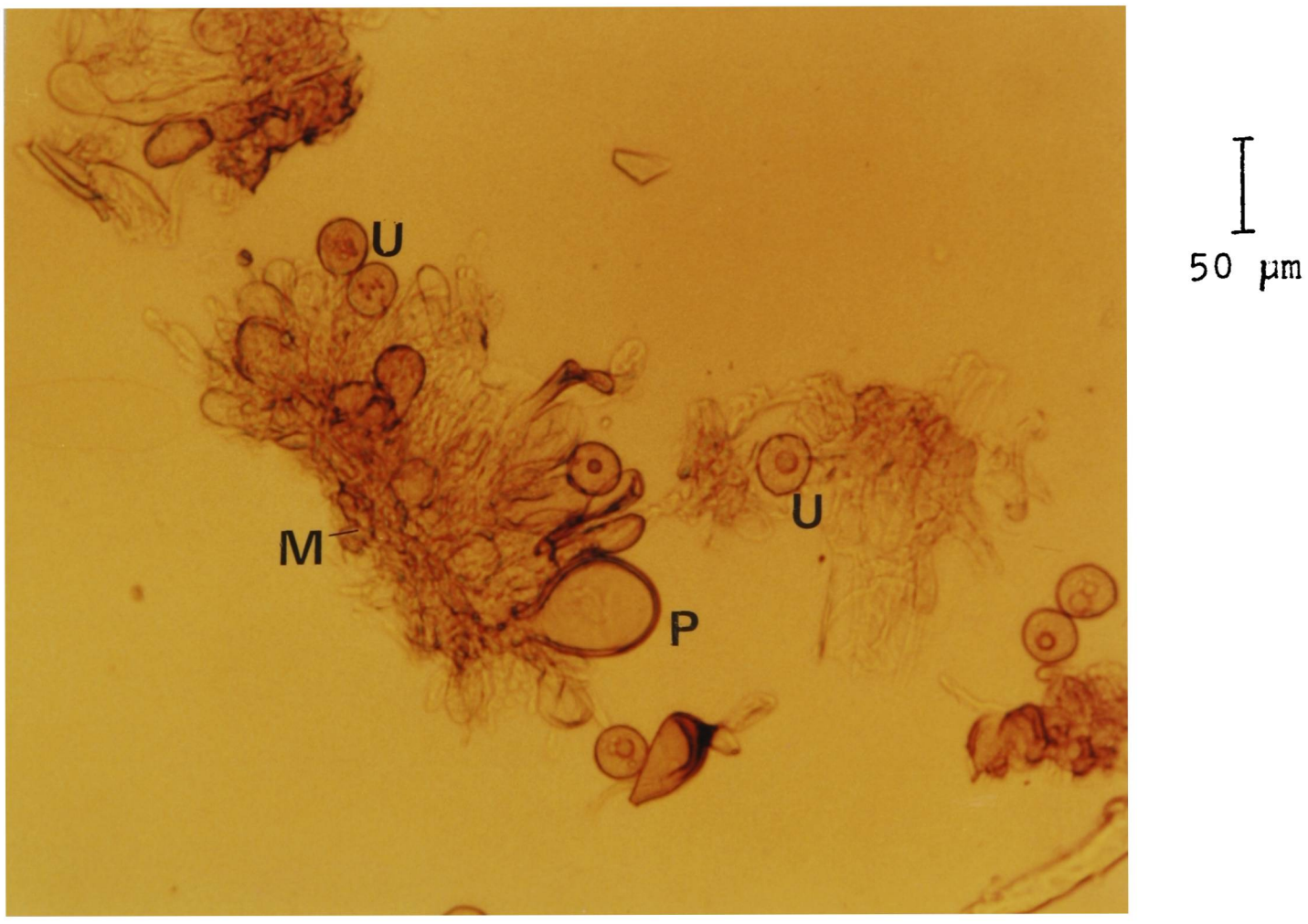


Figure 2.36
T.S. through 'telial region' of pustule in fig. 2.35, showing urediniospores (U), melanized basal material (M) and large, thick-walled pyriform structures (P).

BABNT isolate

Telia formed readily after uredinia, soon after inoculation. Occasionally the telia formed almost directly after the uredinia appeared. The telia were scattered along the flower stalks and leaves, commonly in groups concentrically arranged about uredinia. They appeared as pale grey areas with flattish surfaces, oblong when single, up to 5 mm long by 3 mm wide, but often confluent into large lozenge patches up to 12 mm long by 6 mm wide. The telia often continued to grow for some time after forming, occasionally producing large confluent patches up to 40 mm long and 10 mm wide, especially in those plants maintained in growth chambers. In all cases the teliospores were usually covered by the epidermis.

b) Microscopic study of telia and teliospores

i) Direct Light microscopy

BIRM isolate

Figure 2.36 shows a region of the 'telium initial' on the circumference of a uredinium, dissected out. Urediniospores and their pedicels are clearly visible. Also visible is a base of dark melanised material, to which large thick walled pyriform structures are attached. Figure 2.37 shows the structures in greater detail. The one on the right resembles a pale mesospore, and has a distinct septum at the base. The one on the left has thicker walls but the septum has not formed completely. Figure 2.38 shows mature

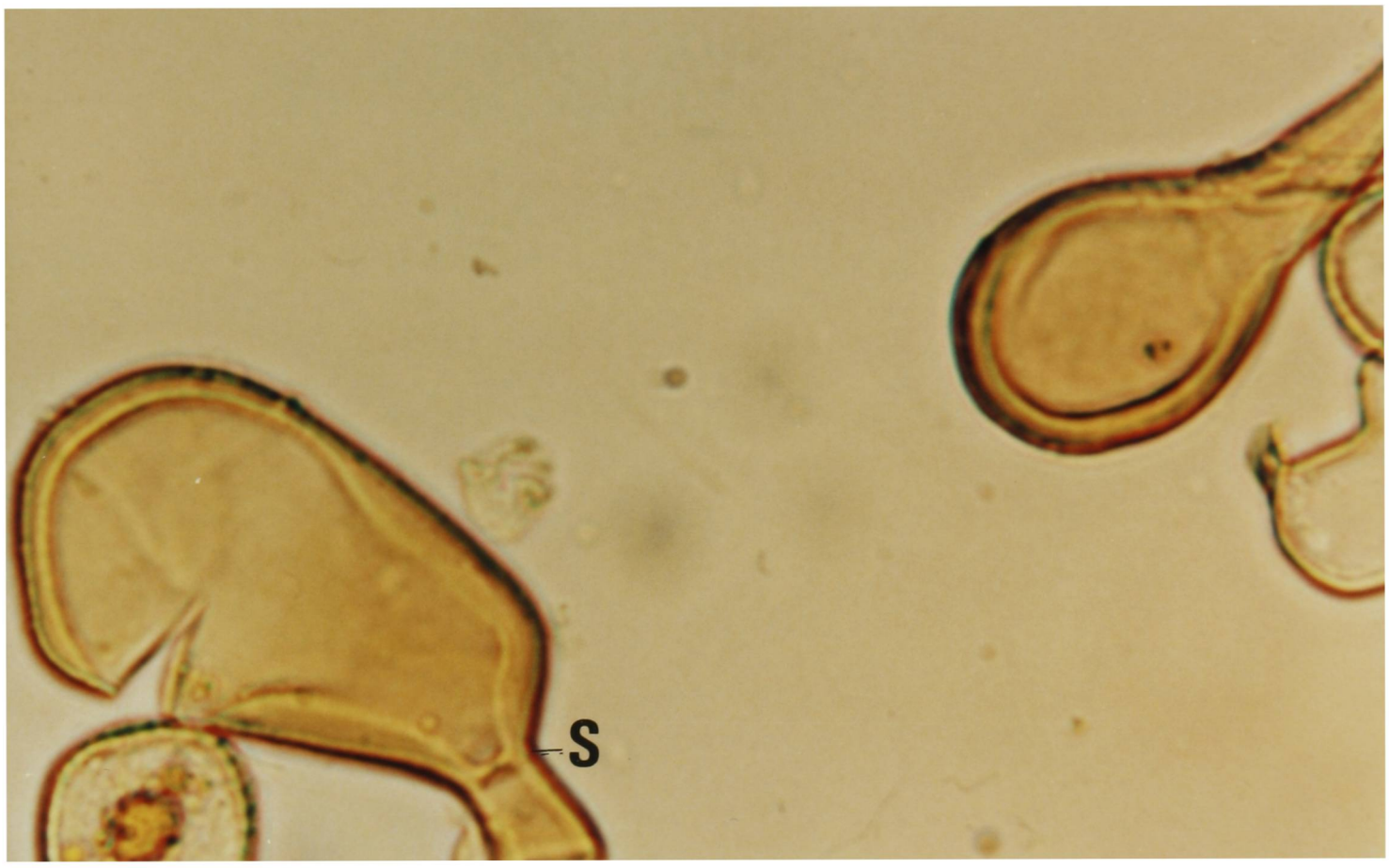


Figure 2.37
Pyriform structures in greater detail, showing incomplete septum at base (S), and walls.

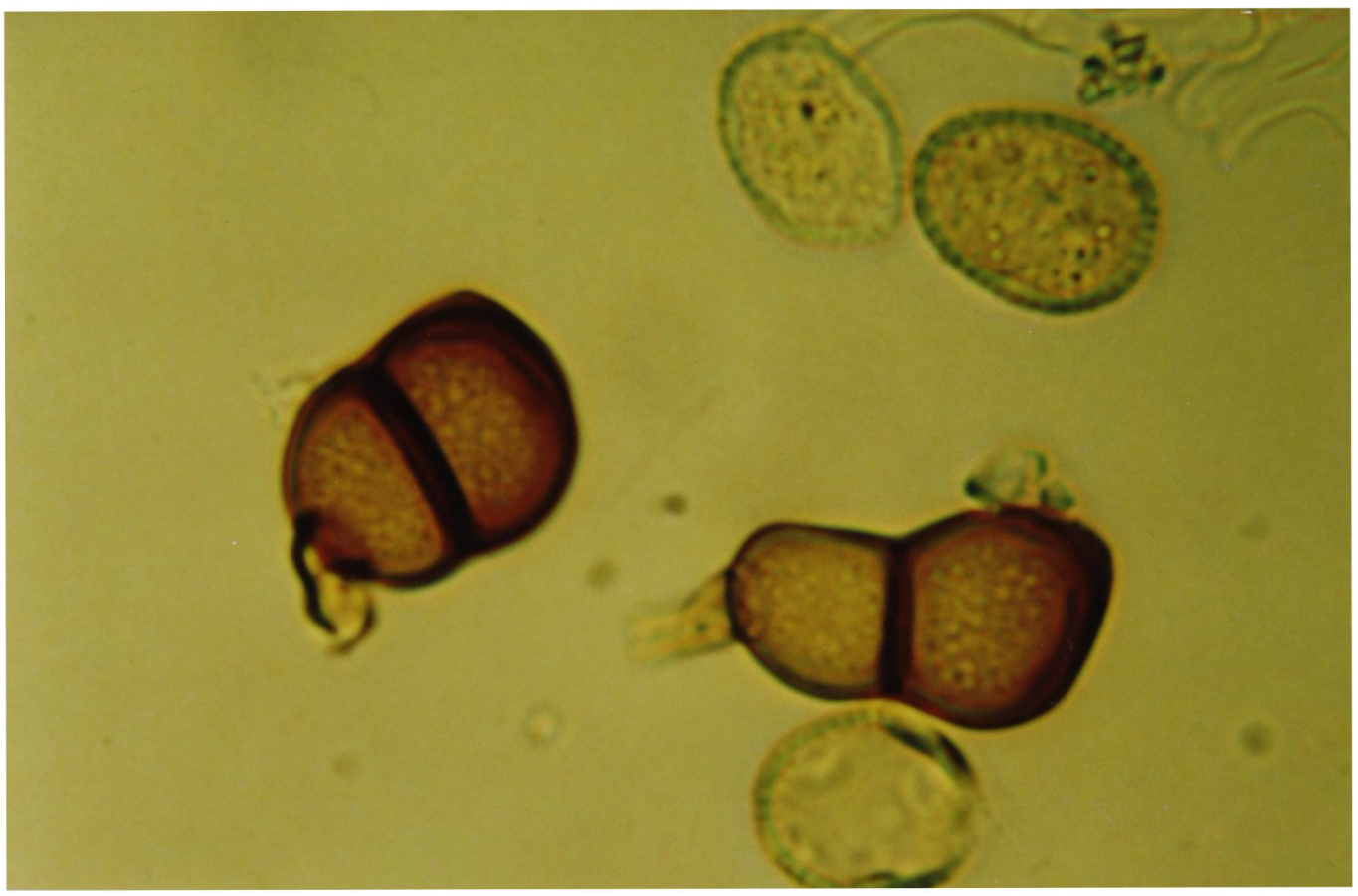


Figure 2.38
Two-celled teliospores from the 'telial region' in fig. 2.39, with thick walls and pedicels still attached.

two-celled teliospores from a different part of the telium, with the pedicels still attached.

NVRS isolate

Figure 2.39 shows a section through a mature telium on Allium porrum. The telium has dark fused paraphyses which form a stromatic, locular structure, with a base of dark melanized material. The teliospores are all two-celled and occur in groups within the locules. The teliospores themselves were usually ellipsoidal to obovoid, often with a distinct constriction at the boundary between the two cells. The upper cell was usually smaller than the lower, and the apices varied from distinctly attenuate to angular and flattened. The detached spores in figure 2.39 retain a pedicel remnant, though spores could be found where the pedicel was completely absent from the detached spore.

CHIVE isolate (also KERR & 6504)

Figure 2.40 shows a section through a mature telium on A. schoenoprasum, of the CHIVE isolate. The telium is still covered by the epidermis. There are no paraphyses present, and the base of the telium is formed from a pale hyphal mass with no obvious melanization. The teliospores were a mixture of one and two-celled teliospores, predominantly one-celled (as in figure 2.41). The proportion of two-celled spores seemed to be higher in the larger and older telia, approximately 40%, compared with 10% in the younger telia. The one-celled spores did not appear to be smaller or have thinner walls than the two-celled spores.

Figure 2.39
T.S. through telium of NVRS isolate on leek, showing fused paraphyses (P) founded in a dark melanized base (B), forming locules (L) containing two-celled teliospores (T) and mesospores (M).

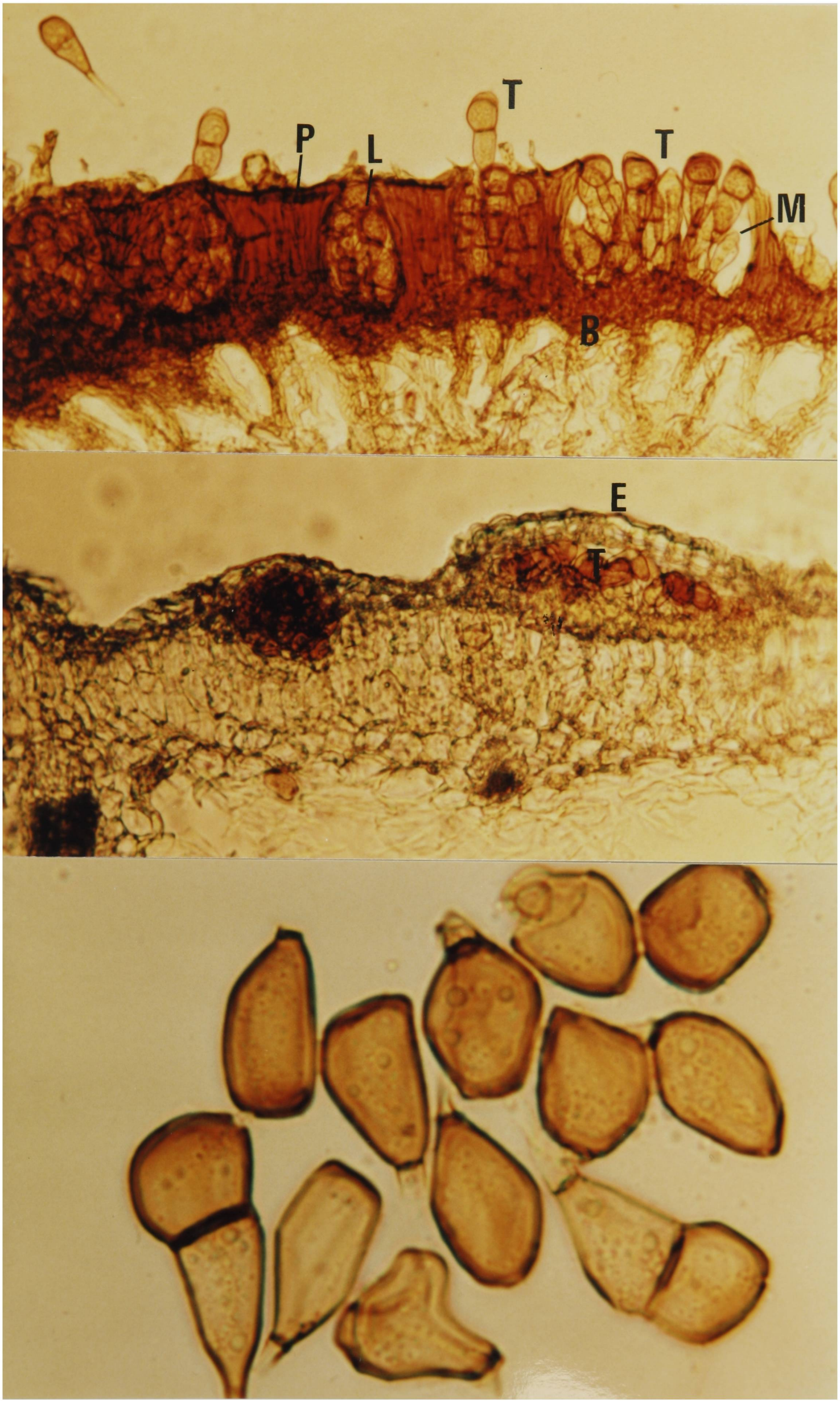
100 µm

Figure 2.40
T.S. through telium of CHIVE isolate on chive, showing teliospores (T) forming in a non-paraphysate telium beneath the epidermis (E).

100 µm

Figure 2.41
One-celled and two-celled teliospores from the telium in figure 2.40, some with pedicel remnants.

50 µm


T
P
L
T
M
B
E
T

BABNT isolate (also H.70 isolate)

Figure 2.42 shows a section through a telium on A. babingtonii. The telium is confined to the adaxial side of the leaf, and has not developed to the abaxial surface. The epidermis remains intact over the telium. There are no paraphyses and the spores are formed over a non-melanized hyphal mass. The spores are all single-celled, and very densely packed in the telium. Figure 2.43 shows detached teliospores. The spores varied in shape but were mainly subglobose, obovoid or rarely pyriform and smooth-walled, and retained a pedicel remnant.

ii) Scanning Electron Studies

Figures 2.44 to 2.46 show representative scanning electron micrographs of teliospores of the NVRS, CHIVE and BABNT isolates.

NVRS isolate

Figure 2.44 shows a two-celled teliospore, with a clear constriction at the point between the upper and lower cell, and a pronounced attenuation at the tip. The spore surface is smooth and lacks ornamentation.

CHIVE isolate

Figure 2.45 shows a detached single-celled spore with the collapsed remains of a pedicel remnant. Although the spore has partially collapsed (due probably to dehydration) the obovoid shape is still apparent. The surface is smooth without any ornamentation.

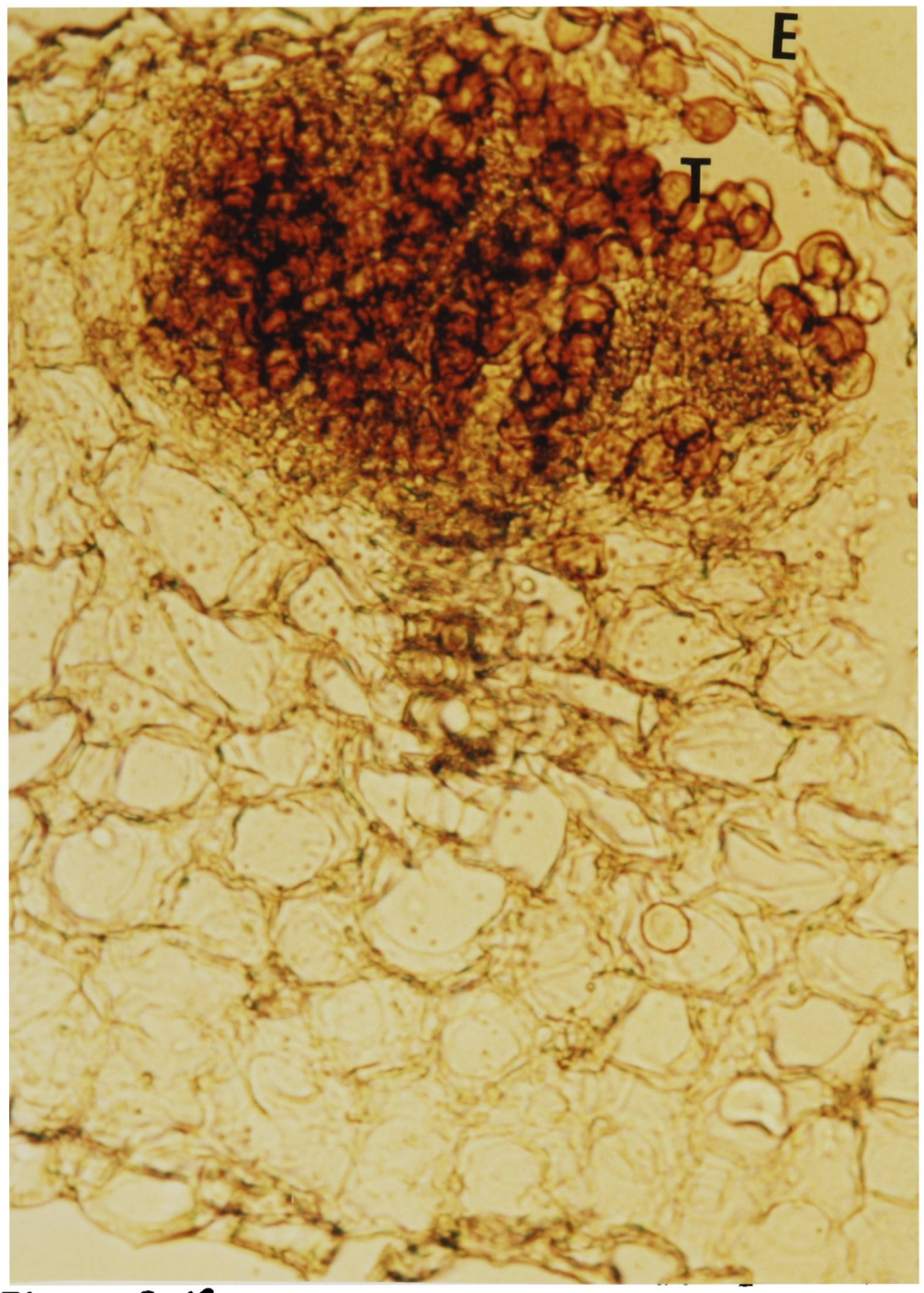


Figure 2.42
T.S. through telium of BABNT isolate on A. babingtonii, showing teliospores (T) forming in non-paraphysate telium, covered by epidermis (E).

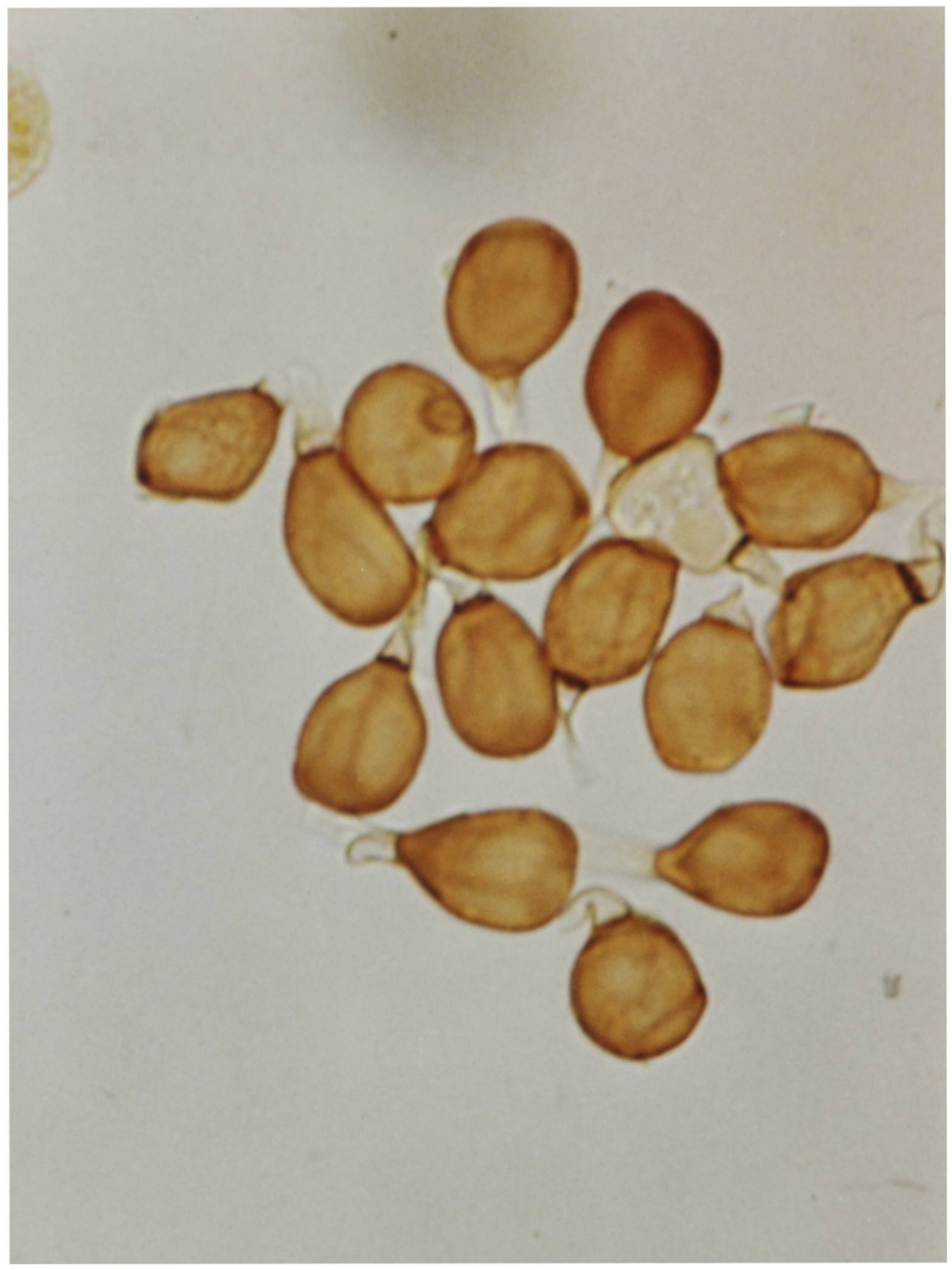


Figure 2.43
One-celled teliospores from the telium in fig. 2.42, all retaining pedicel remnants.

Figure 2.44
SEM of a two-celled telispore of the NVRS isolate, showing sharply attenuated apex, constriction (C) and base broken from the pedicel. Note the smooth surface of the spore.

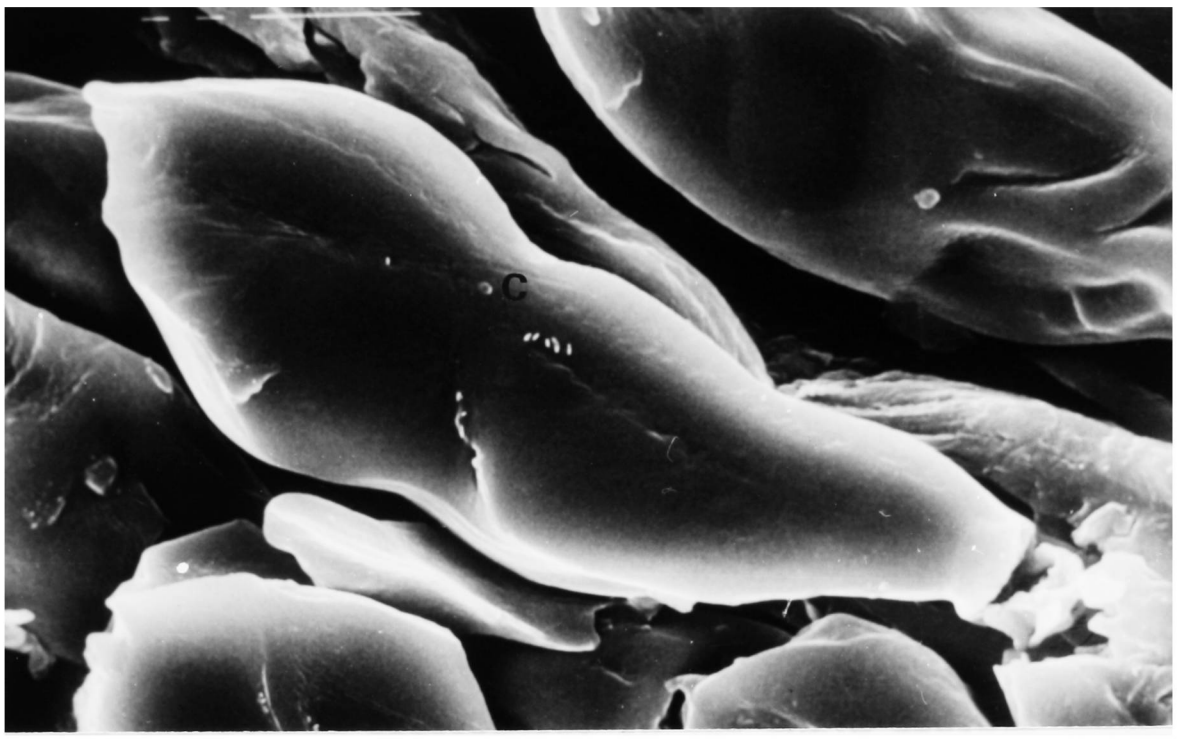
Bar = 10 μm

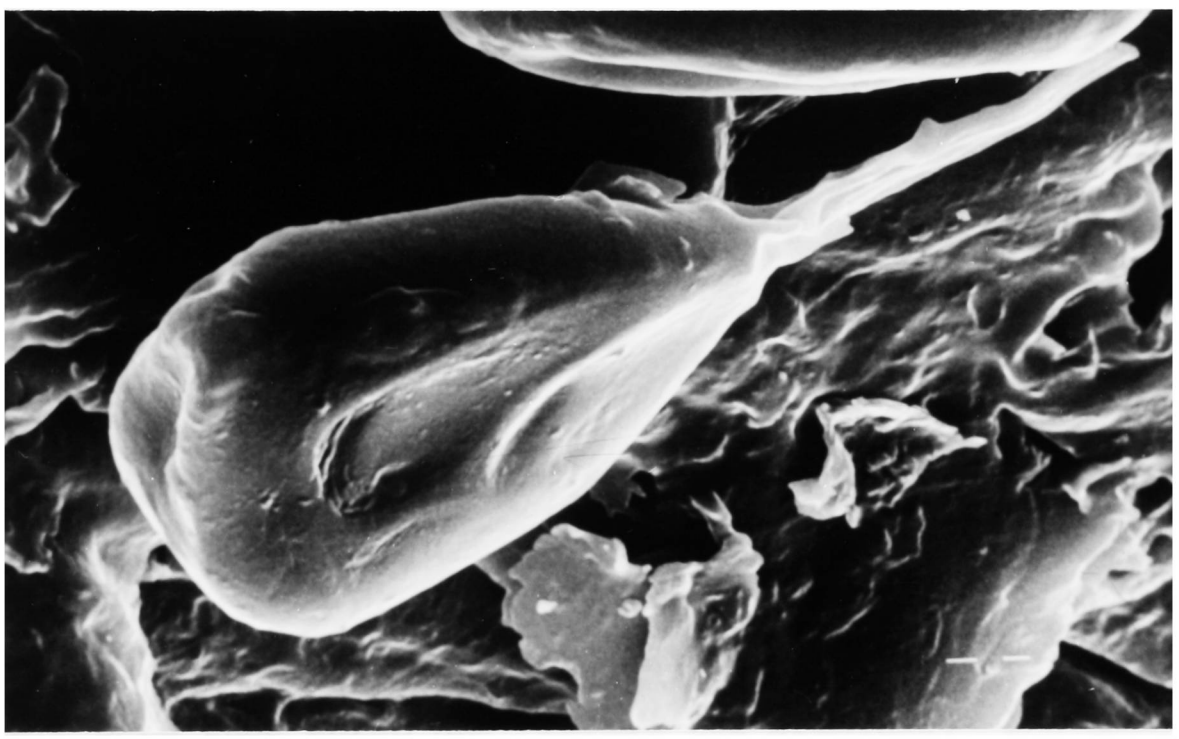
Figure 2.45
SEM of a single-celled teliospore of the CHIVE isolate, showing smooth surface, and collapsed pedicel remnant at the base, and the flattened apex.

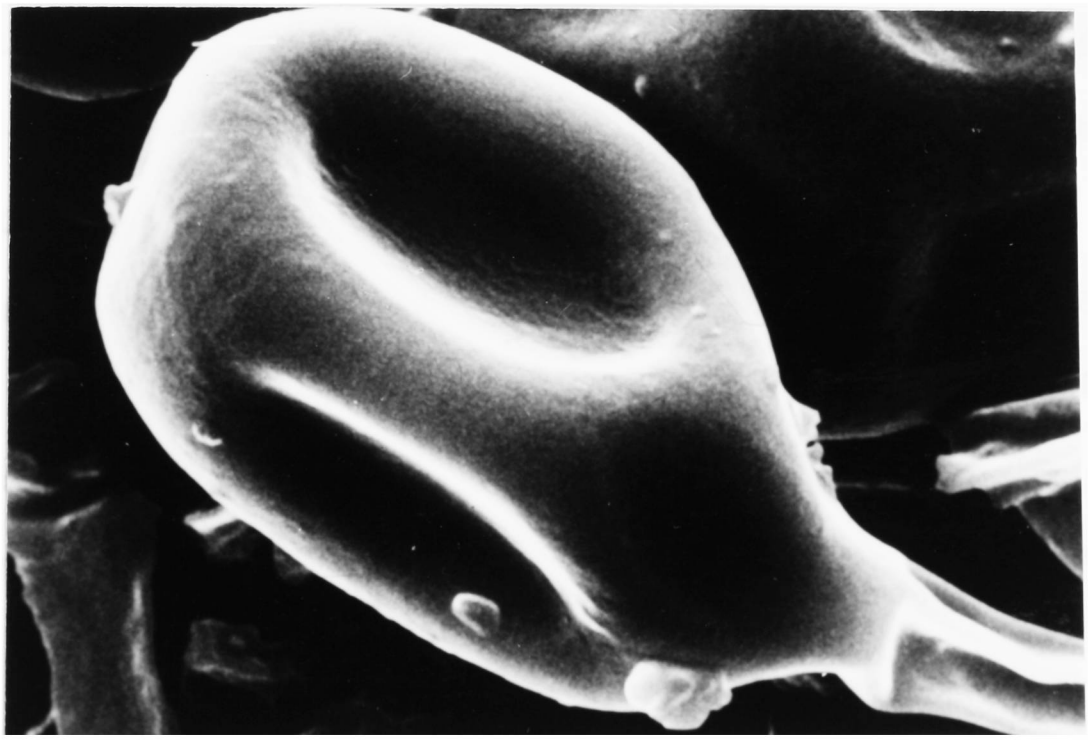
Bar = 10 μm

Figure 2.46
SEM of a single-celled teliospore of the BABNT isolate, showing smooth surface, collapsed pedicel remnant and flattened apex.

Bar = 10 μm


C





BABNT isolate

Figure 2.46 shows a single-celled teliospore which has retained a pedicel remnant. Although collapsed the spore is obovoid in outline and has an exceptionally smooth surface.

d) Germination studies on teliospores

CHIVE isolate

Both single-and two-celled teliospores in the CHIVE isolate germinated freely, though usually only one cell of a two-celled spore would germinate. A variety of septate germinatin structures were seen, but no basidiospores. Branching of the germ tubes occured though with no distinct pattern. Figure 2.47 shows a short, three-septate germ tube emerging from the lower cell of a two-celled spore. Figure 2.48 shows a rather longer non-septate but highly vacuolated germ tube. The tube appears to emerge from an inner layer of the wall. Figure 2.49 shows two teliospores germinating. The single celled spore has produced a three-septate germ tube which has formed two branches. The germ tube from the two-celled spore is also three-septate but has produced three non-septate branches, though one appears to be bifurcated at the tip. Figure 2.50 shows a single celled teliospore viewed under phase-contrast which has produced a three-septate germ tube with three non-septate branches. In all the figures except figure 2.48, the pattern seems to be to produce a three-septate germ tube, with two or more branches. This pattern was seen in most of the spores which germinated.

Figure 2.47
Light micrograph of a two-celled teliospore of the CHIVE isolate with a short three-septate germ tube emerging from the lower cell (septa arrowed).

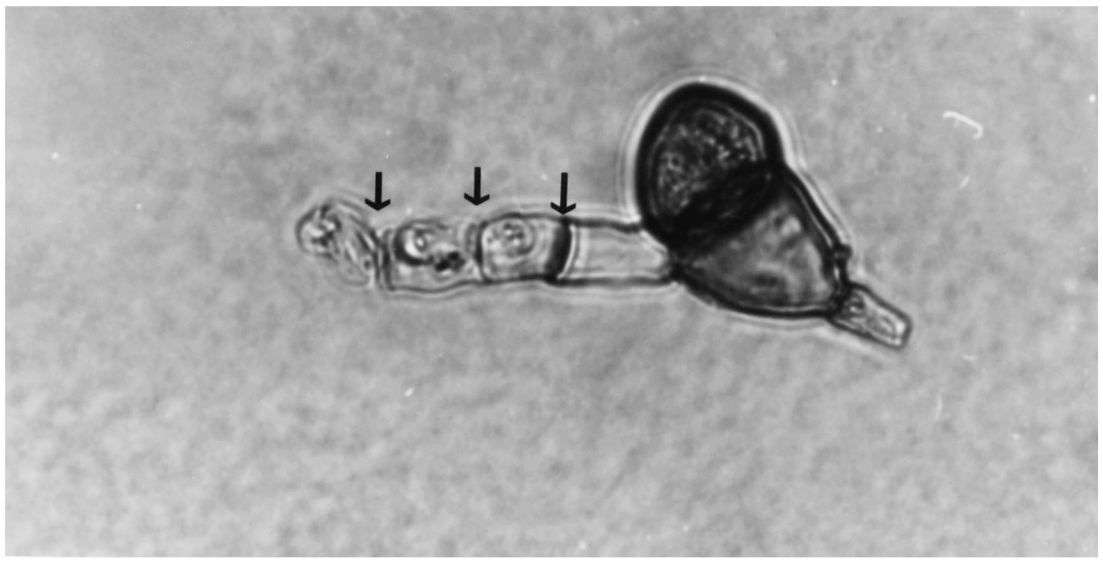
50 µm

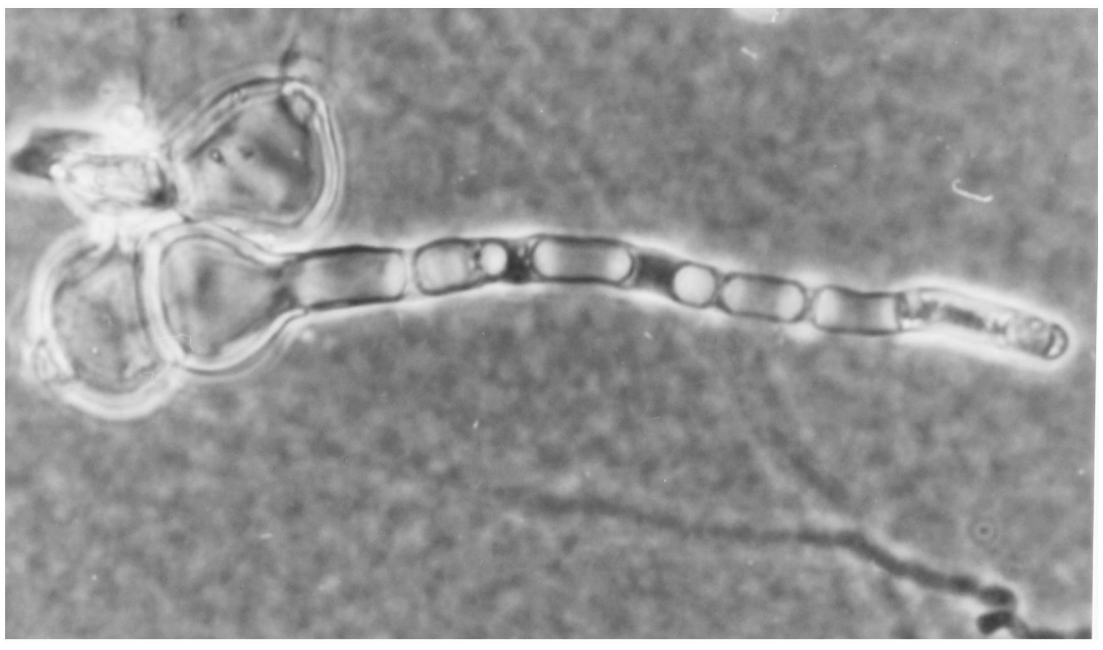
Figure 2.48
Phase-contrast micrograph of a two-celled teliospore of the CHIVE isolate with a long, highly vacuolated but aseptate germ tube emerging from the lower cell.

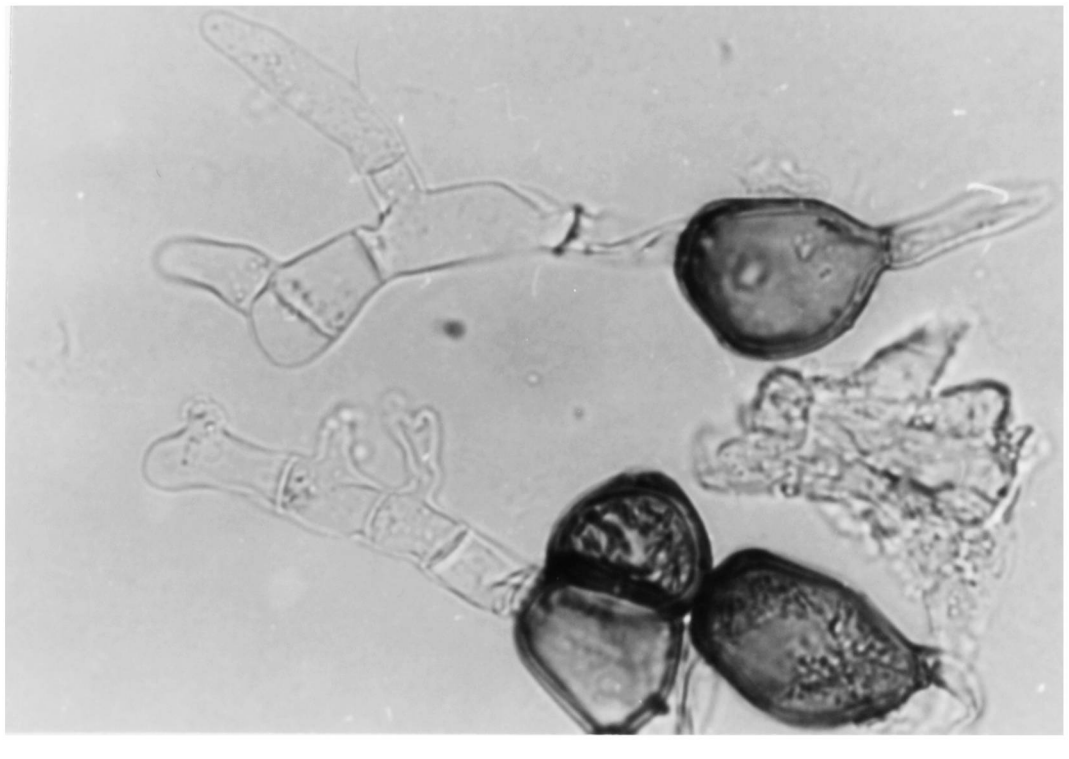
50 µm

Figure 2.49
Light micrograph of two teliospores of the CHIVE isolate showing a single-celled spore with a three-septate germ tube two of which have cut off branches, and a three-septate germ tube from the lower cell of a two-celled spore, with aseptate branches emerging from the three upper cells.

50 µm







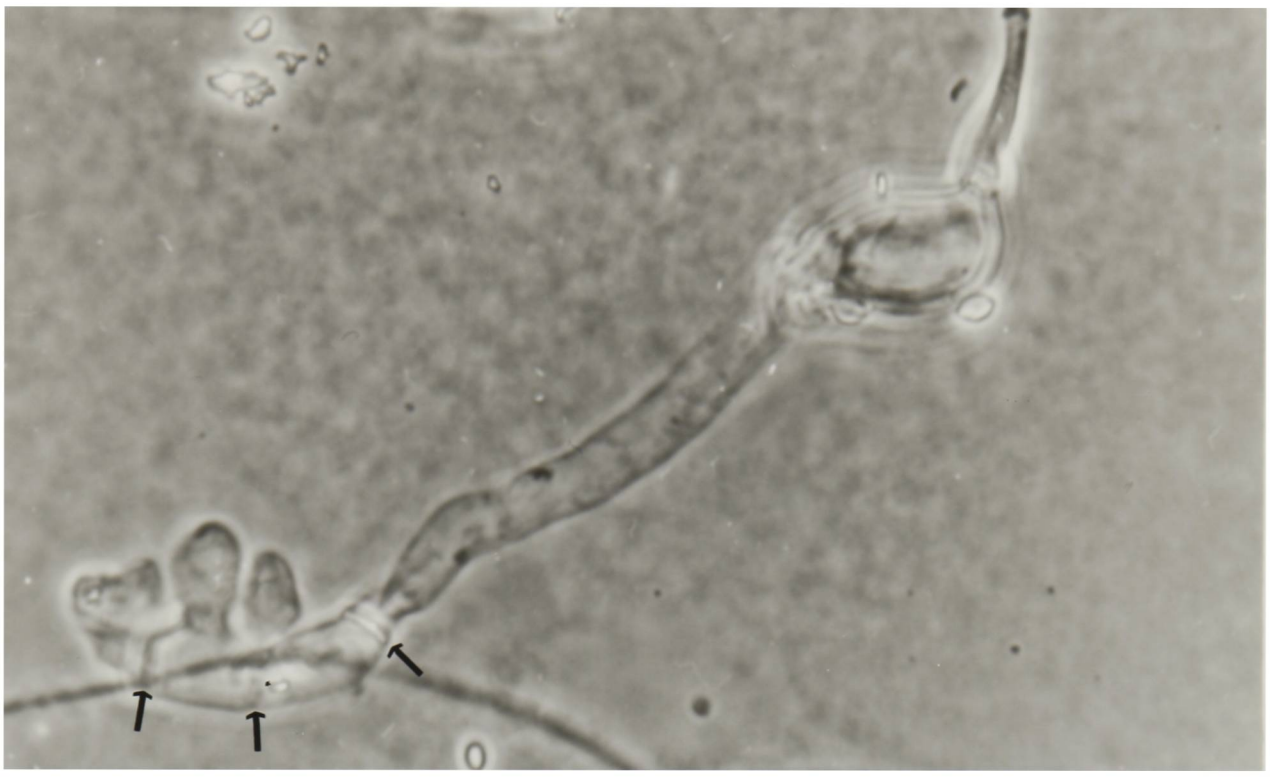


Figure 2.50
Phase-contrast micrograph of a single-celled teliospore of the CHIVE isolate showing three-septate germ tube with three aseptate branches emerging from the upper three cells, (septa arrowed).

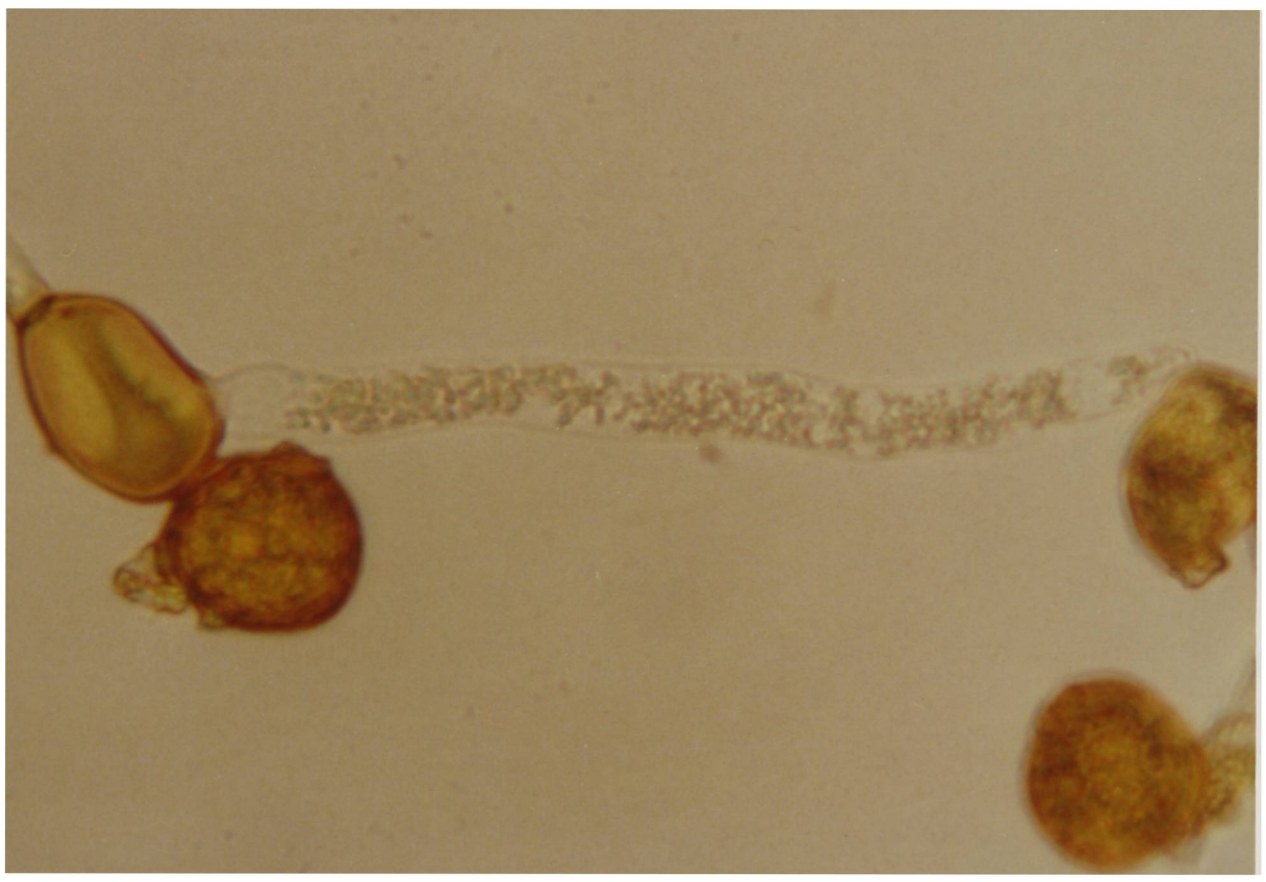


Figure 2.51
Light micrograph of a single-celled teliospore of the BABNT isolate which has produced a long vacuolated aseptate germ tube.

BABNT isolate

Germination was a rare event in the BABNT isolate. Figure 2.51 shows a teliospore of the BABNT isolate which has produced a single, non-septate germ tube, with a very granular appearance to the contents.

2.3.3. Herbarium Material

Details of the material are given in table 2.10

Description of the material by host species

A. ampeloprasum

The telia on the specimens from this species (both from the Mediterranean) were dark and hard in appearance, and up to 4 mm long. The telia both had a locular structure of fused paraphyses, enclosing predominantly two-celled teliospores. The urediniospores on the BU/RNL were large with wide-spaced spines.

A. babingtonii

All the specimens bore large, pale grey telia, up to 12 mm long and 4 mm wide, containing all single-celled teliospores, with no paraphyses. Urediniospores when present, were small, bright orange in colour, with close set spines, and between 11 and 16 germ pores per spore.

A. cepa

Specimens 65568 and 70138 bore dark telia 1 - 3 mm long, with a locular paraphysate structure enclosing two-celled teliospores. Specimen 70362 was similar in structure but had paler coloured telia. The telia on specimen 44167 were very immature, with no paraphyses, and all single-celled telia with pale-coloured walls. The telia on 1307 were locular and contained very thick-walled cells, mostly two-celled, which were very rounded in shape. The urediniospores on 1307 were very small and angular and covered in close-set spines.

A. fistulosum

The telia on 233470 and 233468 were small,pale grey in colour with an approximately equal number of single- and two-celled spores , in a non-paraphysate structure. The uredinia on 233470 were small, pale yellow in colour and contained small spores with densely-packed spines. The telia on specimens on 60765 and 65567 were hard, black and paraphysate, with locules containing two-celled spores. The urediniospores from specimen 60765 were very angular in shape with a low spine density.

A. flavescens

The only specimen on this species bore aecidia in tightly packed groups, the aecidiospores being small, yellow-coloured with a warty surface.

A. flavum

The telia on this specimen were dark, up to 3 mm long, and contained

many paraphyses in a locular structure. The teliospores were all two-celled.

A. obliquum

The telia on this specimen were dark, up to 3 mm long and were highly paraphysate, with predominantly two-celled teliospores.

A. porrum

Specimens 44168 and BU/JN bore dark black telia, with highly paraphyste structure forming locules, containing groups of two-celled teliospores. Specimen 24013 bore large uredinia with spores bearing wide-set spines. The uredinia also contained a few single-celled teliospores but there was no distinct telial structure in this specimen. The uredinia on 4978 were similar, containing mostly urediniospores but a few one-or two-celled teliospores with a dark melanized base, but otherwise no distinct telial structure.

A. sativum

All the specimens bore small black telia 1 - 4 mm long, with paraphyses fused to form locular structures, containing groups of two-celled teliospores, except for specimen RBGE/DSA where there were rudimentary single- and two-celled teliospores in the uredinia. The teliospores on specimen 126910 were notable for their unusually thick walls.

A. sphaerocephalon

The specimen bore dark grey-black telia 1 - 5 mm long, which had a

locular structure of fused paraphyses, containing single- and two-celled teliospores, (over 80% two-celled).

A. schoenoprasum

The specimens on this host fell into two categories, regarding telial morphology. The majority had pale grey telia up to 3 mm long, containing a mixture of single- and two-celled teliospores, predominantly single-celled, varying in proportion from 49% to nearly 100%. Specimen RBGE/JS was unusual in having a high proportion of two-celled spores (77%). Most specimens had 90 to 99.5% single-celled spores, with two-celled teliospores always found in the mature telia (i.e. those not containing urediniospores). There were no paraphyses in any of these telia. The urediniospores, where present, were generally small, thin-walled with close-set spines. The second group of specimens, all from Scandinavia, had hard dark, or black telia with locular structures formed from fused paraphyses, containing mostly two-celled spores, from 50% to 90%. The specimens in this group were CMI/201571/60667/60767/30824, RBGE-FEF 309.

A. scorodoprasum

The specimens from A. scorodoprasum fell into three categories. Firstly, specimen 208320 from Ireland had telia with a locular structure formed from fused paraphyses, containing over 80% two-celled teliospores. The second group, containing most of the specimens, had pale grey telia with low percentages of two-celled teliospores, varying from 5% to less than 1% in the telium (i.e. only a few two-celled teliospores per telium). Telia on the same plant could

vary from having 0% to 2% teliospores. There were no paraphyses in any of these telia. Those plants in the third group, where there were only single-celled teliospores and no paraphyses, included CMI/65583, RBGE-DMH, RBGE/FBME 31/1548, RBGE/MG 2812, RBGE/FBE (Burgas) and RBGE/393/77 (St. Andrews). Specimen RBGE/SAE had 2% two-celled teliospores, but the single celled spores were very similar to those found on A. babingtonii in terms of shape, being all obovoid, rather than a mixture of obovoid, subglobose or pyriform as in the single celled teliospores on A. schoenoprasum, and in all the other specimens of A. scorodoprasum.

A. vineale

All the telia on A. vineale were hard, black, 1 - 4 mm long, and contained fused paraphyses forming locules which contained mostly two-celled teliospores, (varying in proportion from 71% to 91%). No urediniospores were present on the specimens examined.

Table 2.10
Details of host, country or site of origin, herbarium reference number and summary of the major details of the specimens of rust on *Allium* spp. from the herbaria listed below, examined in this project.

Abbreviations
CMI Commonwealth Mycological Institute, Kew, England
RBGE Royal Botanic Garden, Edinburgh
BU University of Birmingham, England

U +/- Uredinia present/absent
1t One-celled teliospores
2t Two-celled teliospores
P Paraphyses in the telium

Ref.	Country/Location	Collection Date	U	1t	2t	P
a) On *A. ampeloprasum*						
CMI/74531	Zirchron-Jackov, Israel	3.4.54	-	+	+	+
BU/RNL	Cala Contra, Ibiza	5.86	+	+	+	+
b) On *A. babingtonii*						
CMI/65584	Newquay, Cornwall, UK	23.5.52	-	+	-	-
BU/DMJ	King Harry Ferry, Cornwall	2.3.85	+	+	-	-
CMI/113013	Tresco, Scilly Is. UK	2.4.65	+	+	-	-
CMI/69112	Tresco, Scilly Is. UK	15.4.57	+	+	-	-
RBGE/7072	Clifden, Conn. Ireland	15.8.63	+	+	-	-
c) On *A. cepa*						
CMI/70362	Carvana, Gatto, Malta	3.4.12	-	-	+	+
CMI/65568	Mparo Kigezi, Uganda	.30	+	-	+	+
CMI/70138	Mistra, Malta	3.5.57	+	-	+	+
CMI/44167	Vallombrosa, Italy	1898	-	+	-	-
CMI/1307	Lyallpur, Punjab, India	15.2.28	+	+	+	+
d) On *A. fistulosum*						
CMI/65567	Italy	1902	-	-	+	+
CMI/233470	Evesham, England	20.11.78	+	+	+	-
CMI/233468	Evesham, England	20.11.78	+	+	+	-
CMI/60765	Jokiniemi, Finland	14. 9.38	+	-	+	+
e) On *A. flavescens*						
CMI/65572	Basarabia, Romania	18.5.30	Aecia only			

Table 2.10 cont'd

Ref.	Country/Location	Collection	U	1t	2t	P
f) On *A. flavum*						
CMI/30809	Aranyosmarot	.8.13	-	-	+	+
g) On *A. obliquum*						
RBGE/FEF307	Turku, Finland	2.9.48	-	+	+	+
h) On *A. porrum*						
CMI/24013	Exeter, UK	26.1.48	+	+	-	-
CMI/44168	Episcopi, Cyprus	.4.36	-	+	+	+
BU/JN	NVRS, Wellesbourne, UK	.7.85	-	+	+	+
RBGE/4978	Midlothian, Scotland	10.11.59	+	+	+	-
i) On *A. sativum*						
CMI/70384	Melbeha, Malta	5.10	-	-	+	+
CMI/126910	Arnsha, Tanganyika	7.42	-	-	+	+
CMI/88518	Harar, Ethiopia	9.60	-	-	+	+
CMI/65576	Dobrogea, Silistra Bulgaria	17.7.39	+	-	+	+
RBGE/IFM	Portugal (UK intercept)	7.1.44	-	-	+	+
RBGE/DSA	Kirkcubright, Scotland	2.9.46	+	+	+	-
j) On *A. sphaerocephalum*						
CMI/50881	Bavaria, Germany	9.2.33	-	+	+	+
k) On *A. schoenoprasum*						
RBGE/Kerr	Aberdeenshire	2.7.48	+	+	+	-
CMI/87660	Vistula, Poland	16.8.58	+	+	+	-
CMI/201574	Viikki, Finland	1.10.73	-	+	+	+
CMI/60766	Jokiniemi, Finland	2.9.35	-	+	+	+
CMI/60767	Karlby, Finland	25.7.38	-	+	+	+
CMI/12702	Zurich, Switzerland	-	-	+	+	-
CMI/65570	Jasenka, Czechoslovakia	.8.24	-	+	+	-
CMI/30824	Tingshogen, Sweden	.10.26	-	+	+	+
CMI/140073	Shrewsbury, England	7.5.69	+	+	+	-
RBGE/DMH	Flotterstone, Scotland	12.9.68	-	+	+	-
BU/DMJ	Birmingham, England	18.10.84	+	+	+	-
RBGE/JS	Orkney, Scotland	8.30	-	+	+	-
RBGE/DMH	Glen Fincastle, Scotland	3.7.69	-	+	+	-
RBGE/BH588	Bramhope, Leeds, England	.10.82	-	+	+	-
BU/GM	Penzance, England	.5.85	-	+	+	-
RBGE/FEF309	Vitfagelskar, Finland	3.9.49	-	+	+	+
RBGE/OJ	Triglitz, Germany(?)	15.8.13	-	+	+	-
RBGE/AL	Westfalen, Germany	30.8.34	-	+	+	-
RBGE/3048.MG	Tamsel, Germany	25.5.36	-	+	+	-
RBGE/661.MG	Rothwasser, Germany	.7.07	-	+	+	-
RBGE/BH828	Cornwall, England	26.7.85	-	+	+	-
RBGE/KS	Orkney, Scotland	.8.39	-	+	+	-
RBGE/WMW	Wye, Kent, England	.12.37	-	+	+	-
RBGE/6504	Aberdeen, Scotland	30.6.84	+	+	+	-

Table 2.10 cont'd

Ref.	Country/Location	Collection	U	1t	2t	P
1) On A. scorodoprasum						
RBGE/DMH	Kircudbrightshire, Scotland	.70	+	+	-	-
CMI/33042	Csepel, Hungary	8.1888	-	+	+	-
CMI/65571	Rosetti, Romania	2.7.27	-	+	+	-
CMI/33066	Huvusvolgy, Hungary	5.27	-	+	+	-
CMI/11547	Moravia, Czech.	-	-	+	+	-
CMI/65583	Caliacra, Romania	25.5.36	-	+	-	-
CMI/207796	Ostergotland, Sweden	14.7.60	-	+	+	-
CMI/171621	Gory, Oltenia, Romania	16.5.69	-	+	+	-
CMI/208320	Kinsale, Cork, Ireland	22.5.76	-	+	+	+
RBGE/Fung. Dk.	Salthomen. Denmark	30.6.1893	-	+	+	-
RBGE/ 31/1548	Brnin Czech.	.5.21	+	+	-	-
RBGE/MF 527	Ido, Finland	24.7.38	-	+	+	-
RBGE/MG 2812	Thuringen, Germany	23.8.36	-	+	-	-
RBGE/FBE	Burgas, Scotland	13.5.59	-	+	-	-
RBGE/B.Ing	Muckross, Killarney, Irlnd.	.7.84	-	+	+	-
RBGE/Jack	Logie, Scotland	1.7.67	-	+	+	-
RBGE/ 393/77	St. Andrews, Scotland	.7.56	+	+	-	-
RBGE/SAE	Forres, Scotland	20.7.54	-	+	+	-
RBGE/2765	Inverbevie, Scotland	23.5.56	-	+	+	-
m) On A. vineale						
CMI/33041	Bergholm, Sweden	.8.28	-	+	+	+
RBGE/DMH	Sandhead, Scotland	18.7.58	-	+	+	+
RBGE/Duncan	Arbroath, Scotland	.8.44	-	+	+	+
CMI/44228	Borth-y-gest, Gwynedd, Wales	.6.41	-	+	+	+
RBGE/-	Kilmory, Arran, Scotland	.24	-	+	+	+
RBGE/JHA	Landulph, Cornwall, England	.7.44	-	+	+	+

2.4. DISCUSSION

Urediniospore Studies

Macroscopic features

It was generally not possible to distinguish the rusts from the macroscopic study of the uredinia, the appearance of which often varied within an individual plant, and was dependent on the pustule density, extent of confluence between pustules etc. It is likely that the differences between the rusts were caused by differences in host anatomy rather than the actual rusts. The most noticeable differences were the bright orange colour and often striped appearance of the wild infections on A. babingtonii. The striped appearance could have been caused by an interaction of host anatomy and environmental conditions, favouring longitudinally adjacent infection from an existing pustule in the leaf angle where the new tissue emerged. Norwood (1985) suggested that in field-grown leeks, subsequent infection within a plant occurred in the leaf sheath where trapped water provided ideal conditions for spore germination.

Spore Morphology

In general the urediniospores of the three rusts were very difficult to distinguish by qualitative light microscopy. The spines in the CHIVE and BABNT isolate did appear to be closer together than those on the BIRM isolate spores, but required comparative study (i.e. on the same slide under adjacent cover-slips) to be clearly noticeable. The lack of clear differences in germ pore cap morphology between the

isolates was in contrast to Henderson and Bennell (1979), who stated that the rust on leeks had more clearly capped germ pores. The extent of 'pore-capping' varied between individual spores, and may thus have been an artifact of the mounting conditions or simply a variation between spores.

The three isolates were difficult to distinguish using interference microscopy, though the walls of the BIRM isolate did appear to be thicker, which agrees with Savile (1961) and Henderson & Bennell (1979). However, in contrast to both authors, there were no clear-cut differences in germ pore cap morphology between the isolates, although this feature was very clear in all the isolates when viewed under interference contrast microscopy. In general, this technique would seem to be more useful for examining certain structural features rather than 'size differences', even qualitatively, since it is difficult to reproduce the same colour background between slides, and the apparent 'size' of features is affected by the lighting conditions.

The scanning electron microscope showed that there were essentially no morphological surface features which could be used to distinguish between the three isolates. The surface features were essentially as described by Uma (1984), except that no structures which could be described as germ pores were found on the surface of unimbibed spores. The circular depressions devoid of spines at the basal end of the spores, interpreted by Uma (1984) as germ pores were interpreted here as pedicel scars. Similar structures were described by

Stanbridge and Gay (1969) on urediniospores of Puccinia striiformis. The spine morphology on all three isolates was similar to the descriptions for most other fungi (Littlefield & Heath, 1979) in being conical and sometimes curved at the tip, with the angle between the spines and the spore surface being variable (Amerson & Van Dyke, 1978). Depressions and raised annuli are common in Puccinia spp. and Uromyces spp. The ones described here on all three isolates resembled those on similarly air-dried preparations of uredinia of Puccinia coronata f. sp. avenae (Corlett, 1970) and Uromyces dianthi (Jones, 1971), in being very shallow in mature spores. The variation in spine morphology between individual spores may reflect varying spore maturity as in Puccinia sparganoides (Amerson & Van Dyke, 1978). The same authors also noted that the surface between the spines was flat but slightly irregular, as described here.

Germ Pores

The use of phase-contrast with the aniline-blue staining technique considerably enhanced the visibility of the germ pores, especially those not in profile, making assessment of their number and their position more accurate. Several authors have commented on the value of germ pore number and position as reliable and consistent taxonomic characters, (Cummins, 1936; Wilson & Henderson, 1966; Hawker & Madelin, 1974; Cummins & Hiratsuka, 1983) although previous authors had described the germ pores of Uromyces ambiguus and Puccinia porri as indistinct (Gäumann, 1959; Wilson & Henderson, 1966). Savile (1984) stated that in urediniospores 'without wall pigment, a full pore count was usually difficult'. This technique may therefore be of

benefit in the study of the germ pores in other taxa. The germ pores in all three species here would be described as 'numerous and scattered' on the definition of Baum and Savile (1985), a primitive feature according to Cummins and Hiratsuka (1983), but one regarded as advanced in graminicolous rusts by Baum and Savile (1985).

From both phase contrast and SEM studies, it appeared that the germ pore regions were covered with spines and not visible or locatable on the exterior of dormant spores. This is similar to the urediniospores of U. dianthi described by Jones (1971), but different from the urediniospores of U. appendiculatus (Hardwick et al., 1975) and the studies of Uma (1984) on an isolate of leek rust. The germ pores of some other Puccinia spp. are clearly visible as 'pores' on the surface, e.g. as in P. triticina (Nilsson, 1983). Further evidence that the germ pores were overlain with spines in the BIRM isolate came from the SEM studies on germinating spores. The germ tubes appeared to peel back the outer wall layer in which the spines were embedded, with the germ tube itself continuous with an inner wall layer; this is consistent with the concept of the germ tube wall being continuous for a short distance inside the spore, (Littlefield & Heath, 1979) and in contrast to the claim of Manocha & Shaw (1967) that the germ tube wall of Melampsora lini was continuous with an outer layer. It may be that the structure varies between taxa. The cause of the rupture may be physical pressure or enzymic activity as the germ tube emerges. Studies with acid-base digestion in P. graminis f. sp. tritici showed that the germ pore differed chemically from the inner wall layer, since the site of the germ pore was left as a void after the

treatment (Ehrlich & Ehrlich, 1969). Jones (1971) noted that the germ tubes of U. dianthi did not rupture the outer wall when emerging.

Fluorescence Microscopy

The specificity of Calcofluor White M2R (CFW) has been shown to be rather broad, binding to B-configurated hexopyranose polymers such as cellulose, carboxymethylcellulose, mannan and chitin, (Maeda & Ishida, 1967). However, preferential binding to actively growing cell walls has been shown by Darken (1962) in hyphal tips and septa of Mucor and Penicillium, and by Hawes and Beckett (1977) when the intercalary and basal regions of conidiogenous cells of Ceratocystis adiposa exhibited greater fluorescence. Johnson et al. (1974) also showed preferential binding to wall structures, with cytoplasmic fluorescence only occurring when the cell wall was damaged. In the work here, the greater part of the urediniospore wall did not fluoresce at all, suggesting that either the spore wall does not contain any suitable binding polymers, or more likely that the wall has non-binding outer layers which exclude CFW. These layers may also be connected with the hydrophobic nature of the intact spore wall. The preferential binding to the hilar region, seen even in fixed spores of the BIRM isolate, suggests that this 'pore' region is the only part of the main wall where suitable binding sites for CFW occur. Ehrlich and Ehrlich (1969) described the wall as completely absent at this site, which suggests that fluorescence is related to accessibility to binding sites. The very distinct preferential binding to the germ pore regions on the 'inner wall' here indicated that these regions differ from the rest of the wall both chemically and metabolically,

supporting the views of Ehrlich & Ehrlich (1969), Bennell & Henderson (1978) and Savile (1984). Williams and Ledingham (1964) found that the main constituent of the germ pore region in P. graminis urediniospores was an electron-transparent 'middle-wall-layer'. The bright fluorescence of the germ tubes and protuberances observed in the BIRM isolate agree with the findings of Darken (1962) and Hawes & Beckett (1977) regarding the preferential binding of CFW to actively growing areas. Mendgen et al. (1985) found that FITC-labelled lectins bound to chitin on the surface of germ tube walls, whereas Ebrahim-Nesbat et al. (1985) described labelled wheat-germ lectin as binding to chitin throughout the entire thickness of the spore walls and the inner layer of the germ tube wall. The results here suggest that there is a wall layer of the germ tube which shows preferential binding to CFW, which is continuous with an inner layer of the spore wall. It appeared in the BIRM isolate, that although several protuberances could develop, only one normally proceeded to form a germ tube. Hardwick et al. (1975) described a similar situation in U. appendiculatus, and proposed that it was the first protuberance to rupture the pellicle that formed the germ tube. However the protuberances here were often substantial structures which then collapsed when a germ tube developed, suggesting that the control mechanism determining which protuberance forms the germ tube acts rather late on in the germination sequence. Formation of protuberances which then collapse seems wasteful and is difficult to explain though it could be related to the germination conditions in vitro. Jones (1971) also described the collapse of germ pores in germinated spores, though in

the SEM vacuum. The control of germ-tube emergence has therefore yet to be fully understood.

Quantitative Studies

Hierarchical Analyses

The results of the hierarchical studies indicated that most of the variation and significant differences were at the pustule or slide level, so for an accurate assessment of the characters as many slides from as many pustules as possible should be used. In practice this was achieved by using bulked spore samples on several slides. The similarity of the means of spore length etc. from the fresh and herbarium samples of the BIRM isolate indicated the remarkable retention of structural integrity long after the spores had lost their viability. However, many specimens in herbaria are considerably older than two years, and slow degradation of cell-wall components could occur after longer periods of dessic ated storage.

Comparison of the different Isolates

The normal distribution of quantitative characters is typical for individuals in a population, (Parker, 1979). However, most taxonomic descriptions give ranges, and it is possible for two populations to differ statistically in a given character despite considerable overlaps. Therefore statistical analyses of certain quantitative characters can be useful in comparing subtle differences between two populations, without necessarily being comparable with traditional taxonomic classification.

The most distinctive differences between isolates were in spine density, splitting the isolates into 'leek' and 'non-leek' groups, but these differences were difficult to see qualitatively. The length and pedicel scar diameter results generally supported these groupings, though both characters were subject to variation, and the actual differences between the means were small. The variation in the character of spore width may have been accounted for because it was a less strictly defined character, defined in terms of another character, spore length. It may also have varied with environmental factors, e.g. crowding of the spores in the pustule. The length/width ratios indicated that shape as well as size varied within the isolates. Very few authors have carried out quantitative or biometrical studies on rust urediniospores. Hiratsuka and Hiratsuka (1966) compared Uromyces durus and related rusts on Allium grayi in Japan, examining both teliospore and urediniospore characters. They measured urediniospore length, width, germ pore number and diameter, but did not analyse their data statistically. They found only small differences between 42 isolates in these characters, despite finding morphological differences in the telia. Goto (1933 & 1934) compared the urediniospores of several rust isolates from different Allium spp. in Japan. He examined spore length, width and length/width ratio. His figures for length and width were generally much lower than the figures here, length varying between 22.78 µm and 32.91 µm, (mean = 27.5 µm) and width 18.99 µm to 26.58 µm (mean = 22.80 µm), although he admitted to measuring immature spores. The length/width ratios varied from 0.9 to 1.8, so he apparently measured spores which were wider than long, though he did not define either length or width. These

large differences could reflect the different mounting medium compared with the^se studies (KOH as opposed to lactic acid), as well as different definitions of length and width and the effect of measuring large numbers of immature spores. It is therefore difficult to compare the results of his studies with those here.

Germ Pore Number

The results showed that this character was also normally distributed, indicating the inadequacy of using only a range to describe a population. This character was of major importance in differentiating between the BABNT and H.70 isolates and the rest, the differences being so large as to be clearly visible without quantitative analysis. However the destructive preparation technique prevented direct comparison with the other characters (e.g. spore length, width) except at the population level. The means and ranges of germ pore number in all the isolates were considerably greater than those previously described by all the authors on these rusts (von Tavel, 1932; Goto, 1933; Gaumann, 1959; Savile, 1961; Wilson & Henderson, 1966), demonstrating the improvement of the technique on previous methods of counting germ pore number.

Multivariate Analysis

The first principal component, which separated the isolates here into 'leek' and 'non-leek' groupings, is often a measure of size differences whereas information on structure can be obtained from a second and third component plot, (Temple, 1968; Dunn & Everitt, 1982). However, the results showed that consideration beyond the second - and

perhaps even the first component - was not justified in this case since the first component accounted for so much of the variation compared with subsequent components. The dendrogram reflected the principal component in producing a stronger 'leek'-'non-leek' split compared with the 'chive'-'non-chive' split. The differences between the fresh CHIVE isolate and the two other chive isolates from the herbaria could have been due to changes having occurred in storage over a long period of time. The H.70 isolate was more recent, and much more similar to the fresh BABNT isolate.

The conclusion from both numerical analyses was the existence of distinct groupings based on the host origin of the isolate, i.e. a 'leek' group and rather less distinct 'chive' and 'babingtonii-scorodoprasum' groups. This ties in with the traditional approach from the study of the quantitative characters, which separated the 'leek' and 'non-leek' groups on the basis of spine density, and split the 'chive' group on the basis of germ pore number. The major shortcomings of the multivariate analysis were the small number of characters, and the degree of linkage between them. Ideally, other characters, such as telial morphology, infection data and physiology could have been used, but these would have prevented comparison with herbarium material. Also, infection data relating to the pathology of the rusts could have been affected by physiological specialisation, based on a few genes, to produce rather different groupings. Another criterion which could have been altered was the weighting of the characters. A higher weighting for germ pore number would have distinguished the 'chive' and 'non-chive' groupings more

clearly, but such weightings are only valid when carried cut *a priori*. The decision of when or when not to weight characters is a difficult one in this type of analysis (Dunn & Everitt, 1982). However, the aim here was to analyse a complex set of data rather than produce a complete classification as Sneath & Sokal (1973) proposed, which ideally requires hundreds of characters.

Until now this technique has been limited in its use in fungal taxonomy to those taxa with few morphological characters, but where there are a large number of cultural characters, which are lacking in most rusts. Even so, despite its early adaptation for work on the fungi imperfecti (Kendrick & Proctor, 1964) and more recently in the genus *Rhizopus*, (Dabinett & Wellmann, 1973), the system is currently used most often to analyse the complex banding patterns produced by electrophoretic biochemical analyses of fungal groups (Chesson *et al.*, 1978; Jackman *et al.*, 1983; Seviour *et al.*, 1985) though agreement with traditional taxonomy is not always good (Shechter *et al.*, 1973). Kendrick and Weresub (1966) attempted to use numerical methods at the ordinal level of the Basidiomycetes, but found that 'haphazardly assembled, equally-weighted characters produce haphazard classification'. They argued that not all characters carry the same amount of information, and that a consideration of homology is important at higher levels of classification. Recently, numerical methods have been successfully applied at the species level in the genus *Drechslera* (Lam & Chapman, 1985) using a very large number of cultural, morphological and pathological characters, and despite the problems of linkage between characters (particularly cultural charac-

ters) the classification produced was very similar to the traditional approach. Therefore, where characters are limited, as in the rusts, multivariate analysis should be seen as a useful additional taxonomic tool, from which useful information can be drawn, rather than a replacement for traditional approaches.

Temperature of Urediniospore Germination in vitro

The high percentage germination recorded for the leek isolates (except the BIRM isolate) between 5°C and 22.5°C has important epidemiological considerations, but the lack of a clear optimum reduced the taxonomic value of this information. The performance of the BIRM isolate over a narrower range from 10°C to 20°C, may have been selected for by culturing the isolate in glasshouse conditions for so long. The sharp optimum of the CHIVE isolate indicated a more distinct temperature specialisation, but again its use as a taxonomic character was limited because it was so similar to the other isolates. Of more potential was the temperature characteristic of the BABNT isolate, with its low optimum of 10°C and distinct decline in its ability to germinate above 15°C, which was clearly different from the other isolates. It is difficult to explain why the BABNT isolate from the mild climate of Cornwall should perform better at low temperatures whilst the leek isolates from further north performed better at higher temperatures, with the rather high optimum of 20°C. This may reflect the separate origins of the rusts, or it could be connected with their life cycle, i.e. the leek isolates are adapted to the warmer temperatures of the late summer/early autumn, whereas the BABNT isolate is prevalent in

the colder months of February to March and is thus adapted to lower temperatures.

Studies on the telial stage

Telial formation

Telial formation on leeks in the U.K. would appear to be a rare event, having only been reported by Uma (1984) and Norwood (1985), (also in Harrison (1987)). The 'pre-telia' formed by leek isolates here appeared, both macroscopically and microscopically to be attempts to form normal telia and indeed normal teliospores were occasionally found. This suggests that the U.K. isolates have the ability to form telia, but are either losing that ability or require very specific environmental conditions. Harrison (1987) suggested that telial formation was triggered after an unusually hot, dry summer, and Uma (1984) reported telia forming abundantly on leeks in the glasshouse for seven months, after which formation became erratic, as well as forming on senescing leaf pieces maintained in vitro. Thus the evidence suggests an environmental factor controlling telial formation.

Macroscopic Study

The hard, black telia of the NVRS isolate were easily distinguishable from those of the other isolates, but the only real difference between the CHIVE and the BABNT telia was the greater size in the BABNT isolate,

which could have been a host-dependent effect, due to the greater size of the leaves of A. babingtonii compared with A. schoenoprasum.

Microscopic Study

The differences in the internal structure of the telia corresponded to the macroscopic differences; the NVRS isolate with its stromatic structure was clearly very different from the CHIVE and BABNT isolates. Although the telial structure of the BIRM isolate was misformed, the production of two-celled teliospores so early on would indicate that it was more closely related to the NVRS isolate than to the others. The structures of the CHIVE and BABNT isolates were very similar and suggested that they are more closely related to each other than the leek isolates. The only real differences between the BABNT and CHIVE isolates were the complete absence of two-celled spores from the BABNT isolate, and the predominance of obovoid teliospores in the BABNT isolate compared with the more often 'angular' spores of the CHIVE isolate. Nevertheless the two-celled teliospores of the CHIVE isolate resembled paler versions of the spores from the NVRS isolate.

SEM Study

The micrographs of the teliospores showed the complete absence of any form of ornamentation in any of the isolates. This is in contrast to the work of Uma (1984) who described irregularly reticulate ornamentation on the surface of teliospores of a leek isolate. Since the teliospores here were examined without any form of chemical preparation before gold-coating, the 'ornamentation' described by Uma (1984) may have been an artifact of the fixing process.

Teliospore germination

Both Norwood (1985), working on the NVRS isolate, and Uma (1984), working on a leek isolate originally obtained from field trials at NIAB, described the germination of teliospores and the formation of normal basidia and basidiospores. Uma (1984) also described the formation of abnormal basidia with only three septa and with elongated sterigmata. In the CHIVE isolate, both single and two-celled teliospores were seen to germinate, indicating that the single-celled 'mesospores' were in fact as mature as the two-celled teliospores. The structures produced varied but often resembled those described by Uma (1984) for leek rust. The 'abnormal' structures observed here on the CHIVE isolate may either have been the normal structures of this rust or induced by the environmental conditions, i.e. high levels of free water. Petersen (1974) described situations where the telia germinated to produce basidia with one, two or three cells, in various species of rust. In Uromyces aloes he describes the formation of one uninucleate and one trinucleate cell. Pavgi (1975) noted that physiological factors like excess water could cause abnormal teliospore germination, resulting in either transverse septa or no basidiospores. It is therefore likely that the CHIVE isolate could produce normal basidiospores in the right conditions. Also, aeciospores have been recorded on chives in the field in the U.K. (Dale, 1970), indicating that the rust on chives possesses a life-cycle of at least three stages.

The rare germination of the teliospores of the BABNT isolate indicates that this may be a redundant stage. The simple germ tubes produced by

those teliospores that did germinate might have some infective ability; again the environment may have influenced the development of the structures. Other spore stages have not been described for the rust on A. babingtonii, or for Uromyces ambiguus on any other host.

Both urediniospore and teliospore studies therefore suggest the existence of three morphologically distinct 'entities' or 'biotypes' of rust on Allium species (sections Allium and Cepa) in the U.K. Broadly, they can be described:-

(A) Isolates on leek, Allium porrum

(including telial specimens from NVRS (Norwood) and Manchester University (Uma).

Uredinia

Pustules rounded-lozenge shaped, along the axis of the leaf, often confluent; single pustules up to 10 mm long; epidermis raised into a 'bubble' with pronounced tear in the epidermis. Urediniospores subglobose to ellipsoidal, orange-rust coloured; (29)31-34(38) µm long, (24)25-28(30) µm wide. Germ pores with low or distinct caps, (8)9-11(14) per spore; distinct spines, with a density of (1.3) 1.5-2.0 (2.5) x 10^{-1} μm^{-2}.

Telia

Pustules scattered, occasionally confluent, dark grey to black in colour, very hard; up to 4 mm long and 2 mm wide. Teliospores long

covered by the epidermis. Interior of telia stromatic, with fused paraphyses forming locules in which spores develop. Teliospores (two-celled) dark brown in colour, ellipsoidal to obovoid with constriction between cells, lower cell longer than upper; apices attenuate to flattened, detached spores usually with pedicel remnant. Mesospores rare in mature telium*, similar in colour to teliospores, subglobose to obovoid. * (mature telia defined as those lacking urediniospores)

(B) Isolate on chive, Allium schoenoprasum

Uredinia

Pustules rounded or elongated along the axis of the leaf, sometimes confluent; single pustules 1-2 mm long, 10 mm long when confluent; epidermis sharply raised above surrounding tissue, with linear tear in epidermis exposing spores. Urediniospores subglobose to ellipsoidal, rarely globose, orange-rust coloured; (27)28-31(33) µm long, (21)24-26(28) µm wide; germ pores with low cap, (8)10-12(14) per spore; spines delicate, closely packed, (1.7)2.0-2.6(2.7) x 10^{-1} μm^{-2}.

Telia

Pustules scattered or confluent, pale grey or dull black, up to 5 mm long and 2 mm wide, with flattened surface. Teliospores (two-celled) rare in young telia, up to 40% in mature* telia, pale to dark brown in colour, ellipsoidal to obovoid, constricted between the cells, lower cell longer than upper; apices usually flattened or rounded, occasion-

ally attenuate. Mesospores predominant in young telia, 60% in mature* telium; pale brown, subglobose to pyriform. Paraphyses absent.

(C) Isolate from A. babingtonii

Uredinia

Pustules often rounded or lozenge shaped, elongated along leaf axis; up to 4 mm long, 1 mm wide, often confluent, up to 10 mm long, and/or in long stripes oblong leaf axis. Epidermis raised with narrow slit revealing bright orange spore mass. Urediniospores subglobose to ellipsoidal, bright orange or yellow coloured; (27)28-33(37) µm long, (23)24-27(30)µm wide; germ pores indistinct, high number per spore, (10)12-15(20). Spines closely packed, (1.6)1.9-2.6(3.0) x 10^{-1} μm^{-2}.

Telia

Pustules scattered or often confluent, pale grey colour, 5 mm long and 3 mm wide, confluent patches up to 40 mm long and 10 mm wide; surface flattened, slightly raised above surrounding tissue. Teliospores all one-celled, pale to chestnut brown, subglobose or pyriform, rarely obovoid. Paraphyses absent.

Thus the rust on leek could be separated from the rust on chives and A. babingtonii by its paraphysate telia containing mostly two-celled spores, and larger urediniospores with a lower spine density. The rusts on chive and A. babingtonii differed in the occurrence of two-celled teliospores in the telium, and in the number of germ pores in the urediniospores.

Herbarium material

Most of the material was in telial form only, and resources only permitted a qualitative study of the uredinial material. In general the rusts fell into the three categories found in the U.K. isolates, with an intermediate category (between the chive and A. babingtonii rusts) on certain specimens of A. scorodoprasum. The rust on A. cepa 1307, which did not resemble the other isolates, was the type specimen of Puccinia allii-cepulae.

The differentiation of the types on A. scorodoprasum would require the study of urediniospore germ pore number to separate those specimens with low percentages of two-celled teliospores into either the 'chive' or 'babingtonii' types. In the meantime, there are several possibilities; the specimens could be immature 'chive type' rusts; 'babingtonii type' rusts with a few two-celled teliospores, or a true intermediate. It is also possible that the host plants may have been misidentified, but at present it appears that A. scorodoprasum is a potential host for both 'chive' and 'babingtonii' type rusts.

Another anomaly was the occurence of the 'leek type' rusts on A. schoenoprasum specimens from Scandinavia, whereas all the other European specimens bore the 'chive type' rust. The 'leek type' predominated in the Mediterranean specimens, and Baum and Savile (1985) stated that the presence of melanized paraphyses forming locules was an adaptation to hot, dry climates. It is therefore difficult to explain why the 'leek' type should be the pathogen on A. schoenoprasum in the Scandinavian area, in preference to the 'chive

type' predominant in the rest of Europe. It is possible that a locular structure is an adaptation to all forms of extreme climate or severe dehydration, which would explain its occurrence in both very hot and very cold climates.

Summary of morphological study

The work here has confirmed the existence of three 'biotypes' of rust, on Allium species in the U.K. based on telial and uredinial characters. The telial descriptions closely match those of von Tavel (1932) and Gaumann (1959) describing three species, Puccinia allii, P. porri and Uromyces ambiguus, and of the descriptions of P. allii and P. porri of several authors (Sydow, 1904; Schneider, 1912; Goto, 1934 & 1935). However, the work here has correlated telial and uredinial characters for the first time, thus solving the taxonomic confusion over the status of the rusts in the U.K. and the apparent errors in the U.S. literature (Savile, 1961). The confusion in the U.K. literature arose primarily because of the absence of telial material on leeks, preventing comparison with the European classification. Savile (1961) working in the United States accepted the European description of P. allii from Hylander et al. 1953 and accepted too that the rust on A. schoenoprasum was different, which he called P. mixta. Then, lacking telial material of on leeks, he separated P. porri from P. mixta on the basis of urediniospores characters. It would appear that this delineation was artificial - the urediniospore characters of his 'P. porri' actually corresponded to those of the European P. allii, and his 'P. mixta' to the European P. porri.

Henderson & Bennell (1982) perpetuated this error for the rust on leeks in the U.K., which could only be corrected with the discovery of the telial stage in the U.K. which occured in the early 1980's (Uma, 1984; Norwood, 1985). Savile did not however distinguish between the American P. blasdalei and the European P. allii, though both produce locular sori with two-celled teliospores on Allium species. A comparison of these two species is therefore necessary to find out if they are actually different species.

Therefore the rust on leeks in the U.K. is the same found on most cultivated Allium species in central and Mediterranean Europe, and on A. schoenoprasum in Scandinavia. The rust on A. schoenoprasum is a distinct entity in central Europe and the U.K. in agreement with Rytz (1923), and a rust exists on A. babingtonii and A. scorodoprasum in the U.K. which is closely related to but distinct from the rust on chives, and forms resembling both types occur on A. scorodoprasum in central and Eastern Europe.

CHAPTER THREE

INFECTION STUDIES ON A WIDE RANGE OF *ALLIUM* SPECIES

3.1. INTRODUCTION

Infection studies have been used by previous workers on the rusts on *Allium* species to help compare and delimit the rust species (Schneider, 1912; von Tavel, 1932; Goto, 1933). This approach effectively uses the host-pathogen interaction as a physiological taxonomic character of the rust. However, such information, if sufficiently detailed, can also be used to locate possible sources of resistance, as well as provide epidemiological information about wild volunteer host species that might act as reservoirs or foci for the spread of the disease to crops, and also indicate the potential of the disease to attack new crop species, such as *A. sativum* in the U.K.

What constitutes a host in this context can be difficult to define. Heath (1980) makes the distinction between 'plant species specificity' (determining host species range) and 'cultivar specificity' (determining cultivar range within a given host species) since she states that the principles and mechanisms determining these levels are different, and may coexist in a plant. Thus she compares 'nonhost' resistance with the highly specific resistance- susceptibility reactions occuring in 'host' species. However her the definition of a 'nonhost' as a 'plant species not usually considered to be hosts for the pathogen in question' leaves a lot to be desired, especially when

the 'host range' and host taxonomy are not fully elucidated. Anikster (1984) compared the physiological specialisation of the forma speciales within a rust species, with the morphological boundaries of rust species. He states that the 'host ranges' of morphological species in the rusts can vary from hundreds of species in dozens of genera to a few species in one or two genera, but again fails to define what a 'host' is. However he does point out that host ranges observed from artificially inoculated plants are often much greater than those seen in the field. The basic problem remains however, of distinguishing a 'nonhost' reaction from a 'resistant-host' reaction in a host-pathogen system where existing information is limited. Previous workers on the Allium-rust interaction have recorded different characters to provide such information. Goto (1933) simply recorded the time from inoculation to uredinial and telial formation, as well as descriptions of the lesions formed, though he did not attempt to classify or compare these reactions. Von Tavel (1932), simply recorded the presence or absence of the spore stages, in extensive infection tests on a large number of Allium species, in several sections of the genus.

It was decided here to attempt to apply a 'lesion-type' assessment to a range of cultivated Allium species and their wild relatives. Such assessments have been developed to record the responses of field crops (Eskes, 1982; Stubbs et. al., 1986) and modified for use in glasshouse work, (Zadoks, 1961). The system used here (see section 1.2.10.) is a simplified version based on the appearance of symptoms rather than a perceived interpretation of what they represent (e.g.

whether brown spots are necrotic or not). In addition, a qualitative pustule quantity key was used to provide additional information. The other characters recorded were infection period and latent period, to separate reactions differing only quantitatively, (see section 1.2.10.), and the formation of telia. From this the aim was to :-

a) Look for differences between rusts originating from different host species,

b) Look for differences between the leek isolates,

c) Investigate the levels of resistance and susceptibility to provide basic epidemiologic information.

3.2. MATERIALS AND METHODS

3.2.1. Materials

a) Inoculum

The urediniospore isolates used to inoculate the test plants are detailed in 1.2.1. The STOCK, CORN, CHIVE and BABNT isolates were subcultured on their respective hosts before the trials. The other isolates were original field isolates.

b) Test plants

Details of the host plants are given in table 3.1. The range was selected to include the major European crop Alliums together with the common wild Allium species from the same sections of the genus. The range inoculated using the BABNT isolate was restricted by the small quantity of inoculum available.

3.2.2. Methods

3.2.2.1. Inoculation

The host plants were inoculated using the 'brush-on' technique (1.2.9.(a)) on a 5 cm long area marked with a waterproof pen in the middle of each of two leaves of each plant. For each cultivar/acession two or three replicate plants were inoculated in

each trial. The inoculation method for the BABNT isolate was modified after initial trials; the plants were inoculated as above but before being covered with a polythene bag, a 0.25 cm^3 drop of Tween-80 solution was smeared onto the marked area to provide the spores with free water. The plants were then maintained horizontally until the bags were removed to prevent the Tween-80 solution from running off.

3.2.2.2. Experimental Conditions

The inoculated plants were placed in a randomised design in a controlled growth room at a temperature of 18 +/- 2 ^{o}C for the leek isolate trials, 15 +/- 2 ^{o}C for the CHIVE isolate trials and 12 +/- 2 ^{o}C for the BABNT isolate for the first two days and 15 +/- 2 ^{o}C for the subsequent 19 days. A daylength of 16 hours was provided by mercury vapour lamps giving a photon flux density in the photosynthetic range of 45 +/- 5 $\mu mol\ m^{-2}\ s^{-2}$ at soil level.

3.2.2.3. Disease Assessment

The plants were examined daily after inoculation for the appearance of disease symptoms. The incubation period and latent period were measured to the nearest day on each plant. After 21 days, assessments of lesion type, pustule density , pustule quantity and telial formation were made.

Table 3.1

List of *Allium* accessions inoculated with the selected rust isolates in the host range trials

Species	Accession/Cv .	Origin
Section *Allium*		
A. ampeloprasum	418	Guernsey XI.82
A. babingtonii	562	RBG Kew 6.VII.84
"	702	Cornwall
A. kurrat	632	IVT Wageningen Accn. 77432
"	664	Egypt I.85
A. porrum	Musselburgh	Sutton's
"	495	Martin Luther Univ. DDR
A. sativum	Blanc de Dome	NVRS
"	Fructador	NVRS
A. scorodoprasum	366	NVRS/BU
Section *Cepa*		
A. cepa	Rijnsburger	Johnson's
"	Imai Early Yellow	Hursts
A. fistulosum	Ishikuro	Thompson & Morgan
A. schoenoprasum	701	BU
A. vineale	706	NVRS

Key

BU - University of Birmingham Gene Bank
NVRS - National Vegetable Research Station

3.3 RESULTS

The results for each component recorded are given in tables 3.2 to 3.6.

In general there was very good agreement between duplicates in all the tests. There were few differences in incubation period or latent period with never more than one days' difference between duplicates. The only major differences were in pustule quantity, especially between duplicates of Allium scorodoprasum. Another feature was the variation in the quantitative characters between leek isolates - for example latent period in cv. Musselburgh varied from 11 days (NVRS) to 15 days (WSCOT). It should be noted that the environments for the CHIVE and BABNT isolate tests were different from the leek tests when comparing such quantitative characters.

Incubation periods varied from 4 days (BIRM isolate on A. sativum cv. Blanc de Dome) to 14 days (CHIVE isolate on A. vineale). In general the results between the leek isolates were fairly uniform; incubation periods were consistently longer for A. ampeloprasum 418, A. schoenoprasum, A. babingtonii 562 and 702, uniformly short (6 to 8 days) for leek isolates on both accessions of A. kurrat; and variable on A. cepa (7 to 11 days), A. fistulosum (7 to 12 days) and A. schoenoprasum (6 to 13 days). The two cultivars of A. cepa had a differential response to the NVRS and CORN isolates but otherwise performed similarly.

Host Plant	Acc./cv.	Isolate								
		BIRM	NVRS	LUDD	NIAB	STOCK	CORN	WSCOT	CHIVE	BABNT
A. ampeloprasum	418	14	10	8	X	10	9	X	-	-
A. babingtonii	562	10	8	9	6	8	7	7	9	12
"	702	9	7	8	6	9	8	7	13	11
A. kurrat	632	8	6	8	6	6	7	8	X	-
"	664	8	6	7	7	6	7	8	7	-
A. porrum cv.	Musselburgh	6	7	8	5	8	7	7.5	7	11
"	495	7	6	7	6	8	7	10	8	-
A. sativum cv.	Blanc de Dome	4	6	8	9	9	7	7	4	-
"	Fructador	6	8	6	6	7	7	7	10	-
A. scorodoprasum	366	7	5	8	7	8	-	7	9	-
A. cepa cv.	Rijnsburger	8	7	7	7	6	11	11	7	-
" cv.	Imai Early Yellow	8	11	7	7	6	7	11	10	-
A. fistulosum cv.	Ishikuro	12	7	7	6	6	12	9	7	11
A. schoenoprasum	701	13	6	X	10	9	8	11	8	13
A. vineale	706	9	7	10	9	9	8	8	14	-

Key X no symptoms (immune reaction)
\- not tested

Table 3.2 Incubation period of the rust isolates on selected species from the genus Allium. Mean time (in days) after inocultion to the appearance of pale green flecks. Means of 2 replicates.

Host Plant	Acc./cv.	Isolate								
		BIRM	NVRS	LUDD	NIAB	STOCK	CORN	WSCOT	CHIVE	BABNT
A. ampeloprasum	418	20	14	13	X	17	X	-	-	-
A. babingtonii	562	17	11	18	12	X	14	15	X	15
"	702	16	12	14	11	12	14	15	X	16
A. kurrat	632	12.5	11	12	10	12	14	14	X	-
"	664	8	6	7	7	6	7	8	7	-
A. porrum cv.	Musselburgh	13	11	13	12	12	14	15	20	X
"	495	14	13	13	11	13	13	13	X	-
A. sativum cv.	Blanc de Dome	22*	12	14	17	18	17	18	12	-
"	Fructador	14	12	12	11	12	13	13	14	-
A. scorodoprasum	366	13	13	12	11	13	-	15	X	X
A. cepa cv.	Rijnsburger	X	X	X	X	X	16	X	X	-
" cv.	Imai Early Yellow	X	17	20	X	X	X	X	20	-
A. fistulosum cv.	Ishikuro	18	11	14	12	14	17	16	10	16
A. schoenoprasum	701	X	X	X	X	X	X	X	13	X
A. vineale	706	15	18	15	14	15	19	18	21	-

Key X no sporulation (immune/resistant)
\- not tested
* pustule visible from day 20 ruptured on day 22

Table 3.3 Latent period of the rust isolates on selected species from the genus Allium. Mean time (in days) after inoculation to sporulation. Means of 2 replicates.

Host Plant	Acc./cv.	Isolate								
		BIRM	NVRS	LUDD	NIAB	STOCK	CORN	WSCOT	CHIVE	BABNT
A. ampeloprasum	418	2	1	2	i	2	0	-	-	-
A. babingtonii	562	3	3	2	3	0	3	3	0	3
"	702	3	2	2	2	3	3	3	0	4
A. kurrat	632	3	3	3	3	4	4	4	i	-
"	664	3	3	3	3	3	3	4	0	-
A. porrum cv.	Musselburgh	3	4	3	3	3	3	3	1	0
"	495	3	3	3	3	3	3	3	0	-
A. sativum cv.	Blanc de Dome	2	2	2	1	2	2	3	3	-
"	Fructador	3	3	4	2	4	3	3	2	-
A. scorodoprasum	366	2	3	3	3	3	-	2	0	i
A. cepa cv.	Rijnsburger	0	0	0	0	0	2	0	0	-
" cv.	Imai Early Yellow	0	1	1	0	1	0	0	0	-
A. fistulosum cv.	Ishikuro	2	2	2	2	1	2	1	3	3
A. schoenoprasum	701	0	0	i	0	0	0	0	4	0
A. vineale	706	3	1	2	2	2	2	1	1	-

Key - not tested

Table 3.4 Lesion type reaction on selected Allium species to inoculation by the rust isolates after 21 days.

Host Plant	Acc./cv.	Isolate								
		BIRM	NVRS	LUDD	NIAB	STOCK	CORN	WSCOT	CHIVE	BABNT
A. ampeloprasum	418	2-3	2	3	i	1-2	0	-	-	-
A. babingtonii	562	4	3-4	1	4	0	4	5	0	3
"	702	4	3	1	4	4	4	4	0	4
A. kurrat	632	4	4.5	4	5	5	4	5	0	-
"	664	4	4.5	4	5	5	4	5	0	-
A. porrum cv.	Musselburgh	5	5	3	4.5	4	4	4.5	1	0
"	495	4	4	3	5	4	4	4.5	0	-
A. sativum cv.	Blanc de Dome	3	4	1	2	3	2	2	3	-
"	Fructador	4	4	4	4	4	4	3	1	-
A. scorodoprasum	366	4	3.5	3	3.5	4	-	2.5	0	i
A. cepa cv.	Rijnsburger	0	0	0	0	0	2	0	0	-
" cv.	Imai Early Yellow	0	2	2	0	2	0	0	1	-
A. fistulosum cv.	Ishikuro	3	4	2	4	2	1	2	3	3
A. schoenoprasum	701	0	0	i	0	0	0	0	4	0
A. vineale	706	4	2	3	4	3	1	1	1	-

Key - not tested

Table 3.5. Pustule quantity measurements on selected Allium species inoculated with the rust isolates, after 21 days. (Figures from both replicates given where different).

Host Plant	Acc./cv.	Isolate								
		BIRM	NVRS	LUDD	NIAB	STOCK	CORN	WSCOT	CHIVE	BABNT
A. ampeloprasum	418	0	0	0	X	0	X	-	-	-
A. babingtonii	562	0	0	0	0	0	0	0	X	+
"	702	0	0	0	0	0	0	0	X	+
A. kurrat	632	0	0	0	0	0	0	0	X	-
"	664	0	+	0	0	0	0	0	X	-
A. porrum cv.	Musselburgh	0	0	0	0	0	0	0	+	X
"	495	0	0	0	0	0	0	0	X	-
A. sativum cv.	Blanc de Dome	0	0	0	0	0	0	0	+	-
"	Fructador	0	0	0	0	0	0	0	+	-
A. scorodoprasum	366	0	0	0	0	0	-	0	X	X
A. cepa cv.	Rijnsburger	X	X	0	0	0	0	0	X	-
" cv.	Imai Early Yellow	X	0	0	0	0	0	0	0	-
A. fistulosum cv.	Ishikuro	0	0	0	0	0	0	0	0	+
A. schoenoprasum	701	X	X	X	X	X	X	X	+	X
A. vineale	706	0	0	0	0	0	0	0	0	-

Key
- \- not tested
- X no sporulation
- \+ telia & uredinia
- 0 uredinia only

Table 3.6 Telial formation on selected Allium species by the rust isolates after 21 days. Presence/absence score.

Latent period followed a similar trend to incubation period but the two were not always related. Many of the infections grew to be quite large without actually sporulating, or sporulated very late. For the leek isolates the shortest latent periods were on the A. kurrat accessions, and were longer on A. sativum cv., Blanc de Dome, A. vineale and A. cepa (both accessions). Many of the infections on the A. cepa accessions failed to sporulate, and the infection of the BIRM isolate on A. sativum cv. Blanc de Dome, visible as a green fleck after 4 days did not sporulate until 22 days after inoculation. Latent period varied considerably between isolates in both accessions of A. babingtonii, A. fistulosum and A. vineale. Latent period in the CHIVE isolate varied from 10 days in A. fistulosum to 20 days on A. porrum cv. Musselburgh, with 12 to 14 days on A. schoenoprasum and both cultivars of A. sativum. The BABNT isolate only sporulated on A. babingtonii and A. fistulosum, after 15 to 16 days.

The responses to attack by all the isolates varied from 'immune' (lesion type 0) to highly susceptible (lesion type 5). In some cases where there was no visible reaction (immune) the leaf surface was examined for signs that the inoculum had germinated successfully and this was always found to be the case. In general the pustule quantity followed the trend of lesion type though there were several examples where there were very few, but very 'susceptible' reactions and where there was a resistant reaction, with quite high levels of sporulation.

One feature of the responses observed in these tests was the presence of large green flecks, either with or without sporulating pustules.

Upon examination such green flecks were found to contain colonies of mycelium, occasionally with a few collapsed cells in the centre. In some cases sporulating pustules would develop in the centre of the flecks after a long latent period to produce a 'resistant' or 'moderate' (lesion type 1 or 2) reaction by 21 days.

All accessions of A. porrum and A. kurrat showed susceptible reactions against all the leek isolates, with correspondingly large numbers of pustules (figs. 3.1 & 3.2). A. scorodoprasum and A. babingtonii showed 'moderate' to 'susceptible' reactions but in both cases there were often large numbers of sporulating pustules despite a large number of large green flecks (fig. 3.3). A. sativum cv. Blanc de Dome showed moderate reactions to all but the WSCOT isolate, whereas cv. Fructador showed 'susceptible' to 'highly susceptible' (lesion type 4 or 5) reactions (Figs 3·4 & 3·5). A. cepa cv. Rijnsburger produced lesion type 2 reactions with the CORN isolate but otherwise produced non-sporulating reactions. A. cepa cv. Imai Early Yellow produced resistant, sporulating reactions (lesion type 1) to NVRS, LUDD and STOCK isolates. In both A. cepa cultivars there were often large areas of necrotic tissue adjacent to infection sites though this did not always prevent sporulation, (Figs. 3.6 &3.7). A. schoenoprasum was highly resistant or immune (lesion type 0 or 1) to all the leek isolates, but A. ampeloprasum showed a variety of reactions from immune (0) to moderate (2).

The CHIVE isolate produced susceptible reactions on A. fistulosum and A. sativum cv. Blanc de Dome, and highly susceptible reactions on A.

schoenoprasum, but moderate to resistant reactions on A. sativum cv. Fructador and A. porrum cv. Musselburgh (Figs 3.8 to 3.11), but resistant or immune reactions on the remaining accessions. The BABNT isolate produced susceptible reactions on A. babingtonii and A. fistulosum, but resistant or immune reactions on A. porrum cv. Musselburgh, A. scorodoprasum acc. 366 and A. schoenoprasum.

The only leek isolate to produce telia was the NVRS, on A. kurrat acc. 664. The CHIVE isolate produced telia on A. porrum cv. Musselburgh, A. sativum (both cultivars) and A. schoenoprasum, but not on A. fistulosum. The BABNT isolate produced telia on all the plants where uredinia were formed, often only a few days after the appearance of the uredinia. The morphology of the telia from each isolate conformed to that described for each one in Chapter 2.

Figure 3.1
A. porrum cv. Musselburgh, 21 days after being inoculated with the BIRM rust isolate, showing pustules with halos (H). (Lesion type 3)

Figure 3.2
A. kurrat acc. 632, 21 days after inoculation with the CORN rust isolate, showing pustules without halos. (Lesion type 4)

Figure 3.3.
A. scorodoprasum acc. 366, 21 days after inoculation with the BIRM rust isolate, showing pustules with numerous green flecks. (Lesion type 2)

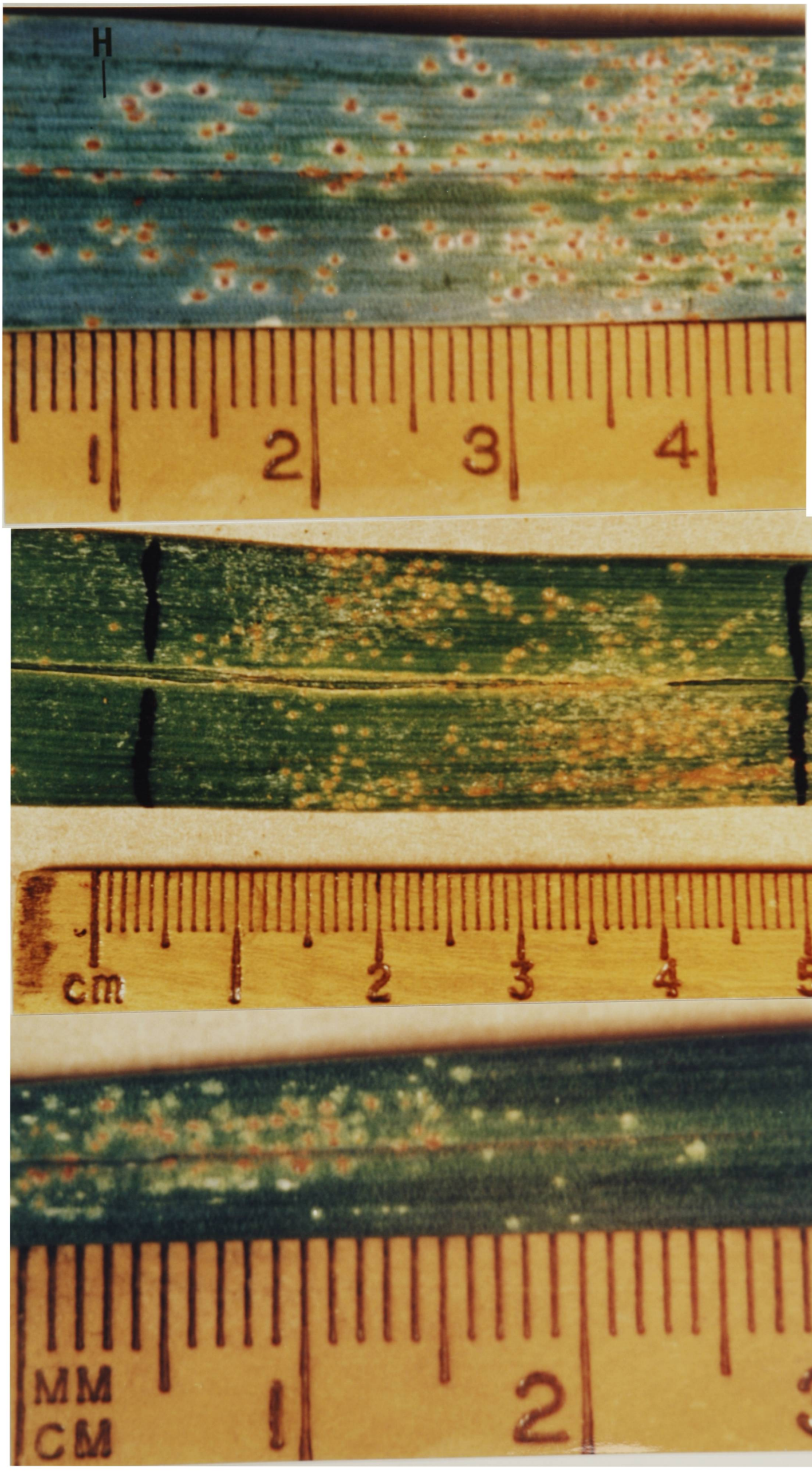
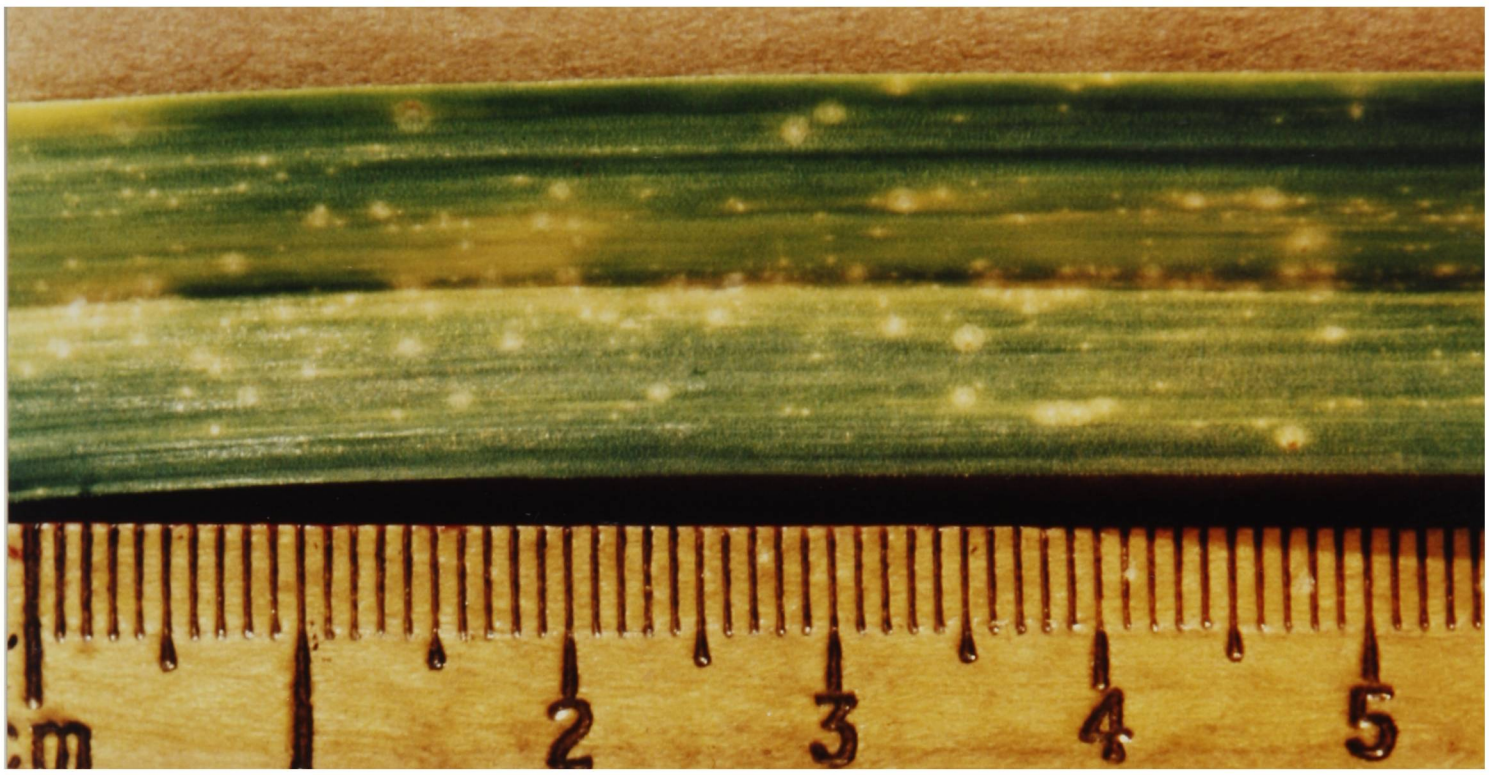

H
1
2
3
4
cm
2
3
4
5
MM
CM
1
2
3

Figure 3.4
A. sativum cv. Blanc de Dome, 23 days after inoculation with the BIRM isolate, with numerous yellow-white flecks, some with white centres, and few pustules, (Lesion type 2)

Figure 3.5
A. sativum cv. Fructador, 21 days after inoculation with the BIRM isolate showing large pustules and distinctive lozenge- shaped halos, (Lesion type 3)

Figure 3.6
A. cepa cv. Imai Early Yellow, 21 days after inoculation with the BIRM rust isolate, showing distinctive yellow flecks. (Lesion type 0)


cm
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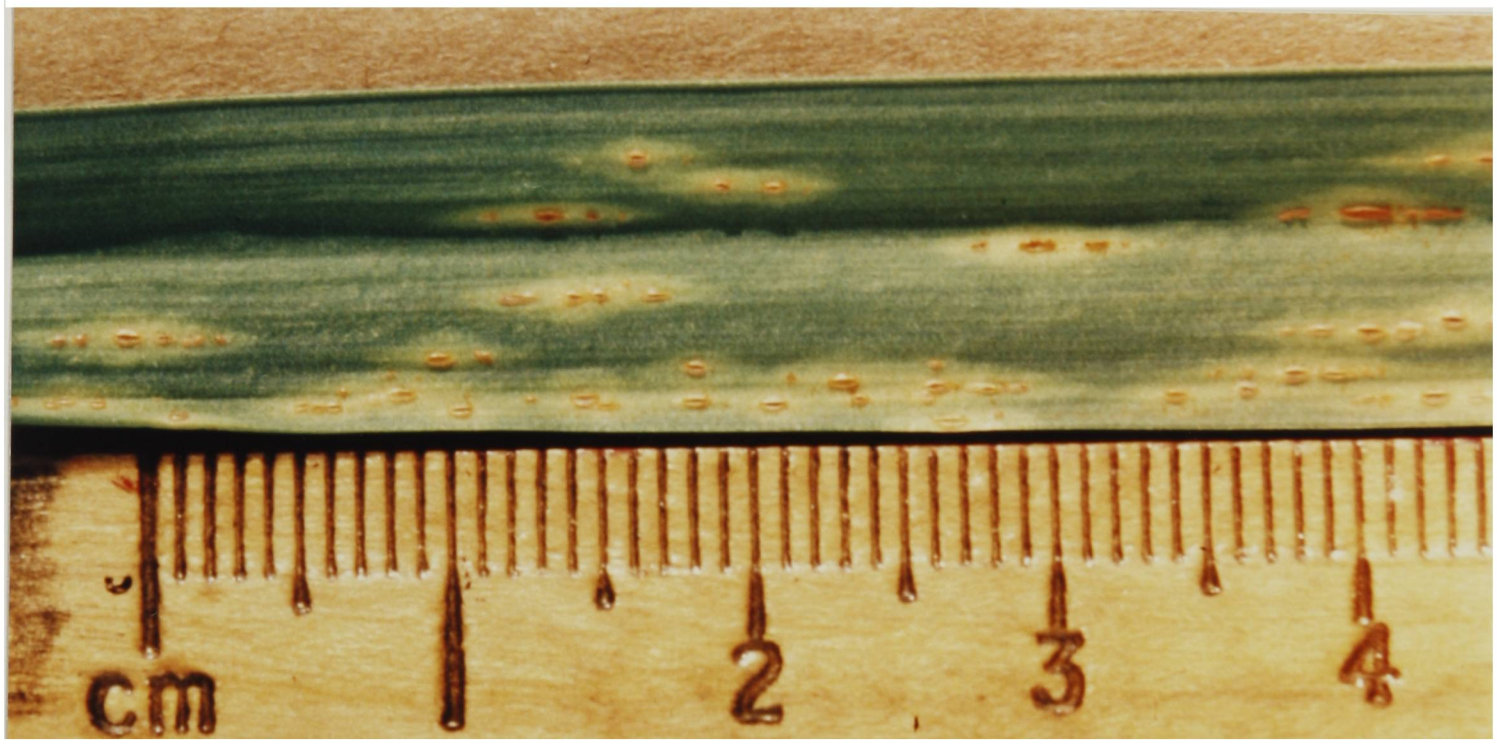
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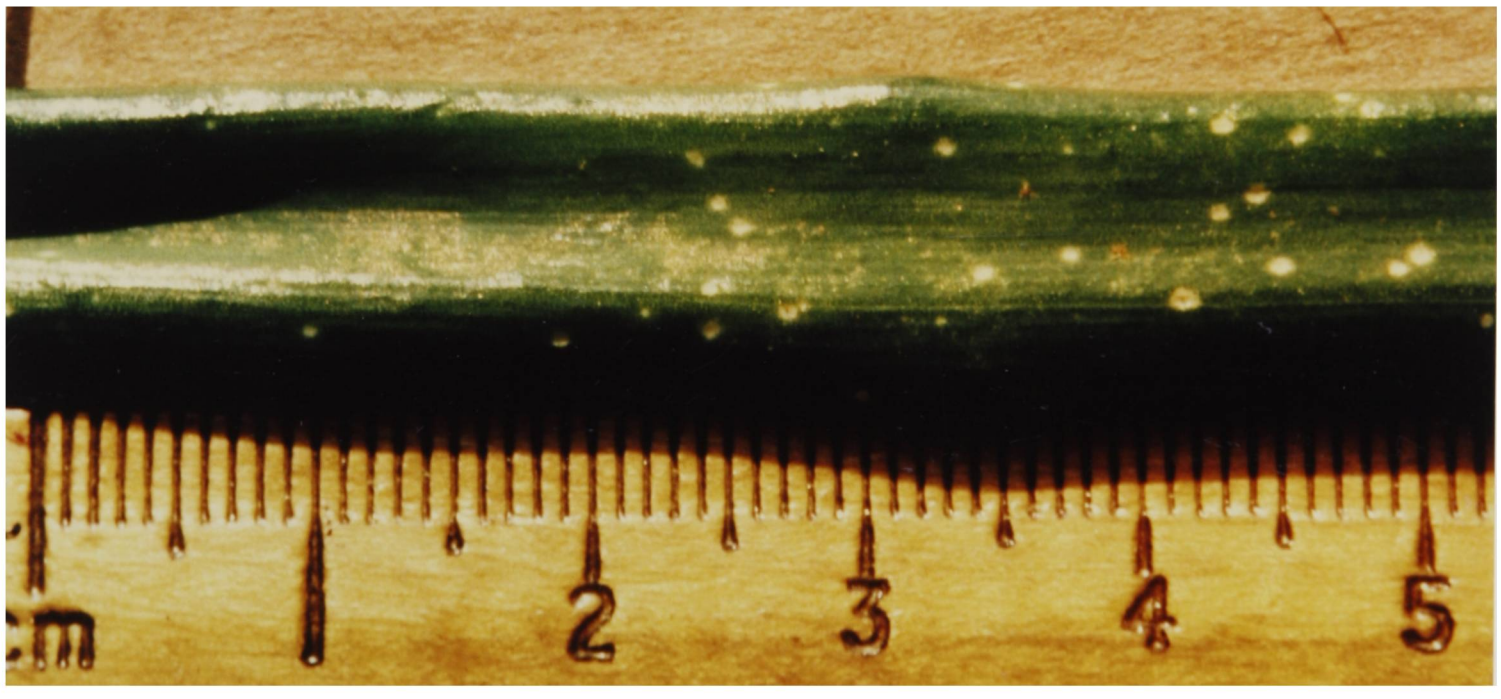
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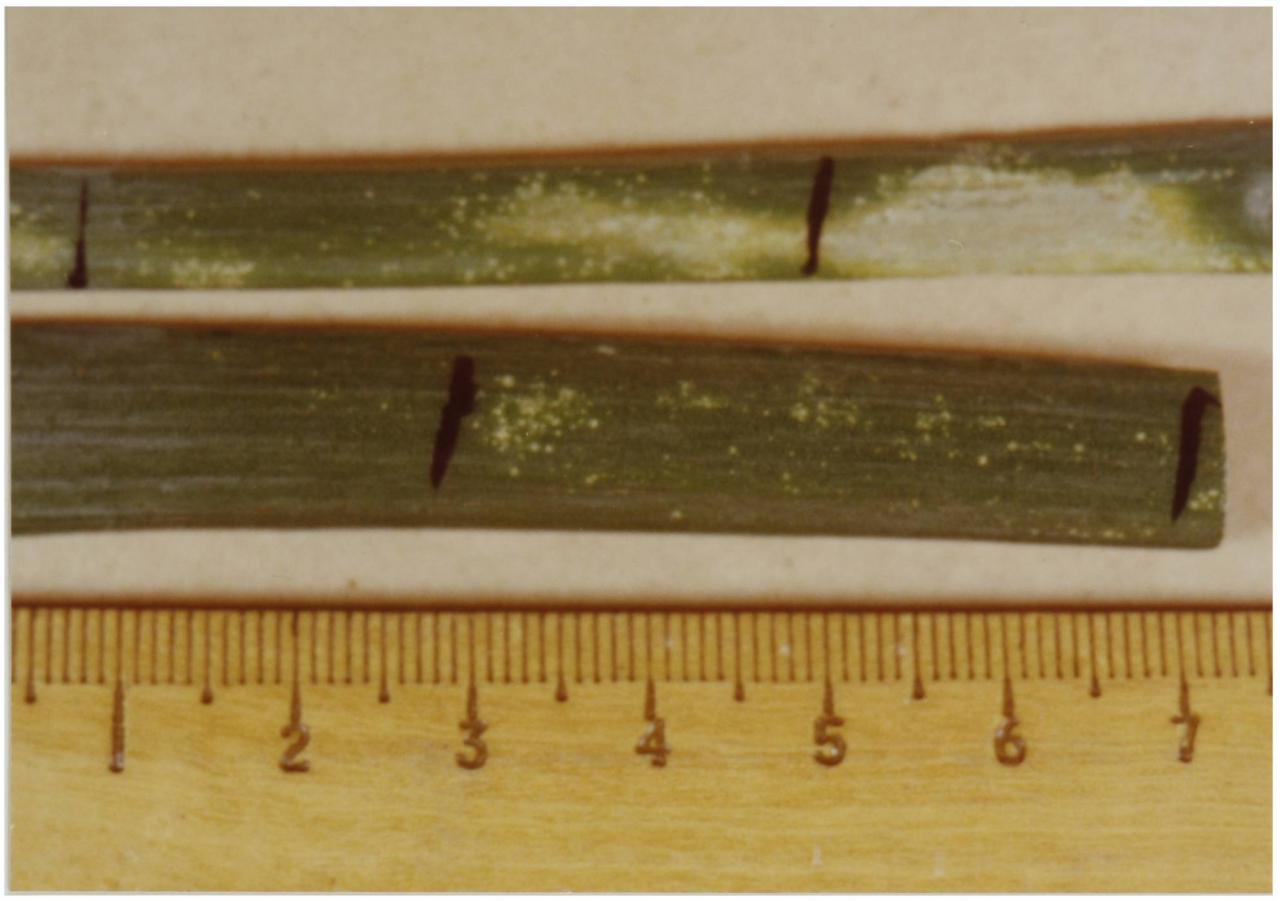


Figure 3.7
A. cepa cv. Imai Early Yellow, 21 days after inoculation with the LUDD rust isolate, showing yellow flecks, large areas of necrotic tissue and small pustules. (Lesion type 1)

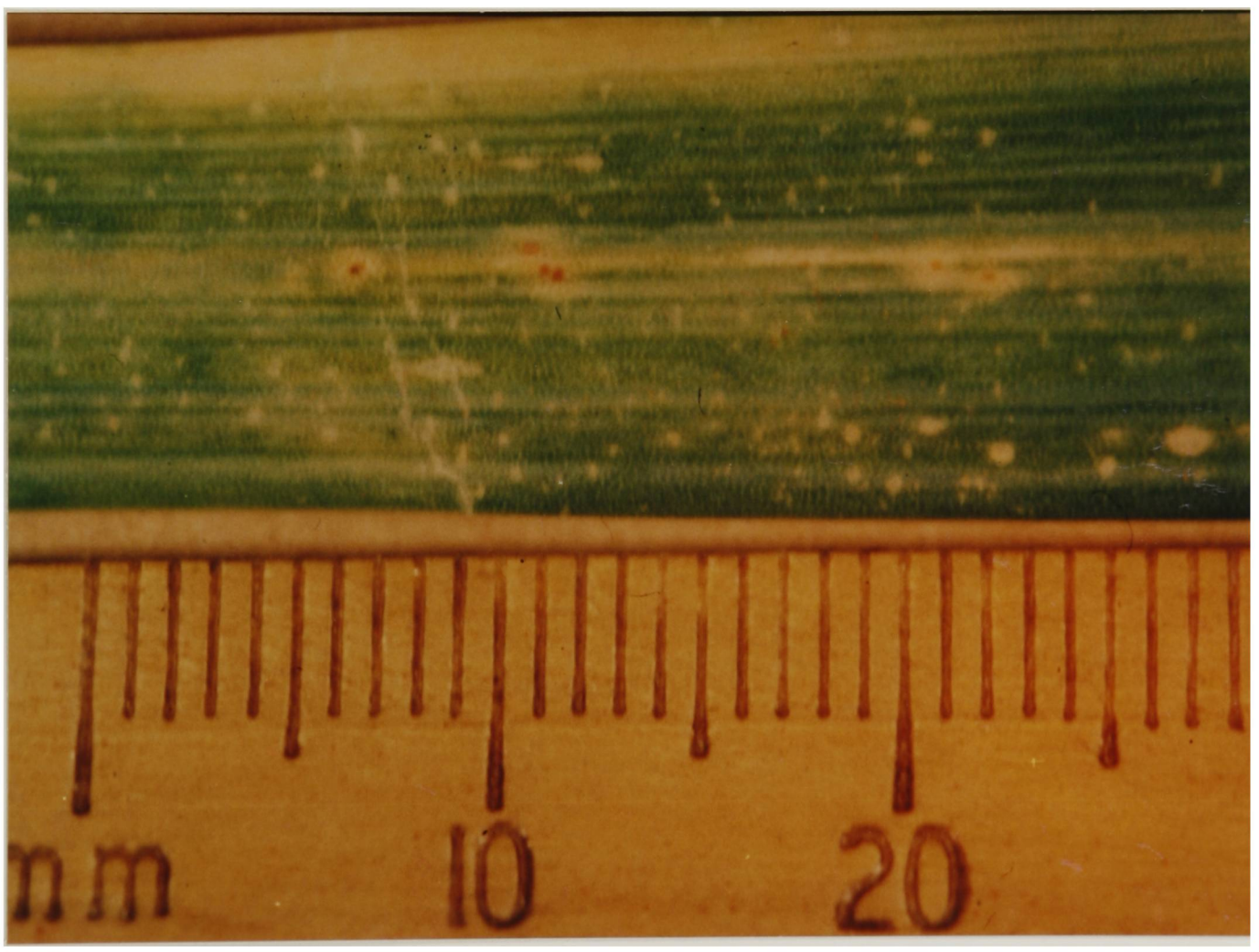
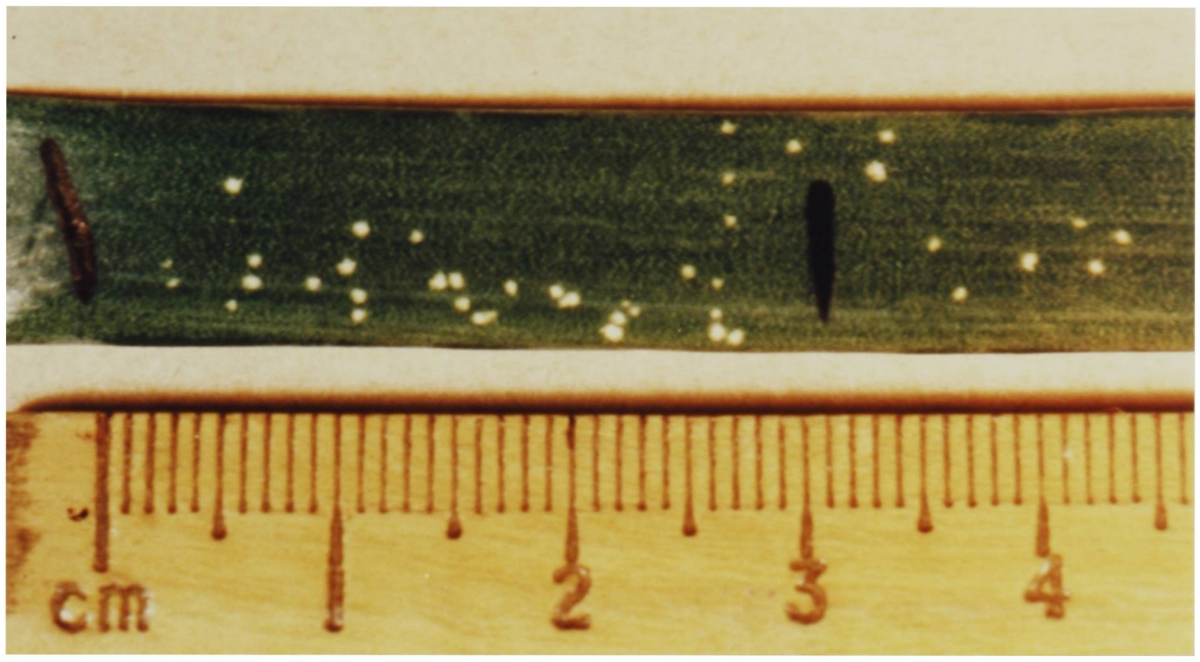


Figure 3.8
A. porrum cv. Musselburgh 21 days after inoculation with the CHIVE isolate, showing numerous yellow-brown flecks and small pustules. (lesion type 1)

Figure 3.9
A. cepa cv. Imai Early Yellow 23 days after inoculation with the CHIVE isolate, showing large yellow-brown flecks, (Lesion type 0)

Figure 3.10
A. sativum cv. Blanc de Dome 23 days after inoculation with the CHIVE isolate, showing young telia with large yellow halos on senescing leaf tissue. (Lesion type 3)

Figure 3.11
A. schoenoprasum acc. 701 21 days after inoculation with the CHIVE rust isolate, showing mature telia and telia with uredinia remaining in the centre. (Lesion type 4)


cm
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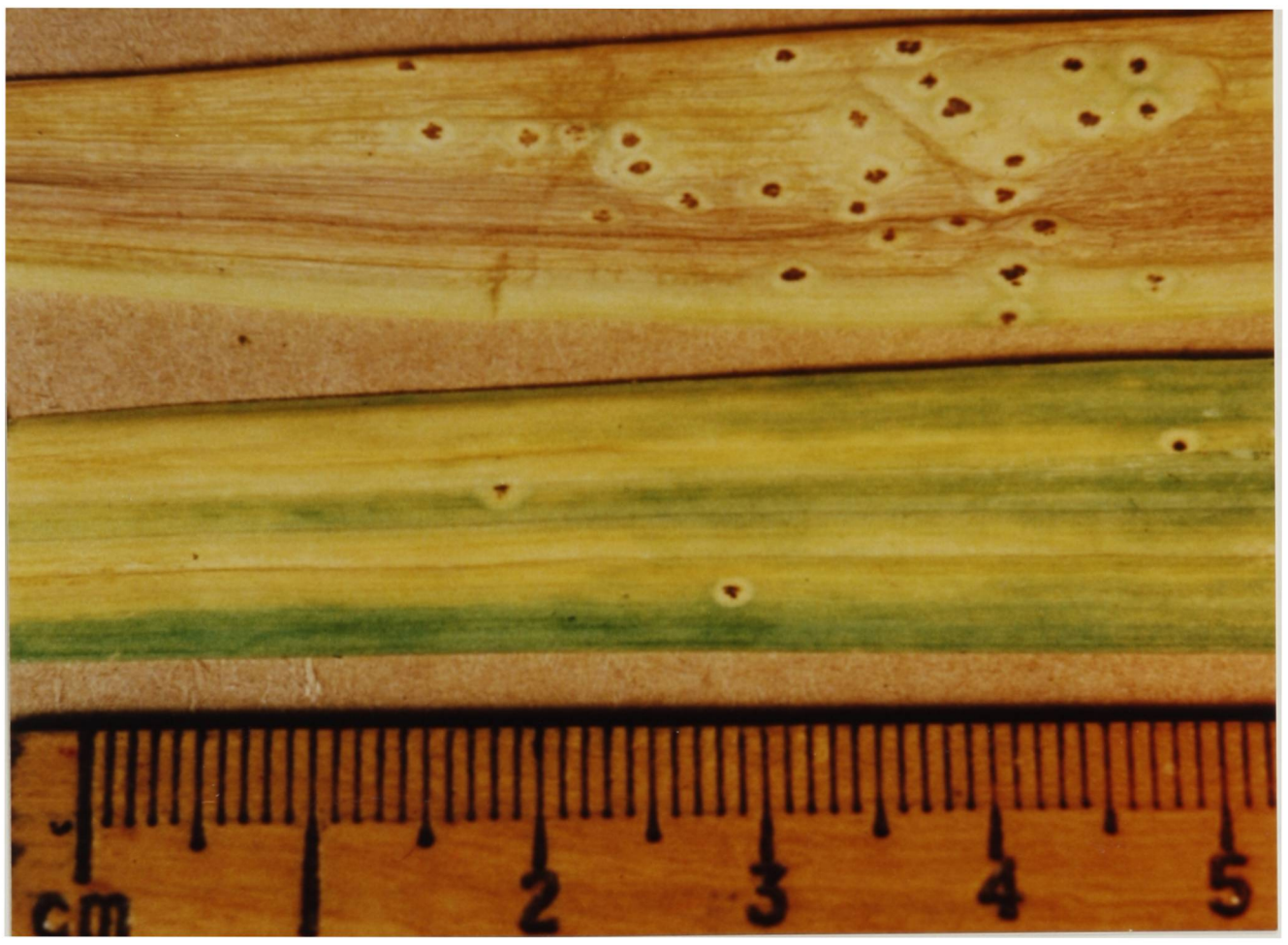
cm
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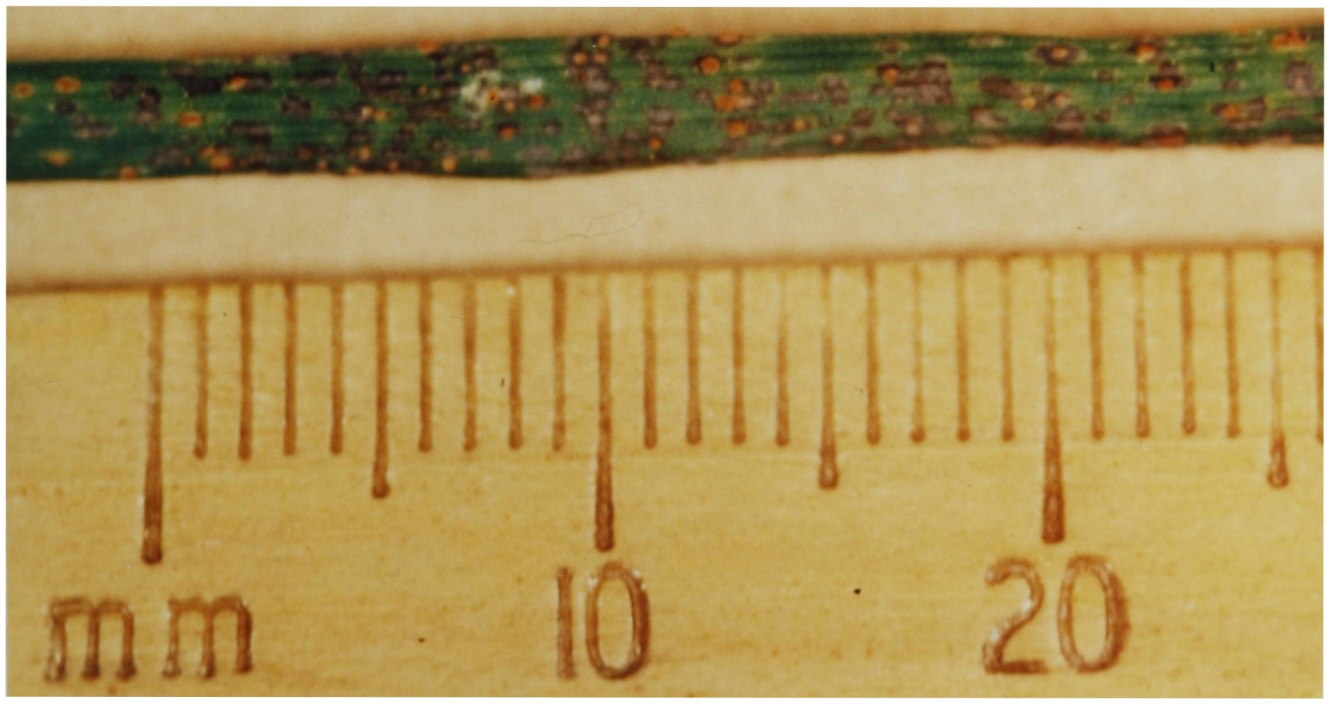

mm
10
20

3.4. DISCUSSION

In general, the trends shown by the different characters were very similar, but the presence of a substantial number of immune (i) and highly resistant (0) reactions indicates the need for more information than incubation period and latent period, for example pre-penetration studies. However, lesion type and incubation type did show differences between several host-isolate combinations with the same lesion type.

There were indications that some of the 'flecks' might have produced pustules given more time or different environmental conditions, and the decision to assess the result in a given time (i.e. 21 days) is a failing of this type of test. The considerable variation between some of the leek isolates, with different responses by the same host to different isolates indicates either genetic differences in the isolates, or environmental differences between tests, or both. Also variation between cultivars of some of the host species indicates the danger of relying on one cultivar or accession as being representative of a species; one may have chosen a resistant member of a 'true' host species. The variation in the responses of A. cepa to the leek isolates and the sporadic nature of telial formation also indicate a large environmental component in the reactions of some of the host-pathogen combinations. Both Uma (1984, 1986) and Norwood (1985) suggested that telial formation was environmentally dependent and Uma (1986) also suggested that only certain isolates might be able to form telia. Benada (1966) suggested that telial formation in rusts was

dependent on host physiology, specifically, internal host pH which was conditioned by light levels. Biali et al. (1972) and Yaniv et al. (1979) found telial formation induced by other fungi. Environmental factors acting on either the rust or host might explain why some of the large 'flecks', particularly on the A. cepa cultivars, did not sporulate. Both Goto (1933) and von Tavel (1932) noted the high level of variation in their studies on the rusts on Allium spp. Goto (1933) mentions also the failure of repeated inoculations and variation between replicates.

Another problem is the interpretation of lesion type assessments on different host species. The appearance, size and density of lesions may be determined by constraints of the host, e.g. the hollow leaves of the section Cepa hosts might not be able to support lesions as large or abundant as those on the section Allium hosts, with their 'double-sided' leaves. Further, in the absence of a type 4 reaction on A. vineale, a type 2 reaction might represent the best performance of the rust on this host, i.e. a susceptible reaction.

Bearing in mind these problems, the results do show major differences between the isolates from different host species. The most consistent difference was the ability of the CHIVE and BABNT isolates to form telia. The conclusions on the host-BABNT isolate studies are restricted by the limited number of hosts tested. However, the failure of the BABNT isolate to infect A. scorodoprasum is notable since herbarium specimens of this host in the U.K., including the H.70 isolate, bear rusts which morphologically identical to the BABNT

isolate. This is in agreement with von Tavel (1932) but not Gäumann (1959) who stated that Uromyces ambiguus could infect A. scorodoprasum.

The performances of the leek isolates were similar to each other but different from the CHIVE isolate. Since each isolate was compared in a separate trial, between isolate differences may be partially or wholly due to minor variations in conditions between experiments.

The differences between the leek isolates and the CHIVE isolate were most clear cut on the members of the ampeloprasum complex. The difference in the reaction of the isolates from the two hosts on A. porrum is clearly marked and shows the value of recording more than a simple 'sporulation' score. The A. cepa cultivars were generally more resistant than average to both rusts, but the results were variable. It may also be that the host reaction in A. cepa varies between the first and second years in this biennial crop. A. sativum was generally more susceptible to the leek isolates than the CHIVE isolate, but the resistance of cv. Blanc de Dome was noticeable, especially to the BIRM isolate. A. sativum cultivars are effectively clones, which reduced the level of between plant variation, which may in turn have highlighted the cultivar effect here, (see also Chapter 7).

A. fistulosum was the only host tested which gave similar reactions to all the isolates. This could be because of its non-European origin, such that it is neither fully compatible nor incompatible with the

genetic system in European rusts, producing a moderate-susceptible response to all the U.K. Allium rusts (Bennell, pers. comm.).

The host ranges of the leek and CHIVE isolates found here correspond reasonably well to those described by previous authors. Schneider found Puccinia allii to infect A. sativum and A. fistulosum among others, and P. porri to infect A. schoenoprasum easily, and A. ampeloprasum and A. fistulosum less strongly. Von Tavel (1930) found P. allii to infect A. fistulosum and A. porrum, and P. porri to A. schoenoprasum, A. fistulosum and A. cepa. Von Tavel (1932) extended her studies and found the rusts from A. carinatum, A. sphaerocephalum and A. pulchellum, resembling P. allii, to infect (and sporulate) A. sativum, A. cepa, A. porrum and A. fistulosum, whereas several isolates from A. schoenoprasum (P. porri) infected A. sativum, A. vineale, A. fistulosum and seedlings of A. porrum and A. ampeloprasum. This infection of A. porrum and A. vineale is in contrast to the results here, but it could be due to differences between isolates, or, in the case of A. porrum, the difference between seedlings and adult plants. Goto (1933) compared northern and southern 'strains' of the rust on A. fistulosum in Japan. The southern 'strain' had paraphyses in the telia, with predominantly two-celled teliospores and he called P. allii, with the northern 'strain' as P. porri. Thus P. allii was able to infect A. fistulosum, A. scorodoprasum and, with longer infection times, A. cepa. P. porri was able to infect A. scorodoprasum with latent periods of 9 days, A. fistulosum with latent periods of 7 to 11 days and A. cepa with latent periods of 8

to 12 days. P. porri formed telia more readily than P. allii, especially on A. fistulosum. Goto also noticed that P. allii often produced 'white necrotic lesions in 9 days, frequently followed by the formation of...small uredia' or that both strains produced 'abundant small uredia with a yellowish halo'. This description of P. allii could equally be used to describe the reaction of A. cepa to the leek isolates found in this study. The description also suggests that this type of reaction is common in the Allium-rust interaction. Both Curthoys (1986) and Boys (1986) found white flecks containing mycelium on A. cepa artificially inoculated with the BIRM isolate. Although the colonies were large they did not sporulate before the leaf senesced. This suggests that in A. cepa at least, there is a form of resistance where growth and differentiation of the pathogen is retarded - rather like an extreme form of slow-rusting. This may explain the observation of Norwood (1985) that both in the field and in controlled environment tests, A. cepa was much more resistant than A. porrum to 'leek rust'. The various descriptions of 'flecks' on Allium spp. in response to inoculation to rusts suggests that this type of reaction is common in the genus. Von Tavel's work on the host range went beyond the sections of Allium studied here (Von Tavel, 1932) and herbarium specimens suggest that the rust is found, if not commonly, on other wild species in the genus Allium. Hylander et al. (1953) actually suggested making A. oleraceum the lectotype host for their description of Puccinia allii.

Thus these studies seem to substantiate the findings of the morphological study that the 'leek' isolates, the CHIVE isolate and the BABNT isolate are three different entities, with extensive and overlapping host ranges, but with physiologic specialisation within the host range of each morphological entity.

There appears to be no real evidence of specialisation within the leek isolates that could not be equally attributed to environmental or host variation in a test such as this; a more detailed study can be found in Chapters 5 and 6. However, the different responses of the cultivars A. cepa and A. sativum does suggest that there may be useful resistance in these species; certainly A. sativum would appear to be vulnerable leek rust, as would cultivars of A. kurrat, (see Chapter 4), and A. fistulosum, vulnerable to potentially all three rusts. Such information should be borne in mind in breeding programmes, especially those attempting to cross A. cepa x A. fistulosum (Gonzalez & Ford-Lloyd, 1987), since the hybrids may be more susceptible than existing A. cepa cultivars.

Also there appears to be potential for the wild species, A. vineale, A. babingtonii and A. scorodoprasum to act as reservoirs of the rust which could act as foci for epidemics on agricultural crops. Specimens of A. vineale are frequently found with rust, but Cornish specimens of A. babingtonii bear only the BABNT entity despite the prevalence of the CORN isolate on cultivated leeks in relatively close proximity. It is difficult to explain why the rust on A. babingtonii in Cornwall is not the CORN isolate since it is susceptible to it, and

the BABNT isolate appears to have very fastidious environmental requirements to infect successfully. It may be a factor of timing, related to the post-winter emergence of A. babingtonii and the absence of this species during the Autumn when leek rust is most prevalent, or that the BABNT performs relatively better in the field. Either way, the BABNT isolate would not seem to pose any immediate threat to leek production in Cornwall, but again should be borne in mind if germplasm of A. babingtonii is used in breeding programmes with A. porrum (see also Chapter 4).

CHAPTER FOUR

STUDIES ON RESISTANCE WITHIN THE AMPELOPRASUM COMPLEX

4.1 INTRODUCTION

Allium ampeloprasum L. forms a variable species complex, including the wild A. ampeloprasum L. and cultivated leek (var. porrum (L.) Gay), kurrat (var kurrat Schf. et Krause, syn. A. kurrat Schf.) and great-headed garlic (var. ampeloprasum). Tutin et al. (1980) considers the leek sufficiently different to merit specific rank as A. porrum L. The kurrat and great-headed garlic are cultivated in the E. Mediterranean area. The wild form in western Ireland and south-west England, var. babingtonii (Borrer) Syme, (syn A. babingtonii Borrer) is distinguished from other forms of the complex by the few flowers and many bulbils in the umbel, and is thought to be a relic of former cultivation (Tutin et al., 1980).

The centre of origin of the complex is thought to be around the Middle East to southern Russia, and wild, often weedy forms occur in this area, forming a polyploid series (2n = 16,24,32,40,48). The tetraploid leek and kurrat reproduce by seed and are interfertile with each other and with tetraploid forms of the wild A. ampeloprasum (McCollum, 1974; Anon, 1983). Great-headed garlic is hexaploid and likewise interfertile with wild hexaploid forms. A wild form classified as A. ampeloprasum in the UK resembles Mediterranean forms and occurs in Cornwall, the Channel Islands and the Bristol Channel

region. The nomenclatural type for A. ampeloprasum L. comes from Steep Holm island in the Bristol Channel, and has 2n = 48 (Tutin et al., 1980). The interfertility between var. babingtonii and the other members of the complex is unknown; some monographers have treated it as a variety of A. scorodoprasum L. (Lousley, 1971).

Rust occurs in the E. Mediterranean on leeks and wild A. ampeloprasum, but has not been reported as a serious problem, except on garlic (A. sativum), (Rabinowitch, 1984). However, rust has proved to be a major problem on kurrats grown in N. Europe used for breeding purposes (van der Meer, 1984; & pers. comm.).

Studies examining the resistance mechanisms operating in wild populations compared with agropopulations of cereals, have been reported by Browning (1974) and Browning et al. (1977). Analysis of the wild cereal populations and their diseases in Israel showed a high degree of stability in the host-pathogen relationship, resulting in a limited amount of disease and the prevention of epidemics. Both 'vertical' and 'horizontal' resistance mechanisms were found to operate; specific resistances in parts of the population were backed up by a high level of background or general resistance. This contrasts with the agricultural systems, where vertical resistance based on a few genes has often broken down in plants with a low background resistance, and were then very susceptible, producing a 'vertifolia' effect (Vanderplank, 1982), leading to epidemics. The alternative concept, of durable resistance, has been reviewed by Johnson (1984). However the interaction between resistance and durability is poorly understood; there are

examples of quantitative resistance which are both race specific and race non-specific, (Jones & Clifford, 1983) and durable and non-durable (Johnson, 1984). Further, certain race-specific resistances have proved durable (Johnson, 1984). Browning & Frey (1969) and Browning (1974) proposed that durable resistance would be obtained by 'mimicking' the situation in wild mechanisms, and proposed the use of multilines - mechanically mixed cultivars of several lines containing specific resistances, with a background of general resistance. This system has proved very successful in protecting oats (Avena sterilis) from crown rust (Puccinia coronata) in Iowa, (Browning & Frey, 1969).

However, there have been few studies of resistance in wild material of the ampeloprasum complex for use in leek breeding programmes. Therefore it was decided here to conduct a trial of one rust isolate on a selection of wild and foreign cultivated members of the ampeloprasum complex from the Birmingham University Allium gene bank to examine the types of resistance available in the complex which could be incorporated easily into leeks.

4.2 MATERIALS & METHODS

4.2.1. Materials

a) Inoculum

The inoculum used was a 12-week old isolate of the BIRM isolate.

b) Host Plants

The host plants were grown either from seed or bulbils (see section 1.2.3.) and were all in the three to six leaf stage. The individual accessions used are described in table 4.1.

4.2.2. Methods

4.2.2.1. Experimental Conditions

A 10 cm long area was marked out on the adaxial surface of a fully expanded healthy leaf using a waterproof non-phytotoxic pen, on each of two replicate plants of each accession. The inoculum was applied to the marked areas using the 'brush-on' technique (1.2.9(a)). After inoculation the plants were placed in a randomised design on a bench in a growth room with a temperature of 19 +/- 2^{o}C, and a 16-hour daylength. Lighting was from mercury-vapour lamps giving a photon-flux-density in the photosynthetic range of 45 +/- 5 μmol m^{-2} s^{-2} at soil level as measured using a Skye solarimeter.

Table 4.1

Accessions of *Allium ampeloprasum* complex in the Birmingham University Gene Bank inoculated with the BIRM leek rust isolate

Varieties of *A. ampeloprasum*	Accession/Cvr.	Material	Origin
A. porrum	Musselburgh	seed	Sutton's
"	714	"	Bulgaria*
"	420	"	TR 37062+
"	421	"	TR 3147+
"	424	"	TR 3740+
"	457	"	Oxford Univ. Acc. 1530
"	495	"	Martin Luther Univ. DDR
A. kurrat	632	"	IVT Wageningen Holland
"	664	"	Egypt (BU)
A. babingtonii	702	Bulbils	Cornwall
"	371	"	NVRS
"	465	"	Inishmaan, Aran Is. Ireland
"	562	"	RBG Kew, London
A. ampeloprasum	418	"	Guernsey

Key

* Cv. Starosagorski kamus
\+ Accession number of the Izmir Gene Bank, Turkey
BU Birmingham University Gene Bank
NVRS National Vegetable Research Station

4.2.2.2. Assessment Methods

The plants were examined daily after inoculation for the appearance of disease symptoms. For each plant, the incubation period (IP), and latent period (LP) were recorded. After 20 days, assessments of lesion type and pustule density were made (section 1.2.10).

4.3. RESULTS

The results are given in table 4.2. In general terms, the reactions were fairly similar between accessions and varieties. The most noticeable difference was in the performance of A. ampeloprasum acc. 418, with longer incubation period, latent period and lower lesion type and pustule density measurements. Otherwise the most noticeable differences were the longer incubation periods and latent periods in and A. babingtonii accs. 562 and 702. The latent period was also longer in A. porrum acc. 495, and pustule quantity lower in accession 421. Pustule quantity was higher in acc. 424 and cv. Musselburgh. Both accessions of A. kurrat and accessions 371 and 465 of A. babingtonii were susceptible. High spore production was noted in the accessions of A. kurrat in this test and in the tests in Chapter 3, but was not measured quantitatively. The characters of incubation period and latent period were not particularly correlated. A. porrum accessions 714 and 495 had the same incubation periods (7 days) but latent periods differed by 1 to 2 days; generally, only when the incubation periods were much longer, in the cases of A. babingtonii and A. ampeloprasum were there correspondingly long latent periods. The longer incubation periods of the accessions of A. kurrat were not reflected in longer latent periods.

Table 4.2

Incubation period (IP), latent period (LP), lesion type (LT) and Pustule Quantity (PQ) assessments of accessions of the *Allium ampeloprasum* complex from the Birmingham University Gene Bank infected with the BIRM isolate of leek rust. (Means of 2 replicates).

Host Variety	Accn/Cvr.	IP (days)	LP (days)	LT	PQ
A. porrum	Musselburgh	6	13.0	3	5
"	714	7	12.5	3	4
"	420	6	13.0	4	4
"	421	6	13.0	3	3
"	424	6	13.0	3	5
"	457	6	12.0	3	4
"	495	7	14.0	3	4
A. kurrat	632	8	12.5	3	4
"	664	8	13.0	3	4
A. babingtonii	702	9	16.0	3	4
"	371	7	13.0	3	4
"	465	7	12.0	3	4
"	562	10	17.0	3	4
A. ampeloprasum	418	14	20.0	2	2-3

4.4. DISCUSSION

The most notable feature of the results was the lack of evidence for 'hypersensitive' or 'vertical' forms of complete resistance. However, there does appear to be a high level of variation in terms of the quantitative characters, particularly in the longer latent periods of the A. ampeloprasum, and some of the A. porrum and A. babingtonii accessions. It is therefore probable that other lines of A. ampeloprasum exist which carry even greater or different levels of quantitative resistance or 'slow-rusting' (Sztejnberg & Wahl, 1977) type resistance, for example differences in pustule size, productivity or longevity of spore production. Slow rusting has been used extensively against the cereal rusts, (Clifford, 1972; Clifford & Clothier, 1974; Luke et al., 1975; Wilcoxson et al., 1975; Johnson & Wilcoxson, 1978). This type of resistance has also been found in the genus Asparagus against Puccinia asparagi, (Johnson, 1986) and in fact is the only form of resistance found in this host-pathogen combination (Thompson & Hepler, 1956; Hepler et al., 1957). This is significant as Asparagus is one of the most closely related crop genera to the Alliums, and Puccinia asparagi has been found on Allium spp. (Walker, 1921; Beraha, 1955). It is therefore possible that the types and levels of resistance to rusts in the two genera are similar.

The susceptibility of A. kurrat agrees with the findings of van der Meer (1984; pers. comm.) who found A. kurrat x A. porrum hybrids to be highly susceptible to rust in the field in the Netherlands. The major gap in this work was the lack of A. ampeloprasum from the

Mediterranean especially in view of the level of resistance in the Guernsey accession of this species. It is possible that wild populations particularly from the E. Mediterranean may have sufficient variation in response to rust infection to be of use in leek breeding. Although the tetraploid forms of the ampeloprasum complex are fully interfertile it may be more difficult to cross A. babingtonii with the other varieties since it is viviparous (Ved Brat, 1965) but wild forms have been observed to flower (pers. obs.) in the glasshouse, and tissue-culture methods may be available to produce somatic hybrids (Ford-Lloyd, pers comm). The relative number of bulbils compared with flowers in the umbels of A. babingtonii may be partially or wholly under environmental control, in which case it may be possible to obtain flowering forms of the desired genotype relatively easily.

Slow-rusting or other types of quantitative resistance have been found to be heritable in other systems, (Gavinlertvatana & Wilcoxson, 1978) but have also been subject to physiological specialisation (Clifford & Clothier, 1974), and are not necessarily more durable than 'hypersensitive' or 'vertical' types of resistance (Johnson, 1984).

Thus, there may be considerable potential for finding and utilising high levels of quantitative resistance to rust in wild ampeloprasum populations.

CHAPTER FIVE

'WITHIN PLANT' STUDIES ON THE LEEK

5.1. INTRODUCTION

There have been several studies investigating the pattern of resistance between leek cultivars, using different types of trials and host material. Norwood (1984) found no evidence for a race structure on leeks and onions, in either field trials using natural inoculum or in controlled environment tests using an isolate from the field on seedlings at the NVRS, whereas field trials at NIAB showed different levels of 'field resistance' between leek cultivars. Uma (1984), using detached leaf pieces from adult plants reported a variety of responses between cultivars, including 'hypersensitive flecking' though some of these symptoms were not found in equivalent intact plants.

Several authors working on other crop plants have commented that the host material must be at the correct stage of plant and leaf development when trying to evaluate components of resistance against pathogens (Umaerus, 1970; Parlevliet, 1979). There are many examples of situations where plants are susceptible at one stage and resistant at another, or where different organs (or different stages of the same organ) show different levels of resistance. Resistance confined to the later-developed parts of the plant is called adult plant or mature plant resistance (Manners, 1982). This type of resistance has been

widely reported (Jones & Hayes, 1971; Ohm & Shaner, 1976; Parlevliet, 1977) and is often very useful in the field, but its existence means that seedlings are often not good predictors of the response of the adult plants or their performance in field conditions.

Farkas (1978) mentioned the difficulty in establishing which leaves were physiologically comparable between plants, a feeling echoed by Parlevliet (1975), and Mares and Cousen (1977). Farkas (1978) suggested using the 'plastochron index' of Erikson and Michelini (1957), which was based on the leaf growth rate and setting rate of new leaves. However, establishing such an index for every plant in a large trial might not be practicable. McGregor and Manners (1985) used a system of 'physiological leaf age' to overcome these difficulties when looking at the effect of temperature on the development of Puccinia striiformis on wheat. In leek plants, leaves are continuously being produced and senescing, such that the number of leaves in the 'standing crop' depends on the age of plant, its rate of growth, etc. Leek plants are also very long lived in the field typically being harvested 9 - 15 months after sowing. Flowering is also dependent on environmental factors and does not occur at a definite stage in the growth of the plant (Yamaguchi, 1983). Figure 5.1 shows a leek development key based on glasshouse measurements. 'Leaf stage' as used here refers not to the number of leaves after the seedling leaf, but to the total number of leaves in the plant.

The components of disease commonly measured when attempting to analyse quantitative resistance have been reviewed by Parlevliet (1979). In

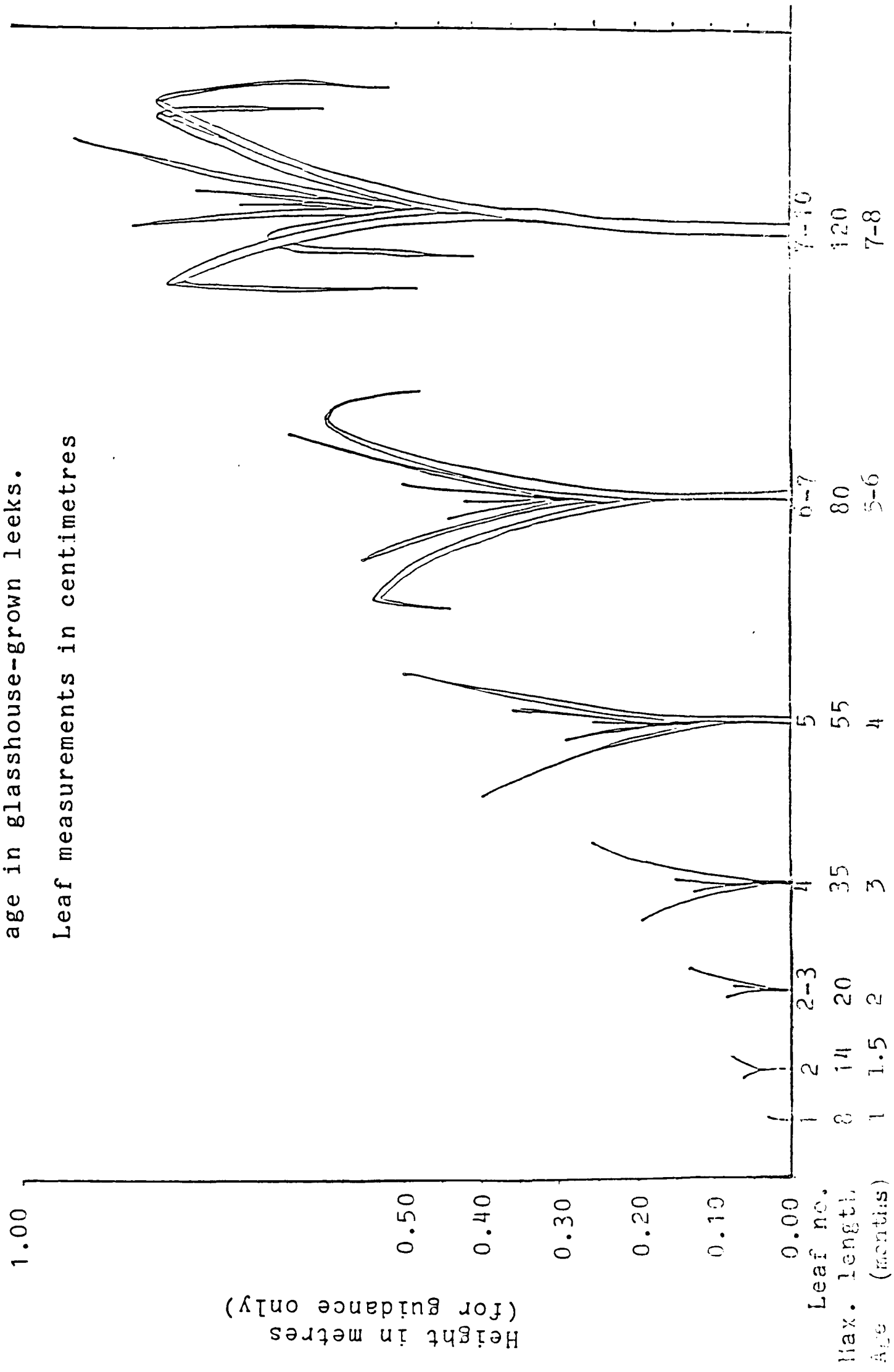


Figure 5.1
Leek development key, relating leaf number and length to approximate age in glasshouse-grown leeks.

Leaf measurements in centimetres

practice latent period has been correlated well with field resistance, though infection frequency (pustule density), lesion and colony size, and infectious period can also provide useful information in monocyclic tests. Spore production can be regarded as being the sum of all the other components (Johnson & Taylor, 1976), but this is only true in monocyclic tests, since other components, e.g. latent period, have been shown to be more important in epidemiological terms in the field. The difficulties in measuring spore production accurately have also been dealt with by Johnson and Taylor (1976), and Parlevliet (1979).

The aim in this study was to examine the differences within plants and within a single cultivar in several components to assess which type of host material and measurements would be most suitable for controlled environment cultivar trials. The main areas examined were :-

a) Host plant material of different age

b) Differences between leaves and leaf tissues of different ages within plants of the same age

c) Comparison of the components of latent period, pustule density, pustule length and colony length.

5.2. MATERIALS AND METHODS

5.2.1. Experiment 1

The effect of host plant age on rust development

5.2.1.1. Materials

a) Inoculum

A 4 - week old sample of the BIRM leek rust isolate, with a mean spore viability of 84% (5 samples of 100 spores, s.e.=0.875) was used.

b) Host Plants

Three ages of leek plant were chosen, using cv. Musselburgh. Different numbers of plants were selected for each category to provide similar total leaf area for comparison of pustule densities, pustule length and colony length, (details in table 5.1).

5.2.1.2. Method

A 5-cm area was marked out on the adaxial surface of the oldest three leaves 15 cm from the leaf tip (10 cm in the case of the young plants) as in figure 5.2. The marked leaves were then inoculated using the spray technique (1.2.9.b) with an inoculum concentration of 0.5 mg cm^{-3}. The plants were maintained in a growth room in a randomised design with a temperature of 18 +/- 2^{o}C, and lighting conditions as in 3.2.2.

Table 5.1

Description of the plants of leek cv. Musselburgh used to determine the effect of host plant age on rust disease development (Experiment 1).

Designation	Age from sowing (months)	No. plants (replicates)	No. leaves (range)	Length of longest leaf (cm)
Old	8	6	5-6	80.0
Medium	4	10	3-4	30.0
Young	2	15	2-3*	20.0

* Plants with three leaves may include the cotyledon

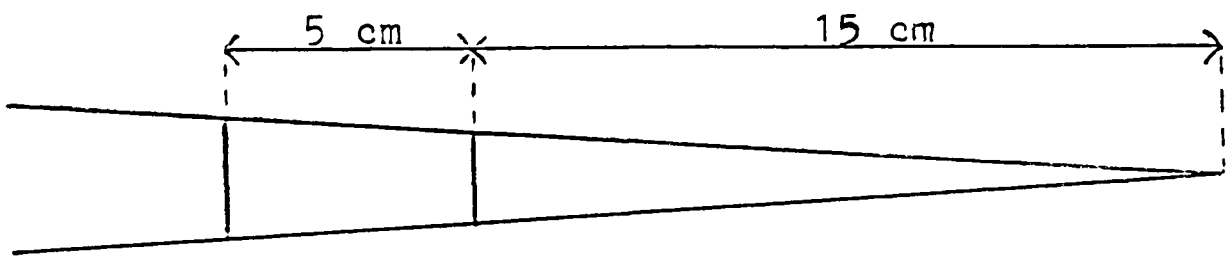
Table 5.2

Details of inoculum and host plants used in experiments 2 to 5.

Expt.	Inoculum			Host Plants		
	Age (weeks)	Viability %	S.E.	Replicates	Age (months)	Leaf stage
2	8	59	0.70	10	8	7-9
3	10	77	0.53	10	8	7-10
4	12	81	0.40	10	8	9-12
5	8	90	0.50	10	8	9-11

Figure 5.2.

Diagram showing the area marked out on leaves to facilitate disease assessment in experiment 1.

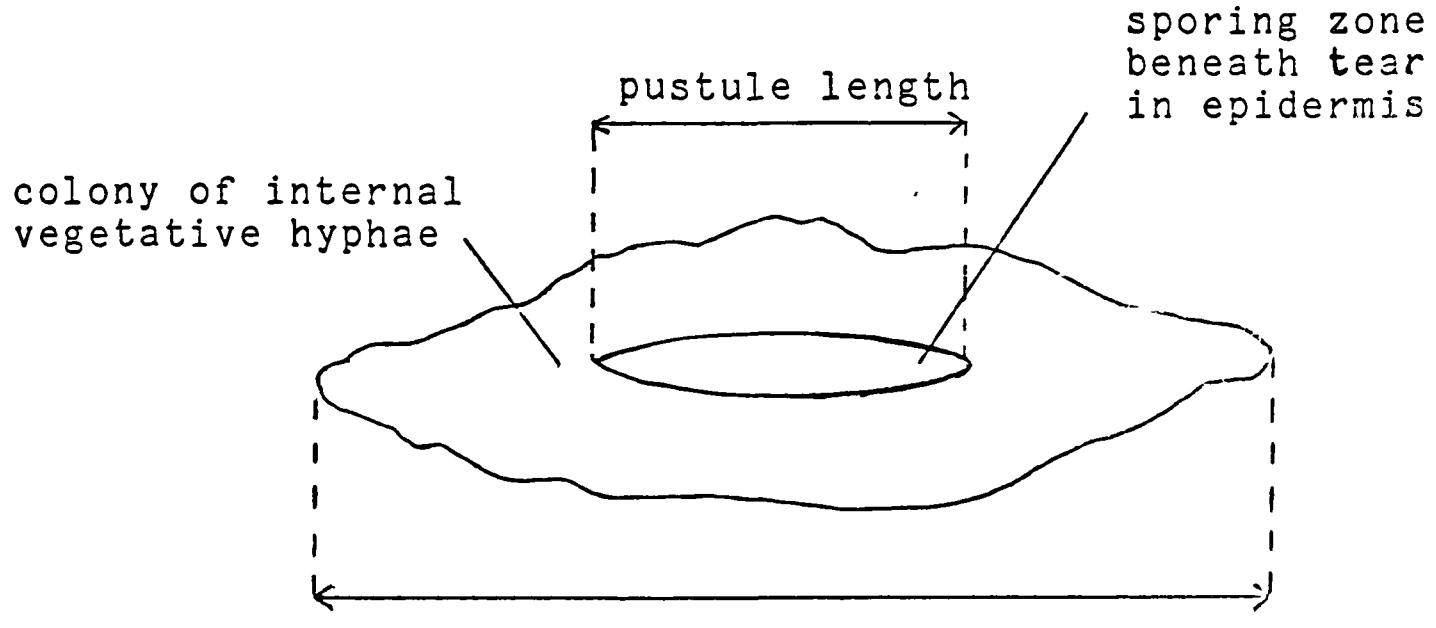


5.2.1.3. Assessment of Experiment 1

The plants were examined daily after inoculation, and latent period recorded for each plant. After 21 days, the number of pustules in the 5 cm marked areas were counted, and the width of the leaves in the middle of the segments were measured to calculate the segment areas, so that pustule density could be calculated for each leaf segment. The segments were then cut from the leaves and stained using the modified Bruzzese and Hasan (1983) technique (1.2.11). The stained segments were examined under the microscope, and pustule length and colony length were measured using an eyepiece graticule for up to ten separate, (non-overlapping) colonies, (see fig. 5.3).

Figure 5.3

Definition of colony length and pustule length measured in stained leaf material.



5.2.2. Experiment 2

The development of disease over time

The materials used in experiments 2 to 5 are given in table 5.2. In all the experiments the inoculum used was the BIRM isolate and the host plants were leek cv. Musselburgh.

5.2.2.1. Materials

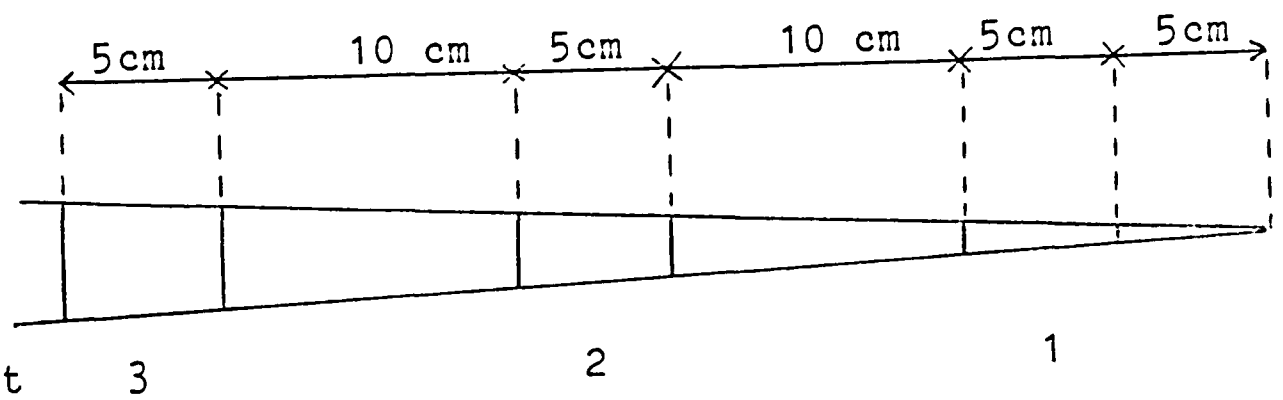
As above and in table 5.2.

5.2.2.2. Method

The oldest three leaves of plant were marked on the adaxial surface as in figure 5.4. The marked leaves were then inoculated using the spray technique (1.2.9.b) with a spore concentration of 1 mg cm^{-3}.

Figure 5.4.

Diagram showing the layout of segments marked on the leaves to facilitate disease assessment in experiment 2.



5.2.2.3. Assessment

The upper surface was examined every 3 days and the number of pale green flecks (infections) and erumpent pustules in each of the 5 cm long segments was counted, and the density calculated as in 5.2.1.3.

5.2.3. Experiment 3

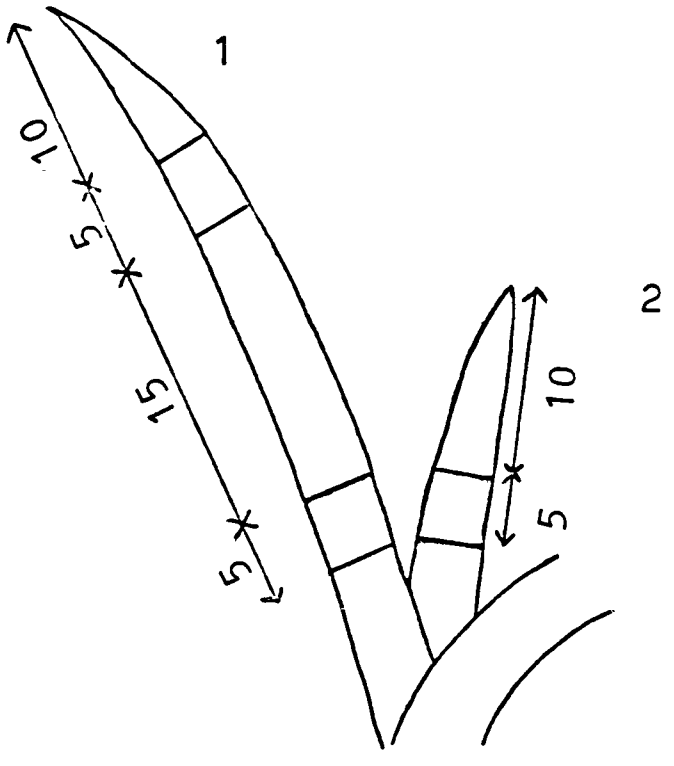
The effect of leaf tissue age on rust development

5.2.3.1. Materials

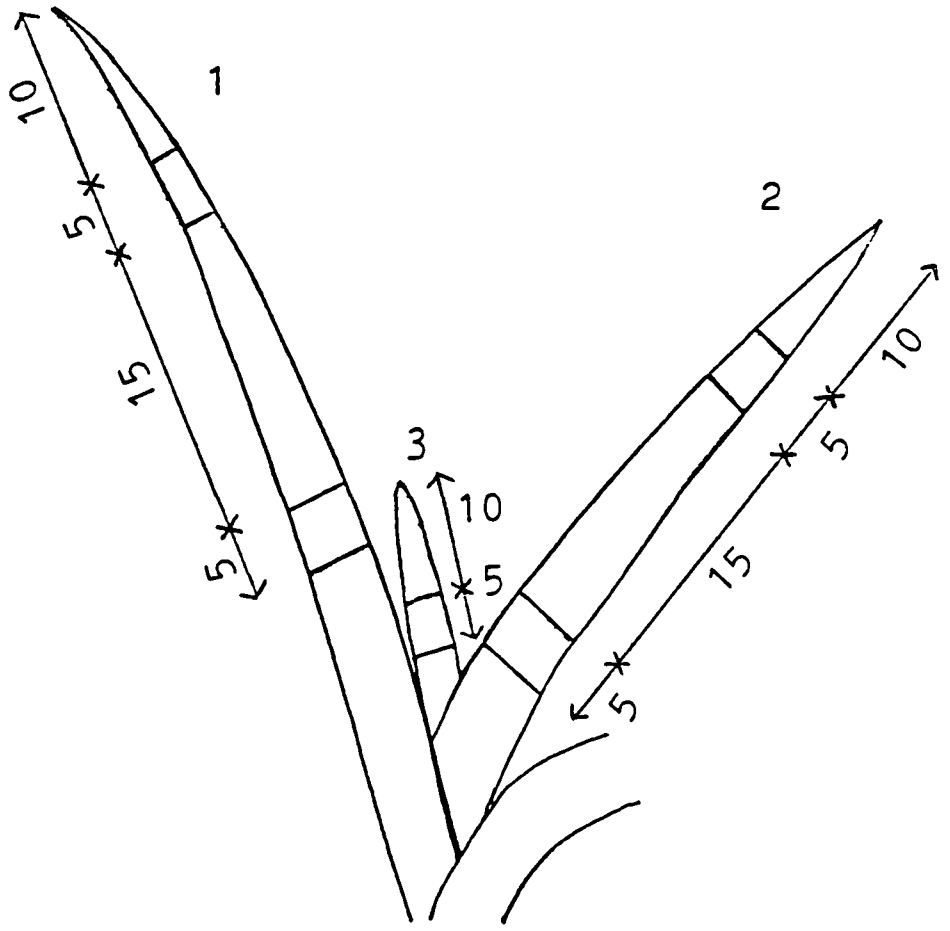
Details of the inoculum and host plants used are given in table 5.2.

5.2.3.2. Method

As the plants were growing the youngest three leaves on each plant were marked out as in figure 5.5. At time 1, segments were marked out 10 cm from the tips of the two youngest leaves. On the older leaf, another segment was marked out at the base ('non-tip') adjacent to the tip segment of the younger leaf, so that the two segments were the same distance from the basal meristem. At time 2, segments were marked out on the second and third leaves in the same way, so that the 'non-tip' segment on the second leaf was the same distance from the basal meristem as the 'tip' segment of the youngest leaf. All the 'tip' segments were 10 cm from the leaf tip. Thus the 'non-tip' and 'tip' segments of successive leaves were of the same age, leaf expansion after emergence being negligible. The rate of leaf growth was such that the 'tip' and 'non-tip' segments were about 15 cm apart in all the plants. The marked leaves were inoculated using the spray technique (1.2.9.b.) with an inoculum concentration of 0.5.mg cm^{-3}. The plants were maintained in a growth room as in 5.2.1.2.



time 1



time 2

Figure 5.5
Diagram showing marking of 'tip' and 'non-tip' segments on successive leaves for experiment 3.
Measurements in centimetres.

5.2.4. Experiment 4

A comparison of 'tip' and 'non-tip' areas on rust development on leeks.

5.2.4.1. Materials

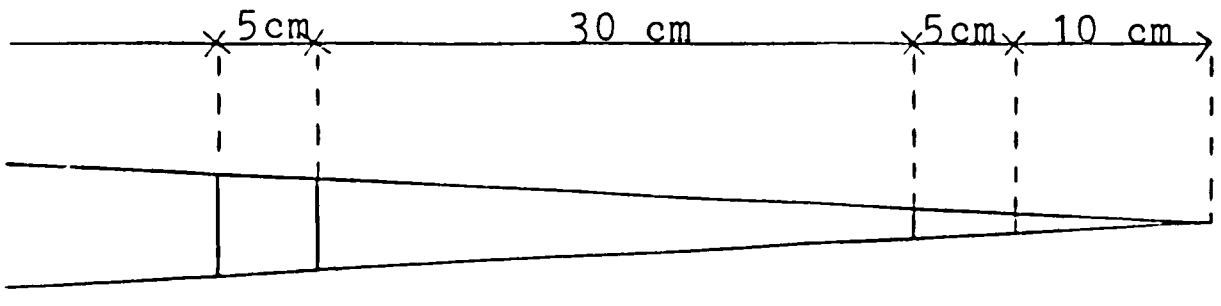
Details of inoculum and host plants are given in table 5.2.

5.2.4.2. Method

After any senescing leaves had been removed from each plant, the oldest healthy leaf was left uninoculated. The next three leaves were marked as in figure 5.6. to give two 5 cm segments, one 10 cm from the tip and the other 45 cm from the tip. The marked leaves were inoculated using the spray technique (1.2.9.b.) using an inoculum concentration of 0.5 mg cm^{-3}. The plants were maintained as in 5.2.1.2.

Figure 5.6

Diagram showing layout of segments marked on the leaves to facilitate assessment in experiment 4



5.2.4.3. Assessment

After 21 days, the pustule density for each segment was calculated as in 5.2.1.3.

5.2.5. Experiment 5

A comparison between 'tip' and 'non-tip' areas of leaves with a greater age difference between them (i.e. non-adjacent leaves) on the development of rust on leeks.

5.2.5.1. Materials

Details of inoculum and host plants are given in table 5.2.

5.2.5.2. Method

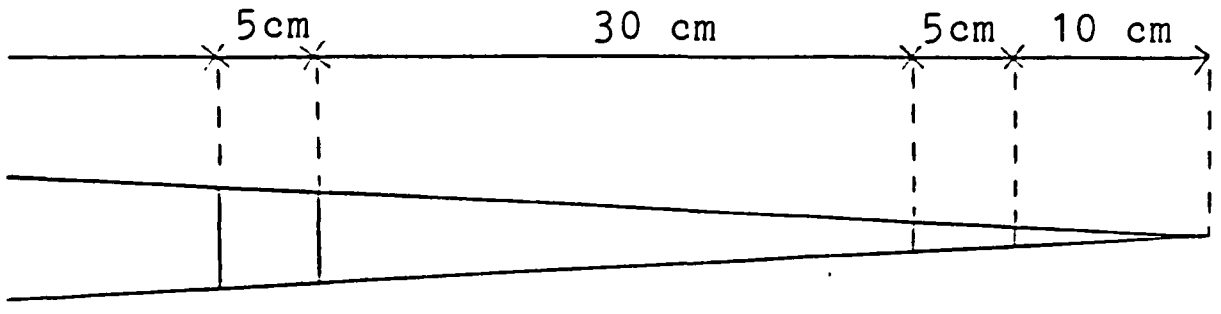
The oldest healthy leaf was left uninoculated, and then every other leaf was marked as in figure 5.7. (three leaves per plant) on the adaxial surface. The marked leaves were then inoculated using the spray technique (1.2.9.b.) using an inoculum concentration of 0.5 mg cm^{-3}. The plants were maintained as in 5.2.1.2.

5.2.5.3. Assessment

Pustule density, colony and pustule length were calculated for each segment as in 5.2.1.3.

Figure 5.7.

Layout of the segments marked on every other leaf of the plants used in experiment 5.



5.2.6. General note on assessments

In all the experiments, where the oldest inoculated leaf of an individual plant died, the next oldest inoculated leaf was considered as the oldest leaf for comparison between replicate plants.

5.3. RESULTS

5.3.1. Experiment 1

The effect of host plant age on rust development

The results are given in table 5.3. There were very highly significant differences between the different age-groups of plants in the parameters tested. The 'old' plants had longer latent periods, lower pustule densities and smaller pustules and colonies than the 'medium' or 'young' plants. The 'medium' plants, although intermediate between the 'young' and 'old' plants, behaved more like the 'old' plants, the differences therefore being between 'young' and 'non-young' plants. Colony length and pustule length were significantly correlated, ($r=0.285$, $P > 0.01$) for all plant ages.

5.3.2. Experiment 2

The development of disease over time

a) Timing of appearance of flecks and uredinia

The first symptoms appeared after 6 days in the form of pale green flecks. After 12 days, uredinia began to form in the centre of some of these flecks, producing a brown pustule with a surrounding pale green halo. Figure 5.8. shows the change in density of flecks and pustules over time (data pooled from all segments). After 15 days the mean density of flecks and pustules declined as some of the older, more heavily infected leaves died; however the mean pustule density

Table 5.3

Effect of age of host plants of leek cv. Musselburgh on selected disease parameters of the BIRM leek isolate (21 days after inoculation)

Plant age	Mean Latent Period (days)+	Pustule Density (cm^{-2})	Pustule Length (mm)	Colony Length (mm)
Old	14.50	3.75	0.691	2.003
Medium	14.10	6.17	0.709	2.169
Young	13.93	15.75	0.791	2.571
Analysis of Variance	N.S.	***	***	***

+ mean of 6, 10 and 15 plants respectively
*** significant at $p<0.001$

Table 5.4

Pustule Density (PD), Pustule Length (PL) and Colony Length (CL) for the leaves of the 'old' and 'medium' plants in Experiment 1

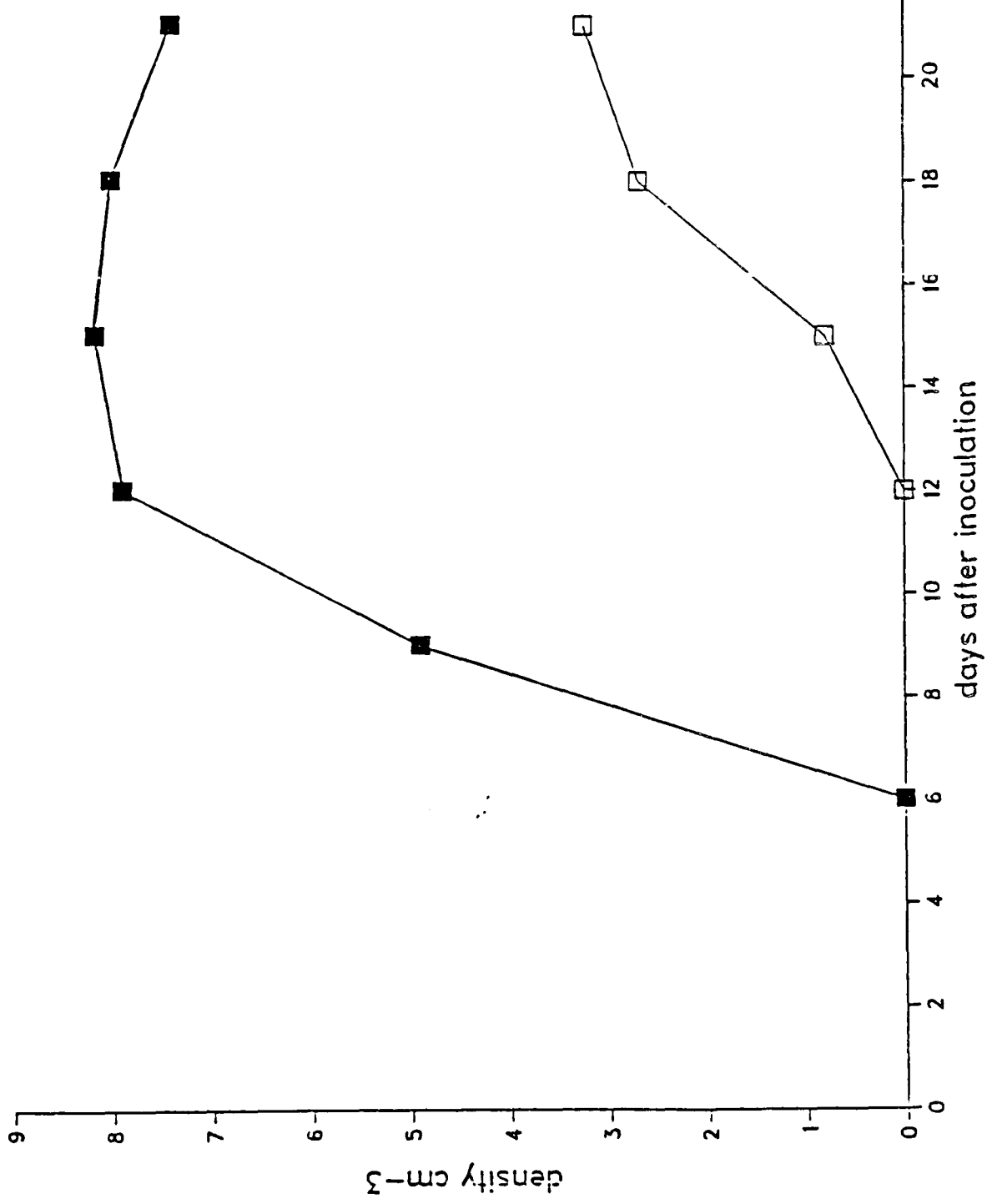
Leaf	PD (cm^{-2})	PL (mm)	CL (mm)
1 (oldest)	7.07	0.693	2.14
2	4.08	0.711	1.85
3	2.86	0.714	2.23
Analysis	N.S.	N.S.	N.S.

Table 5.5

Mean Pustule Density (cm^{-2}) of leaf segments of equivalent age tip to non-tip between successive leaves of leek cv. Musselburgh infected with the BIRM isolate of leek rust

	Leaf One		Leaf Two		Leaf Three
	tip	non-tip	tip	non-tip	tip
Mean	3.42	1.77	2.49	1.76	4.14
S.E.	0.98	0.51	0.54	0.43	1.57

Figure 5.8
Mean density of chlorotic flecks and pustules on the adaxial leaf surfaces of leek cv. Musselburgh up to 21 days after inoculation with the BIRM isolate.
(Mean of all segments).



was continuing to increase after 21 days, although less than half the flecks had actually formed uredinia.

b) Pustule density on the various segments after 21 days.

Figure 5.9 shows the mean pustule density of the various segments on the leaves (pooled from replicate plants). The mean pustule density of the tips (segment 1) was greater than the middle or lower segments (2 & 3) in any of the leaves, and was slightly higher in the older leaves (leaf 1) than the younger (2 & 3). The effect of age of leaf was sufficiently marked for the pustule density of the youngest segments of the middle leaves to be similar compared with the oldest segments (tips) of the youngest leaves. The differences between the leaves (segments pooled) were not significant ($P > 0.05$) when the segment 2 was included, but the difference between segments 1 and 3 (leaves pooled) was just significant ($P < 0.05$). The differences between the between the segments (leaves pooled), were not significant ($P > 0.05$).

c) The effect of leaf age

(Data on leaves from plant age Experiment 1, for comparison with (a) and (b)).

Table 5.4. shows the pustule density, colony and pustules lengths for the individual leaf data pooled from the 'old' and 'medium' plants in experiment 1, (between which there were no significant differences, $P > 0.05$). Pustule density decreased from the older to the younger

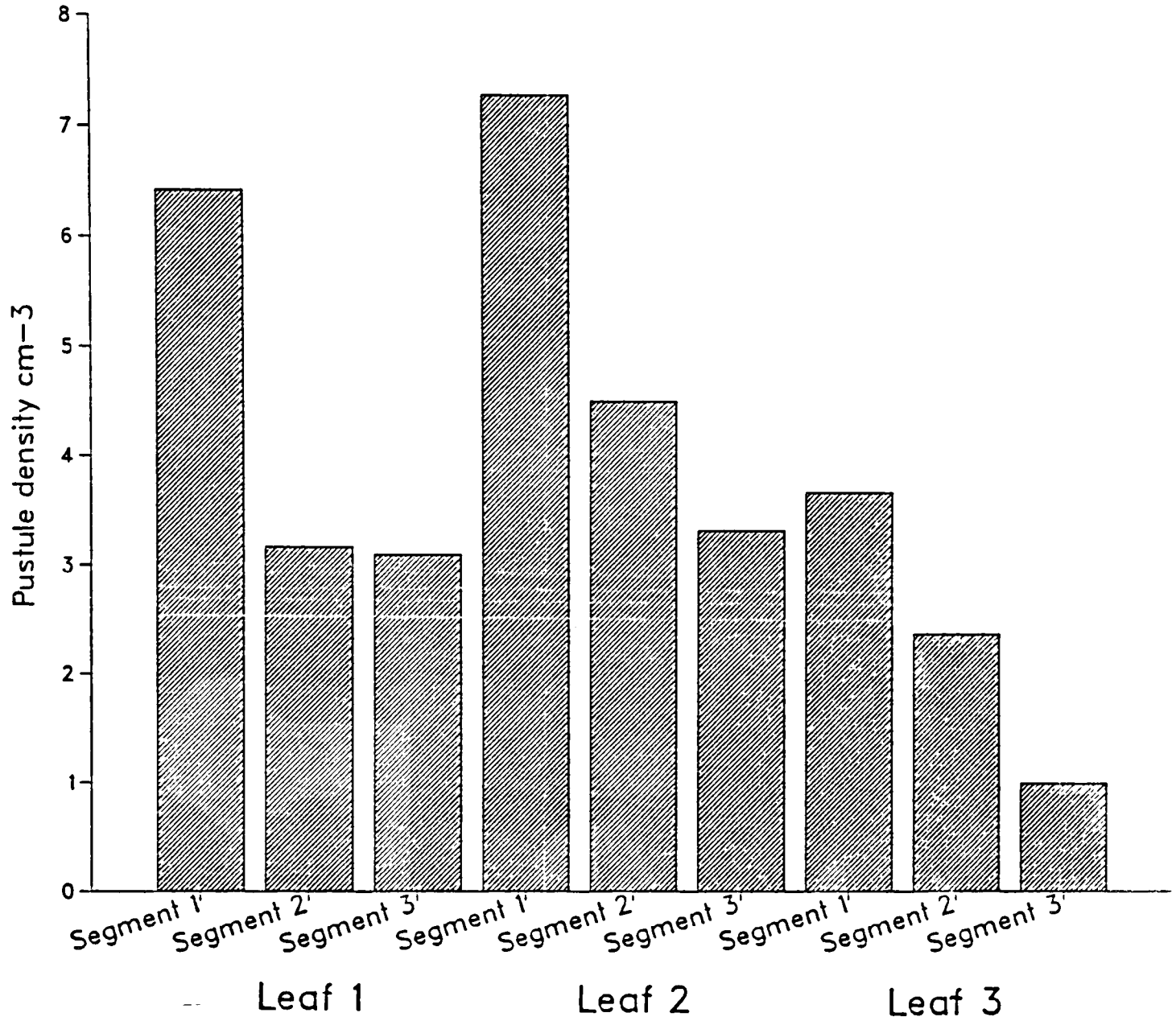


Figure 5.9
Mean pustule density on the leaf segments of leek cv. Musselburgh 21 days after inoculation with the BIRM isolate. (Leaf 1 = oldest leaf, segment 1 = oldest segment (leaf tip)).

leaves, and there was a slight increase in pustule length from older leaves. Neither effect was statistically significant for all the leaves, though the difference in pustule density between the oldest and youngest leaves was just significant ($P < 0.05$). There was no pattern in colony length between the leaves, and there were no significant differences ($P > 0.05$).

5.3.3. Experiment 3

The effect of leaf tissue age on rust development

The results are given in table 5.5.

The major feature was the greater pustule density of the tip segments compared with the low pustule density of the 'non-tip' segments. However, the level of variation in the segments between plants was very high, and none of the differences were significant, either between segments within leaves, or between the pooled tip and non-tip segments ($P > 0.05$). In treatments designed to give the same tissue age, there was no apparent similarity in pustule density, (i.e. in tips and bases of successive leaves).

5.3.4. Experiment 4

A comparison of 'tip' and 'non-tip' areas on rust development

The results are given in table 5.6.

In the oldest leaves, the mean pustule density of the non-tip segments was higher than that of the tip segments but this pattern was reversed in the third (youngest) leaves, with little difference between the segments

Table 5.6

Mean Pustule Density (MPD) (cm^{-2}) in the tip and non-tip segments of leek cv. Musselburgh infected with the Birm isolate of leek rust (means of ten segments)*

Leaf	I (oldest)		II		III	
Segment	tip	non-tip	tip	non-tip	tip	non-tip
MPD	2.65	3.69	3.08	3.16	2.43	2.25
S.E.	0.72	0.88	0.55	0.85	0.67	0.52

*Some figures are means of less than ten segments due to leaf or segment senescence

Table 5.7

Pustule Length (PL) and Pustule Density (PD) on tip and non-tip segments of leaves of leek cv. Musselburgh inoculated with the BIRM isolate of leek rust. Each leaf is the next-but-one in age to the previous leaf (i.e. adjacent leaves)

Leaf	1		2		3		Mean	
Segment	tip	non-tip	tip	non-tip	tip	non-tip	tip	non-tip
PD (cm^{-2})	2.71	5.91	1.85	2.70	3.71	2.61	2.55	3.97
PL (mm)	0.625	0.584	0.593	0.820	0.509	0.857	0.585	0.727

Means of ten replicates per segment; some segments absent due to leaf death

in the second leaf. None of the differences was statistically significant. ($P > 0.05$).

5.3.5. Experiment 5

A comparison of 'tip' and 'non-tip' areas of non-adjacent leaves.

The results are given in table 5.7.

In the older two leaves, pustule density was higher in the non-tip segments, whereas in the youngest leaf, pustule density was higher in the tip segment. The differences between tip and non-tip segments was only significant in the oldest leaf, ($P < 0.01$). The mean pustule density for tip segments was significantly less than non-tips ($P < 0.01$). Conversely, pustule length was greater in the tip segment in the oldest leaf, but greater in the non-tip segments of the younger leaves. Overall, pustule length was greater in the tips compared with the non-tips; this was highly significant ($P < 0.01$) but the significance is dependent on the data from the third leaf non-tip segments - when excluded, there were no differences between tip and non-tip segments ($P > 0.05$).

5.3.6. Comparison of Experiments 2 to 5.

Mean pustule densities for tip and non-tip segments of the three leaves in each of the experiments are given in table 5.8.

Table 5.8

Comparison of pustule densities between 'tip' and 'non-tip' portions of leaves in experiments 1 to 5, of leek cv. Musselburgh inoculated with the BIRM leek rust isolate (means of 10 replicate plants*)

	Pustule Densities (cm^{-2})					
Leaf	I		II		III	
Area	tip	non-tip	tip	non-tip	tip	non-tip
Experiment						
1		7.07		4.08		2.86
2	6.42	3.11	7.29	3.33	3.68	1.00
3	3.42	1.77	2.49	1.76	4.14	-
4	2.65	3.69	3.08	3.16	2.43	2.25
5	2.71	5.91	1.85	2.70	3.71	2.61

* Fewer where leaves had died

The density of pustules on the tip segments was greater than the 'non-tip' segments in the third (youngest) leaf all the experiments. In experiments 2 and 3, the pustule density of the first (oldest) and second leaves was greater in the tip than the non-tip segments, whereas in experiments 4 and 5 the situation was reversed, with higher pustule densities in the in the non-tip segments of this leaf. In all cases, the pustule density of the non-tip segments increased with age, (i.e. highest in leaf I, lowest in leaf III). This effect was more pronounced in experiment 5, where the age difference between the leaves was greater since alternate leaves were inoculated. There was no pattern to the pustule density of the tip segments between leaves in any of the experiments. The major differences between experiments 1 and 2, and 3 and 4 was the greater number of leaves on the plants in the latter experiments, and the inoculation of alternate leaves in experiment 5.

In all the experiments the level of variation was high between plants. such that the few of the differences between segments, leaves or segments within leaves were statistically significant.

5.4. DISCUSSION

The results from experiment 1 clearly showed that the mature plants possessed some form of quantitative resistance, expressed in several disease components, compared with plants just past the seedling stage. This type of resistance usually called 'adult-plant' resistance has been described by many authors for other crops (Jones & Hayes, 1971; Manners, (1950); Mares & Cousen, 1977; Parlevliet, 1975; Ohm & Shaner 1976; Parlevliet & Kuiper, 1977), but may be of less epidemiological importance here since the disease is usually prevalent in the U.K. in the autumn when the leek crop plants are mature - the disease is not reported as a problem on seedlings earlier in the year. However, it is of importance in experimental work since such resistance may not be detectable in seedlings in this system. Parlevliet (1975) stated that seedlings were often not good predictors of adult plant performance in terms of disease resistance, going so far as to say 'research into the components of partial resistance carried out with seedlings should be approached with extreme caution'. He added that seedlings could provide useful information but not in isolation. Jones and Hayes (1971) found that resistance in Erysiphe graminis f. sp. avenae in mature plants could not be predicted from seedling performance. They also found that the resistance developed gradually in successive leaves as the growth rate accelerated, as opposed to being an abrupt seedling-mature plant effect. The results here would agree with that statement, though the 'medium' plants were closer to the adult plants than to the seedlings. Ohm and Shaner (1971) found that 'slow

rusting' in wheat against Puccinia recondita f. sp. tritici was reduced in the seedling stage and after flowering.

Although the results here do not preclude that this type of resistance may be absent in other leek cultivars, its occurrence in cv. Musselburgh suggests that it might occur in others, and that a more realistic comparison between cultivars might be gained by using 'mature' plants rather than seedlings.

Also in this experiment, the high pustule density in the young plants did not appear to affect pustule size. This is in contrast to several authors (Yarwood, 1961; Mehta & Zadoks, 1970; Johnson & Taylor, 1976) who found that increased pustule density caused pustules to to be smaller and produce fewer spores, presumably through competition effects - the result of this being that total spore production per unit leaf area was found to be relatively independent of pustule density. Mehta and Zadoks (1970) stated that the limiting factor was apparently the productivity of the photosynthetic apparatus. Therefore, the density and size of the pustules in the younger plants here may not have reached the critical limiting level.

Experiment 2 showed clearly that pustule break was not simultaneous, but occured over a period of time from 12 days onwards. Many of the infection sites failed to form sporulating pustules before the leaf senesced. However in the field, the mechanism of natural infection would tend to inoculate the leaves at the bases, rather than the older tissues at the tips (Norwood, 1985), giving the rust longer to develop

before the leaf senesced. These results also suggested that very heavy infections of the rust might accelerate leaf senescence - in effect acting as a form resistance by limiting the infectious period. Mehta and Zadoks (1970), Ohm and Shaner (1976) and Shaner and Hess (1977) all noted that pustules of Puccinia recondita on wheat did not open simultaneously despite a 'simultaneous' inoculation, and Mehta and Zadoks (1970) noted that pustule break was affected by both pustule density and environmental factors. This presents a problem here since traditional measures of latent period measure it as the time from inoculation to 50% pustule eruption. Clearly this point would seldom be reached in many leek leaves before senescence, so a lower figure may be desirable, say, 25%, or the onset of sporulation could be used to define latent period and has been used elsewhere (Parlevliet, 1977).

The data from experiment 1 analysed in terms of leaf age showed the necessity of further testing to elucidate the effect of leaf or tissue age, or tissue type on rust infection. The subsequent experiments revealed substantial levels of variation between plants and experiments on this, such that trends within plants were often not statistically significant. However, within each experiment it is possible to explain the results in terms of leaf 'tips' which either do not alter substantially in terms of resistance, or which vary considerably without a pattern to the variation; and non-tip areas (from the median to the base of the leaf) which becomes more susceptible with age, since in all cases, pustule density increased in the non-tip areas in the older leaves. Mares and Cousen (1977)

observed the effect of 'tip variability' in wheat, noting that tip segments gave atypical and irreproducible results, in terms of infection rates and lesion types compared with mid-leaf sections. Several authors working in older crops have found older leaves to be more susceptible than younger leaves. Jones and Hayes (1971) noticed a marked trend in oats for successive leaves to be more susceptible to Erysiphe graminis and also the same 'number' leaves were more susceptible if inoculated later in the year. Cole (1966) reported similar effects. The information on variation in pustule length between leaves (experiments 1 and 5) suggests a reversal of the trend in pustule density in that where younger leaves had lower pustule densities, they produced larger pustules. This suggests that older leaves are more susceptible to infection but that once colonised, younger leaves support greater colony growth. This could be due to the relative health of the younger leaves. Ohm and Shaner (1976) found that pustules of Puccinia recondita on wheat were larger on the youngest leaves as these had not begun to senesce as soon after inoculation as the other, older leaves. It is possible that the earlier stages of the process of senescence limit the size of the pustules before there are any signs of visible senescence. Parlevliet (1975) and Parlevliet and Kuiper (1977) found latent period decreased but infection frequency increased with leaf age. They stated that it was important to use leaves of comparable physiological condition in replicate plants, but also remarked that this was difficult to control in adult plants. Mares and Cousen (1977) suggested using leaves of 'equivalent age', but again this can be difficult, especially in a plant as slow growing as leeks. However, they also found old wheat

leaves were more poorly infected by P. striiformis, and many colonies of the rust did not sporulate. Johnson (1986) also found asparagus shoots to be more resistant with age to Puccinia asparagi, with lower pustule densities on older leaves. Omar et al. (1986) reported that older leaves of broad bean to be more susceptible to the necrotrophic Botrytis cinerea and B. fabae, expressed in greater lesion development and increased sporulation.

Thus the problem of which leaf tissue to choose has been one for many plant pathologists. Parlevliet and Kuiper (1977) also warned that glasshouse-grown plants often differed in growth habit from those in the field, influencing infection studies, and that the 'above plant' inoculations usually used in laboratory tests were in contrast to the field situation where inoculum often comes from below or the side. Nevertheless, they found strong correlations between components of resistance studied in the glasshouse and field resistance, particularly latent period.

Thus in work on leek rust, it would appear sensible to :-

a) Measure latent period from the first appearance of sporulating pustules, since pustule break may not reach 50%.

b) Use adult or mature plants past the seedling and immediate post-seedling stages

c) Avoid using leaf tip areas, and preselect areas at specified distances from the tip.

d) Inoculate replicate leaves within a plant, to cover a range of leaf ages.

e) Inoculate several replicate plants within a cultivar

f) Measure several components e.g. latent period, pustule density and pustule length since they did not always correlate with each other.

CHAPTER SIX

CULTIVAR - ISOLATE TRIALS

6.1. INTRODUCTION

Relatively few studies have been carried out on the leek - leek rust interaction compared with other host - pathogen systems. Field trials at the National Institute of Agricultural Botany (NIAB) have been carried out to look for resistance against the rust in leeks in the field, (Dixon, 1976; Anon., 1986). Dixon (1976) described both uredinia and 'chlorotic spots' as symptoms, and a comparison of cultivars was carried out measuring levels of 'chlorotic spotting' using a cereal yellow rust (P. striiformis) key. However, there are many other possible causes of 'chlorotic spotting' on leeks in the field, and subsequent NIAB trials have concentrated on more reliable assessments based on uredinial quantity. The data from these latter trials have been used to produce a series of resistance ratings for leek cultivars against rust (Anon., 1986).

Uma (1984) compared one isolate of rust on seven leek cultivars using both intact plants and detached leaves. She assessed rust infection in terms of both 'macro' components (e.g. latent period, infection frequency, etc.) and 'micro' components (e.g. percentage spore germination in vivo, appressorium formation and penetration). She found differences between cultivars in most of the components, though very few of the components were significantly correlated, and those

that were, were mainly the 'macro' components. Uma (1984) also reported a hypersensitive-type reaction in some of the cultivars in trials using detached leaf material. However, the general pattern of resistance is that, if present at all, it is quantitative in nature e.g. of the 'partial' or 'slow-rusting type', (Dixon, 1976; Uma, 1984; Norwood, 1985; Anon., 1986).

Parlevliet (1979) is a major review of the components that reduce the rate of epidemic development, applicable to cases of 'partial' or 'slow-rusting' resistance where little or no qualitative (e.g. lesion type) resistance can be found. These components include assessments of latent period, infection frequency and spore production, and associated ways of measuring these components indirectly, e.g. lesion size assessments instead of spore production. All these components act cumulatively to reduce the reproductive capacity of the pathogen, but may have different effects in the field on the epidemiology of the disease (Zadoks & Schein, 1979; Parlevliet et al. 1980; Eskes & Toma-Braghini, 1981). Analysis of such components is therefore helpful in determining efficient selection criteria for practical breeding work as well as helping to elucidate the mechanisms of resistance (Eskes & Toma-Braghini, 1981). It is difficult to predict which components analysed in the laboratory give the best indication of performance in the field, bearing in mind the differences between the monocyclic nature of the disease in experiments and the polycyclic nature nature of disease epidemics in the field. Spore production is an example of a character which gives a good indication of the sum of all the components in a monocyclic test, but which is not always well

correlated with cultivar performance in the field (Parlevliet, 1979). Nevertheless, Johnson and Taylor (1976) have reviewed its usefulness in investigating race-specificity in controlled environment tests.

Although such resistance components have been studied in many other crop-pathogen systems, they have usually been in cereals or other major crops which tend to be inbreeding, and for which there are defined growth stages in the life of a plant. As already discussed in chapter 5, leeks are an outbreeding and consequently variable crop, without defined growth stages, making comparisons with work on other crops difficult. However, the interaction between the most closely related crop plant, asparagus, and the rust P. asparagi, makes an interesting and relevant comparison with the leek- leek rust interaction. Johnson (1986) found no evidence for complete or qualitative resistance, only quantitative differences between cultivars. He compared latent period and infection frequency successfully by correlations with the 'area under the disease progress curve' data from field trials. Blanchette et al. (1982) also found high levels of field resistance in germplasm of Asparagus officinalis, and emphasized the desirability of obtaining pure lines of asparagus through the production of male-sterile hybrids as an aid to study into the genetics of this resistance. Such a development might also further the study of the leek - leek rust interaction.

Therefore, the aims of this study were to:-

a) Analyse the usefulness of components of disease resistance in the study of disease resistance in the leek - leek rust interaction

b) Investigate the extent, if any, of quantitative resistance in a selection of leek cultivars

c) Compare the performance of these cultivars against a range of geographically selected leek rust isolates, looking for broad levels of specialisation which would also indicate the homogeneity of the pathogen population.

6.2. MATERIALS AND METHODS

6.2.1. Materials

a) Inoculum

Urediniospore samples of the BIRM, NIAB, LUDD, STOCK and WSCOT isolates were used, as detailed in section 1.2.1. The BIRM, STOCK and WSCOT isolates had all been subcultured as in 1.2.6. to provide sufficient quantities of inoculum.

b) Test plants

Details of the cultivars used are given in table 6.1. The cultivars were a selection from those which had shown different levels of susceptibility to rust in initial field trials at NIAB. They were also chosen to give range of cultivars from the major 'breeding types' listed in Anon. (1982), which refer to hardiness, stem length and leaf colour. These details are listed in table 6.1. The plants were all in the 4-8 leaf stage and were 5.5 to 7.0 months old.

6.2.2. Methods

6.2.2.1. Experimental Conditions

Each isolate was tested in a separate experiment. A 5 cm long area was marked on the adaxial leaf surface 20 cm from the tip of the

Cultivar	Type (production period, shank length and flag colour)	Origin
Odin Longstanton	Autumn, long, with medium green flag	Harrison
Glennvilliers Splendid	as Odin (above)	Harrison
Winterrreuzen	Jan.-March, medium, medium green flag	Harrison
GW Starina	Dec.-April, short-medium, medium dark flag	Yates
BW Blizzard	Spring, short, with dark green flags	NIAB
BR Longa	Autumnn, very long, pale green flags	NIAB
BW Alaska	as Blizzard	NIAB
BW Derrick	April-May, short, with dark green flags	NIAB/Bejo Zaden
BW Durano	as Blizzard	NIAB
Ludovicus	as Blizzard	NIAB Nickerson-Zwaan
Rolan	Jan.-March, medium, dark green flags	NIAB
Tivi	Sept.-Dec. long to very long, medium green flags	NIAB
AM Walton Mammoth	Oct.-March, medium, dark-medium green flags	A L Tozer Ltd.
Musselburgh	Feb.-May, short with pale-medium green flags	Sutton's
Albin Star	Jan.-March, medium with medium green flags	Enza Zaden (Holland)
AM Goliath	Autumn-Spring, medium, medium green flags	Harrison

Table 6.1

Details of the leek cultivars used in the trials with leek rust isolates from different geographical locations (Anon. 1982; Chowings, J. W., 1983)

Abbreviations: AM Autumn Mammoth; BR Bulgaarse Reuzen; BW Blaugroene Winter; GW Giant Winter; SG Swiss Giant.

oldest leaves of each of five replicate plants for each cultivar in each experiment. Plants of leek cv. Longa were only available for the BIRM and NIAB isolate trials. The plants were inoculated using the spray technique (1.2.9.b.) with the inoculum concentration adjusted to give 0.3 - 0.4 mg cm^{-3} of viable spores (depending on the viability of the isolate). After inoculation the plants were placed in a randomised design in a growth room, with conditions as for 4.2.2.1.

6.2.2.2. Assessment Methods

The plants were examined daily after inoculation and the latent period was measured for each plant. After 20 days, an assessment of lesion type was made for each plant, and pustule density measured for each segment. The leaf segments were then cut from the plants and stained using the Bruzzese and Hasan method, section 1.2.11. and the lengths of up to ten pustules per segment measured.

6.2.2.3. Analysis methods

a) Hierarchical Analysis of Variance

To analyse the variation within the experimental system a hierarchical analysis of variance (Sokal & Rohlf, 1981) was performed on both the pustule density and pustule length data for selected cultivars in the BIRM isolate trial. This type of analysis requires 'balanced' data, i.e. complete data with no missing values in each level.

b) One-way analysis of variance

A standard one-way analysis of variance technique (Parker, 1979) was used to analyse the differences between cultivars in each trial, for each component.

c) Correlation coefficients

For comparison of relative cultivar performance between isolate trials, and between components, product-moment correlation coefficients were used (Parker, 1979).

d) Ratings

To facilitate comparison between the cultivars within and between isolate trials, a rating system was developed. For each component in each isolate trial, a scale of nine equal divisions was formulated, with the upper and lower limits of the scales set by the highest and lowest mean cultivar component values within that trial. The scale was arranged so that a score of 1 was susceptible (e.g. high pustule density, short latent period) and 9 resistant (e.g. low pustule density, long latent period). A rating for each component for each cultivar in each trial was then assigned.

6.3. RESULTS

6.3.1. Latent period

There were no significant differences between the cultivars in any of the trials ($P > 0.05$) with a small magnitude of absolute differences between cultivars. However, several features can be seen in comparing both isolates and cultivars. Figure 6.1. shows the mean latent periods for each of the cultivars in each trial.

The mean latent periods of the cultivars were generally more uniform in the NIAB and LUDD isolate trials, longest in the NIAB trial and shortest in the WSCOT trial. The only significant correlations for cultivars between isolates was between LUDD and WSCOT isolates, ($P < 0.01$, table 6.2) but there was a significant negative correlation between the BIRM and STOCK isolates.

Comparing individual cultivars, no cultivar performed consistently between isolates. Rolan had the most consistently long latent period, and Odin Longstanton and Gennevilliers Splendid had longer than average latent periods in all but the BIRM isolate. Goliath had long latent periods except in the WSCOT isolate, and Albin Star and Musselburgh also had longer than average latent periods. Starina varied the most between isolates, with a long latent period in the BIRM, NIAB, LUDD and WSCOT trials but the shortest latent period of any cultivar in the STOCK trial. Durano and Ludovicus had shorter latent periods than average. These points are summarised by the

Figure 6.1
Histograms of mean cultivar latent period in the isolate trials. Means are from up to 5 plants per cultivar per isolate, bars give standard errors for each combination. (Error bars absent indicates standard error of zero).

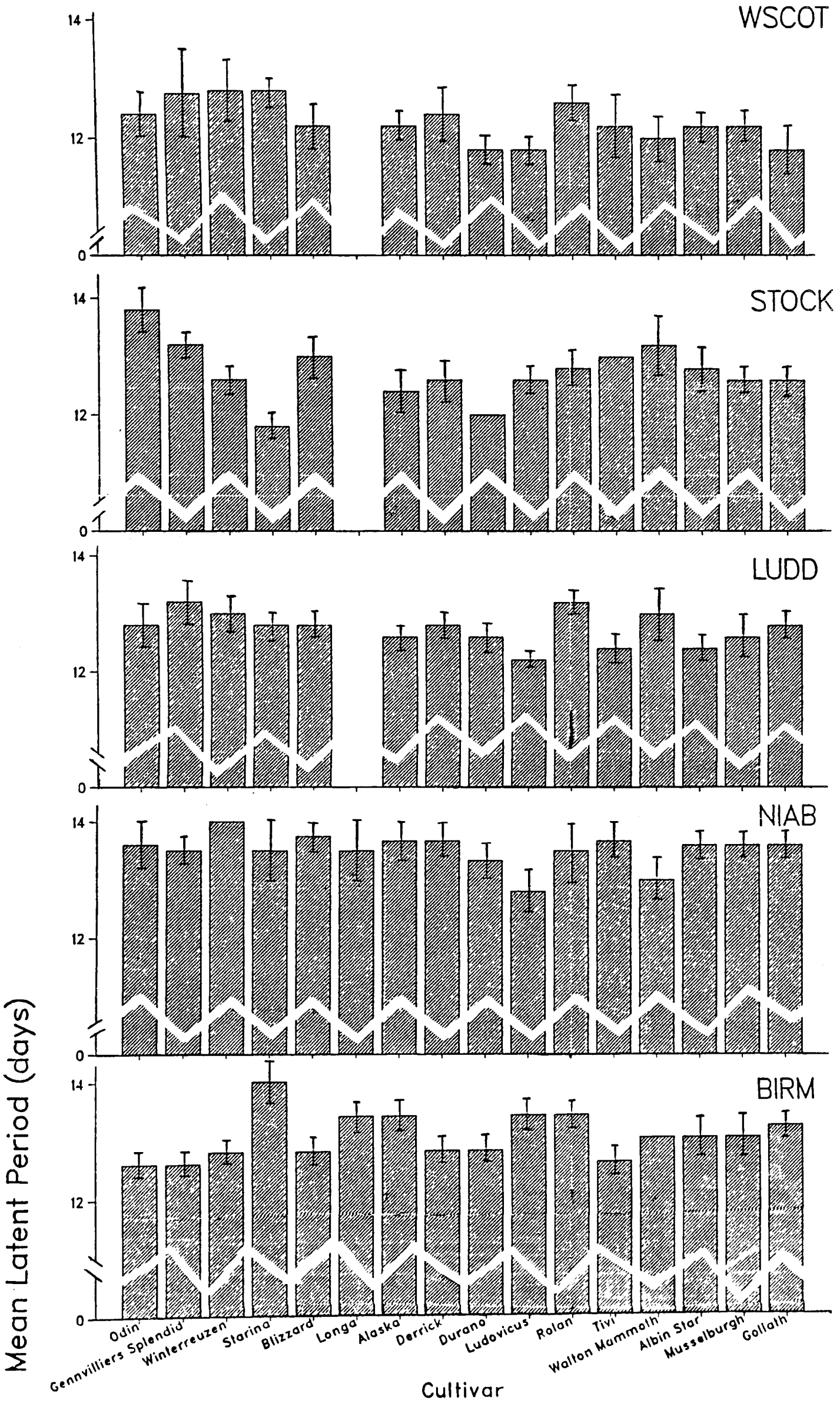

WSCOT
STOCK
LUDD
NIAB
BIRM
14
12
0
Mean Latent Period (days)
Odin
Gennvilliers Splendid
Winterreuzen
Starina
Blizzard
Longa
Alaska
Derrick
Durano
Ludovicus
Rolan
Tivi
Walton Mammoth
Albin Star
Musselburgh
Goliath
Cultivar

Table 6.2
Correlation coefficients for the cultivars between isolates, for mean latent period, (cv. Longa excluded except for the BIRM:NIAB comparison).

	BIRM	NIAB	LUDD	STOCK
NIAB	-0.285			
LUDD	-0.085	0.261		
STOCK	-0.633*	0.019	0.238	
WSCOT	0.057	0.518	0.601*	0.058

* significant at 0.05 level

Table 6.3
Rating for cultivars based on mean latent period.
1 = susceptible (short latent period)
9 = resistant (long latent period)

Cultivar	Isolate					
	BIRM	NIAB	LUDD	STOCK	WSCOT	'mean'
Odin Longstanton	1	7	6	9	6	6
Gennevilliers Splendid	1	6	9	7	9	6
Winterreuzen	2	9	8	4	9	6
Starina	9	6	6	1	9	6
Blizzard	2	8	6	6	4	5
Longa	6	6	-	-	-	-
Alaska	6	7	4	3	4	5
Derrick	2	7	7	4	6	5
Durano	2	4	4	1	1	2
Ludovicus	6	1	1	4	1	3
Rolan	6	6	9	5	8	7
Tivi	1	7	2	6	4	4
Walton Mammoth	3	2	8	7	2	4
Albin Star	3	7	2	5	4	4
Musselburgh	3	7	4	4	4	4
Goliath	4	7	6	4	1	4

ratings given in table 6.3. Only Durano has ratings consistently below average and Rolan consistently above average.

However, the main feature of the results is the large level of variation within cultivars, and the lack of correlation between isolates.

6.3.2. Pustule density

There was considerable variation in the mean pustule density for each isolate, so to facilitate between-isolate comparisons the mean pustule density for each cultivar-isolate combination was divided by the appropriate isolate mean pustule density, producing a 'pustule density ratio', (Figure 6.2).

A hierarchical analysis of variance was performed on the raw data to analyse the sources of variation within the tests. Data from four cultivars (Blizzard, Durano, Walton Mammoth and Goliath) in the BIRM trial were examined, since the analysis required balanced groups (i.e. cultivars with no leaves missing from the plants). For each cultivar, three leaves from each of four plants were analysed (48 leaves in all). The summary analysis is given in table 6.4. The analysis shows a significant added variance component at the the plant level ($P < 0.01$), with cultivars not significant.

When the cultivars were analysed (one-way ANOVA on raw data), there were significant differences between cultivars within the BIRM isolate

Figure 6.2 ratio
Histograms of mean pustule density on the cultivars in the isolate trials. Means are from up to 15 leaves per cultivar per isolate (3 per each of 5 plants), bars give standard error.

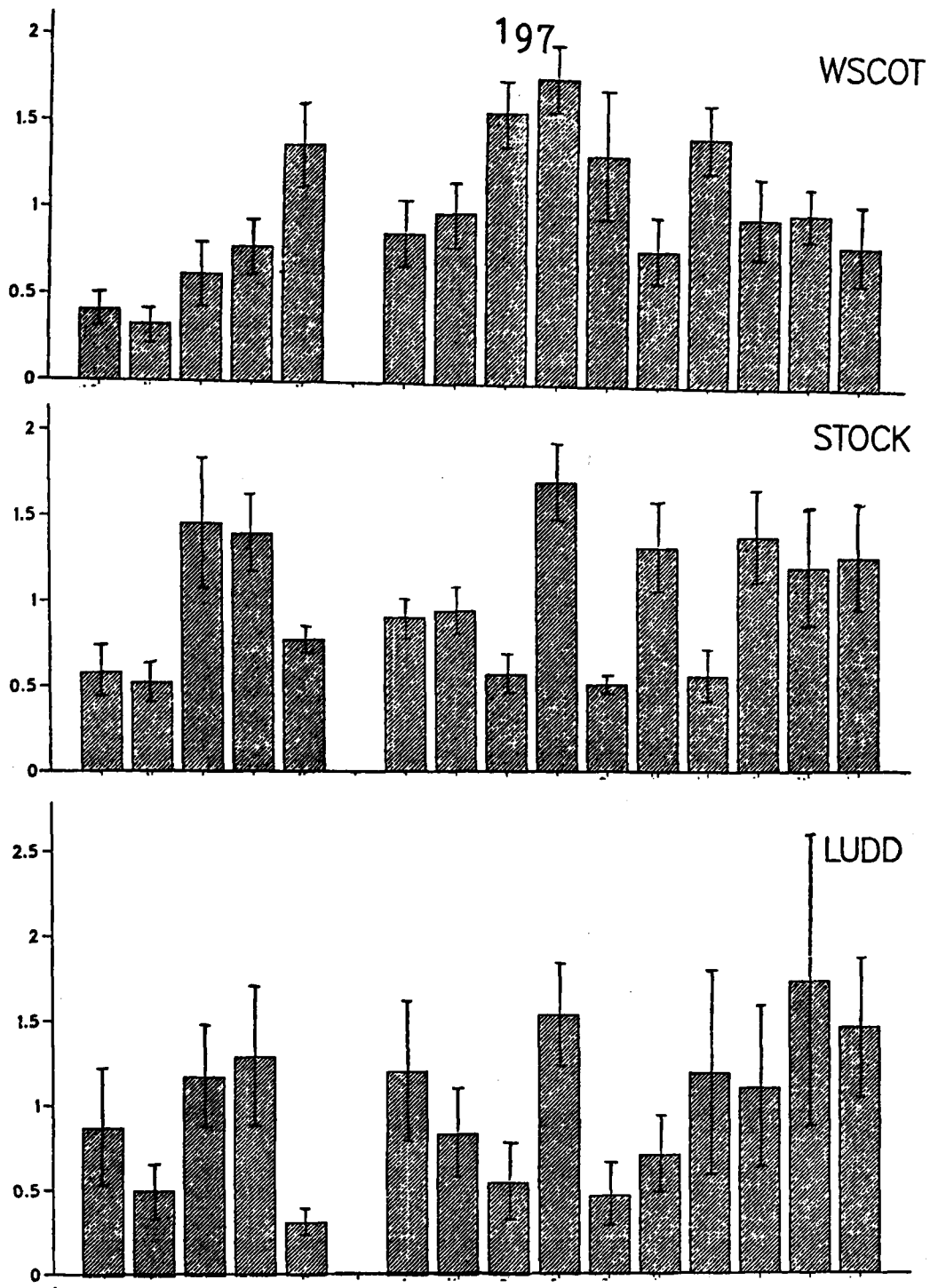

WSCOT
Isolate Mean = 3.12
(pustules cm^{-2})
STOCK
Isolate Mean = 9.91
(pustules cm^{-2})
LUDD
Isolate Mean = 1.60
(pustules cm^{-2})
NIAB
Isolate Mean = 2.87
(pustules cm^{-2})
BIRM
Isolate Mean = 5.45
(pustules cm^{-2})
Pustule Density Ratio
Odin
Gennvilliers Splendid
Winterreuzen
Starino
Blizzard
Longa
Alaska
Derrick
Durano
Ludovicus
Rolan
Tivi
Walton Mammoth
Albin Star
Musselburgh
Gallulh
Cultivar

Table 6.4
Heirarchical analysis of variance of pustule density with levels ; leaves within plants, within selected cultivars, in the BIRM isolate trial.

Sources of variation	df	SS	MS	F	P
Cultivars	3	49.08	16.36	0.227	N.S.
Plants	12	815.59	67.97	2.290	< 0.01
Leaves	32	948.76	29.65	-	-

Table 6.5
Correlation coefficients for the cultivars between isolates, for mean cultivar pustule density (cv. Longa excluded except for the BIRM:NIAB comparison).

	BIRM	NIAB	LUDD	STOCK
NIAB	-0.173			
LUDD	-0.318	0.426		
STOCK	-0.106	0.156	0.657*	
WSCOT	0.282	0.007	0.019	0.024

* significant at 0.05 level

Table 6.6
Rating for cultivars based on mean pustule density.
1 = susceptible (high pustule density)
9 = resistant (low pustule density)
( ) 'mean' of less than 5 isolates

Cultivar	Isolate					
	BIRM	NIAB	LUDD	STOCK	WSCOT	'mean'
Odin Longstanton	6	8	6	9	9	8
Gennevilliers Splendid	7	9	8	9	9	8
Winterreuzen	2	5	4	2	8	4
Starina	9	9	3	3	7	6
Blizzard	2	9	9	8	3	6
Longa	4	9	-	-	-	(7)
Alaska	8	1	4	7	6	5
Derrick	4	3	6	6	5	5
Durano	1	9	8	9	2	6
Ludovicus	6	6	2	1	1	3
Rolan	6	9	9	9	3	7
Tivi	5	8	7	3	7	6
Walton Mammoth	4	5	4	9	3	5
Albin Star	5	8	5	3	5	5
Musselburgh	8	6	1	4	5	5
Goliath	2	8	2	4	6	4

($P < 0.01$) and the STOCK and WSCOT isolates ($P < 0.01$). There were no significant differences between the cultivars in the NIAB and LUDD isolates ($P > 0.05$) where the mean pustule pustule density for the isolates were low but the level of variation high. However there was little correlation between the cultivars in different isolate trials, as shown by the correlation coefficients (table 6.5). Only the LUDD and STOCK isolates were significantly correlated, ($r = 0.657$, $P < 0.05$) the remaining correlations being low, or negative in the case of the BIRM isolate. Thus the cultivars did differ in their response within certain isolates, but not consistently between them. Table 6.6 shows the results summarised in rating form. Again, the mean feature here was the lack of consistency within given cultivars to different isolates. Gennevilliers Splendid and Odin Longstanton were the only cultivars with consistently low pustule densities, with none having consistently high densities. Derrick and Tivi had pustule densities in the middle range, and Ludovicus varied from middling to high pustule density. Winterreuzen generally had high pustule densities except in the WSCOT isolate, and similarly for Goliath except in the NIAB isolate. The ratings for the NIAB isolate were disproportionately affected by the very high pustule density of Alaska, compared with the other cultivars. In fact, Alaska had middling to high pustule densities except in the BIRM isolate, and was a major component of the negative correlation of the BIRM and NIAB isolates. The other principal feature of the results was the large level of error present in the NIAB trial, and to a lesser extent, the LUDD trial.

6.3.3. Pustule Length

An hierarchical analysis of variance was performed on the pustule length data from four cultivars in the BIRM isolate trial, to examine the level and nature of variation within the trials system. The cultivars were the same as those analysed in 6.3.2. for pustule density. For each cultivar, pustule length on each of 10 pustules on each of three leaves on each of three plants was analysed, (360 pustules in total). The summary analysis is given in table 6.7. The analysis shows a significant added variance component in the leaves, ($P < 0.001$) although the cultivars were only just insignificant ($P > 0.05$). The absolute differences between the leaf means were quite large, even within a given plant, (table 6.10). However, the standard errors for each cultivar were relatively small when the data were pooled from leaves and plants. The data were pooled in this way to give an overall picture of each cultivar, since individual leaves were not necessarily of equivalent physiological age between plants or between cultivars.

The analyses of variance within each isolate show that there were very highly significant differences between some of the cultivars ($P < 0.001$ in each case, table 6.8). However there were no significant correlations between the isolates (table 6.9). Thus the cultivars differed significantly from each other within a given isolate but not necessarily in the same direction between isolates. It is therefore necessary to examine the data from individual isolate-cultivar combinations in more detail. Figure 6.3. shows the mean pustule lengths

Table 6.7.
Hierarchical analysis of variance on pustule length data from three leaves, on three plants from the cultivars Blizzard, Durano, Walton Mammoth and Goliath, in the BIRM isolate trial.

Source of variation	df	SS	MS	F	P
Cultivars	3	2.40	0.80	2.35	N.S.
Plants	8	2.72	0.34	0.87	N.S.
Leaves	24	9.37	0.39	19.50	< 0.001
Pustules	324	6.49	0.02		

Table 6.8
Analysis of variance between cultivars within each isolate-pustule length data

	F	d.f.	P
BIRM	8.24	15/1691	< 0.001***
NIAB	15.85	14/923	< 0.001***
LUDD	13.17	14/1065	< 0.001***
STOCK	24.09	14/1967	< 0.001***
WSCOT	16.20	14/1646	< 0.001***

Table 6.9
Correlation coefficients (r) of the cultivar pooled mean pustule length between the isolates.

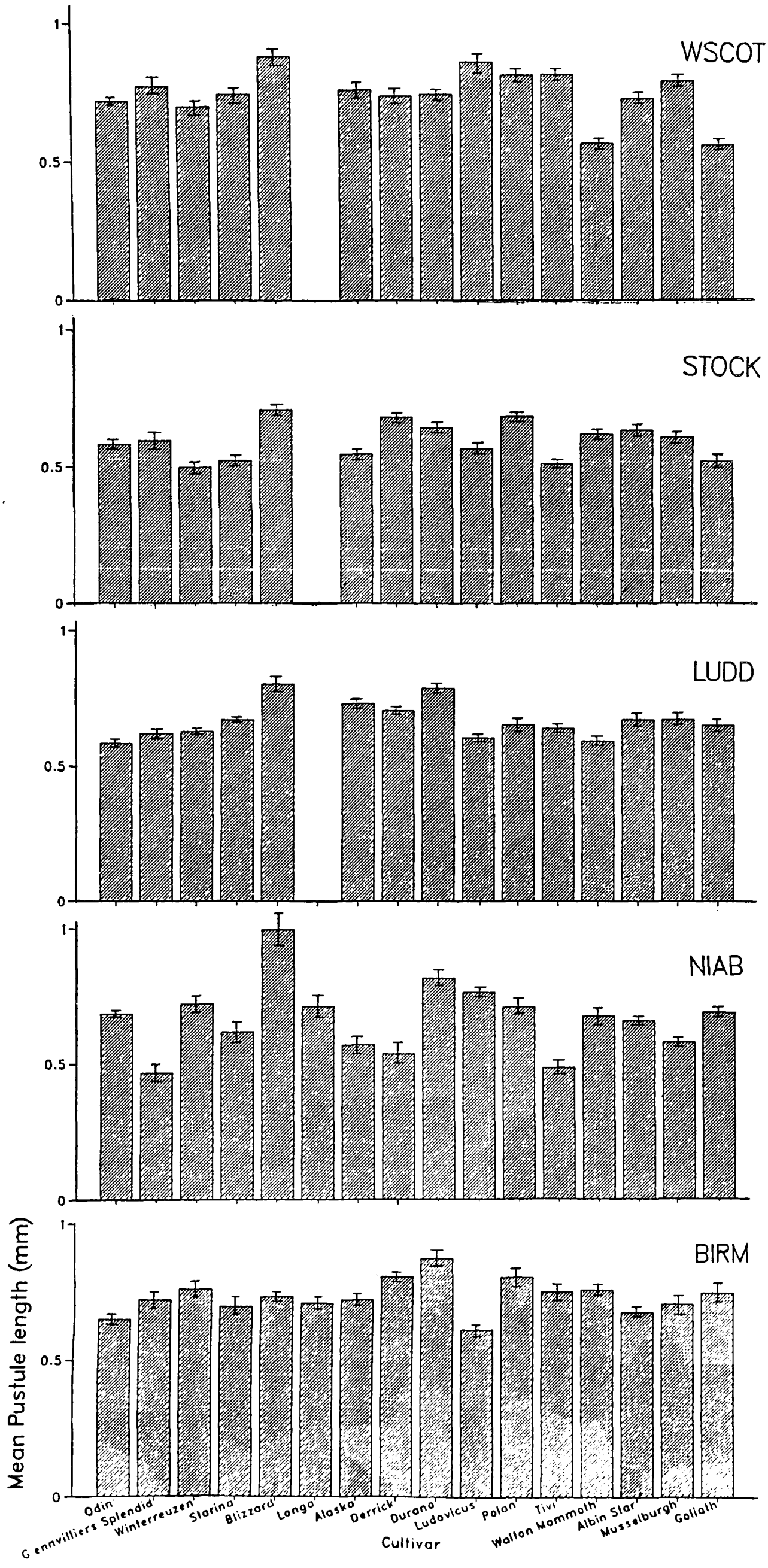
	BIRM	NIAB	LUDD	STOCK
NIAB	0.065			
LUDD	0.481	0.438		
STOCK	0.302	0.387	0.479	
WSCOT	-0.211	0.159	0.333	0.281

Table 6.10
Means of pustule length of leaves within plants, within cultivars Blizzard, Durano, Walton Mammoth and Goliath in the BIRM isolate trial.

Cultivar	Leaf*	Plants			Cultivar mean
		1	2	3	
Blizzard	1	0.825	0.624	0.568	
	2	0.618	0.735	0.585	
	3	0.664	1.063	0.843	
		0.702	0.807	0.665	0.725
Durano	1	0.728	0.628	0.902	
	2	0.799	0.723	0.934	
	3	1.366	1.052	1.295	
		0.964	0.801	1.044	0.936
Walton Mammoth	1	0.624	0.802	0.584	
	2	0.711	0.820	0.635	
	3	0.683	1.114	0.918	
		0.673	0.912	0.712	0.766
Goliath	1	0.579	0.745	0.581	
	2	0.664	0.804	0.653	
	3	1.075	0.994	0.773	
		0.773	0.994	0.773	

*leaf 1 = oldest 3 = youngest
plants = 3 replicates

Figure 6.3
Histograms of mean pustule length on the cultivars in the isolate trials. Means are from up to 150 pustules per cultivar per isolate, bars give standard errors.


WSCOT
STOCK
LUDD
NIAB
BIRM
1
0.5
0
Mean Pustule length (mm)
Odin
Genvilliers Splendid
Winterreuzen
Starina
Blizzard
Longa
Alaska
Derrick
Durana
Ludovicus
Polan
Tivi
Walton Mammoth
Albin Star
Musselburgh
Goliath
Cultivar

for each cultivar with each isolate. Table 6.11 shows the rating for each cultivar based on the mean pustule length, derived as in 6.2.2.3(d). Note that in the trial, the exceptional performance of Blizzard gives all the other cultivars an 'average-to-short' rating. The cultivar with the longest pustules was Blizzard, which performed consistently in all except the BIRM isolate trial. Durano also had consistently longer than average pustules, with a less extreme response to the STOCK and WSCOT isolates. At the other end of the scale, Odin Longstanton and Goliath had consistently shorter than average pustules. Winterreuzen also had shorter or average length pustules, with the shortest in the Stock isolate trial. Walton Mammoth had exceptionally short pustules in the LUDD and WSCOT trials, and Gennevilliers Splendid, although middle ranking generally, had exceptionally short pustules in the NIAB trial. This pattern of 'average' performance overall with short pustules in the NIAB trial was also found in cultivars Tivi, Alaska and Derrick. Starina was also average overall with short pustules in the NIAB and STOCK trials. The overall picture is therefore of relatively uniform performance within cultivars to the different isolates, with average-to-longer or average-to-shorter pustules, despite the low arithmetical correlation between isolates. The major exception is Ludovicus with very short pustules in the BIRM, LUDD and STOCK trials and long pustules in the NIAB and WSCOT trials. The ratings also serve to illustrate these points, bearing in mind the distortion of the scale in the NIAB trial.

Consistency of the within plant variation

The data used for hierarchical analysis of variance also showed the

Table 6.11
Rating for cultivars based on mean pustule length.
1 = susceptible (long pustules)
9 = resistant (short pustules)
( ) 'mean of less than 5 isolates

Cultivar	Isolate					
	BIRM	NIAB	LUDD	STOCK	WSCOT	'mean'
Odin Longstanton	8	6	9	6	5	7
Gennevilliers Splendid	6	9	8	5	3	6
Winterreuzen	5	5	8	9	6	7
Starina	7	7	6	8	4	6
Blizzard	6	1	1	1	1	2
Longa	6	5	-	-	-	(6)
Alaska	6	7	3	7	4	5
Derrick	3	8	4	3	5	5
Durano	1	4	1	3	4	3
Ludovicus	9	5	8	6	1	6
Rolan	3	5	6	2	2	4
Tivi	5	9	7	9	2	6
Walton Mammoth	5	6	9	4	9	7
Albin Star	7	6	6	4	5	6
Musselburgh	6	7	6	5	3	5
Goliath	5	6	6	8	9	7

Table 6.12
Lesion type assessments for each cultivar-isolate combination using the key in 1.2.10 (a). The results are the range of lesion type on the replicate plants of each cultivar

Cultivar	Isolate				
	BIRM	NIAB	LUDD	STOCK	WSCOT
Odin Longstanton	3	3	3	2-3	3
Gennevilliers Splendid	2-3	2-3	3	3	2-3
Winterreuzen	3	3	3	3	3
Starina	2-3	2-3	3	3	3
Blizzard	2-3	3-4	3	3	3
Longa	3	3	-	-	-
Alaska	3	3	3	3	3
Derrick	3	3	3	2-3	3
Durano	2-3	2-3	3	3	3
Ludovicus	3	2-3	2-3	2-3	3
Rolan	3	3	3	2-3	3-4
Tivi	3	3	3	2-3	3
Walton Mammoth	3	3	2-3	3	2-3
Albin Star	3	3	2-3	3	3
Musselburgh	3	3	3	3	3
Goliath	3	3	3	3	3

direction of the variation between leaves in each plant of the cultivars examined (table 6.10). In most cases the older leaves had smaller pustules than the younger leaves, and this variation was consistent between cultivars.

6.3.4. Lesion Type

The lesion type assessments for the cultivars are given in table 6.12. The main feature was the lack of variation between cultivars and isolates. Despite the variation in lesion size and number, the appearance of infected tissue in all the cultivars was usually very uniform, with the only differences being the number of pale green flecks (non-sporulating colonies) and the size of the pustules (measured more accurately by lesion length). There were no 'resistant-type' reactions or necrotic (brown) flecks, or areas of necrotic tissue associated with the pustules. In a few cases, the whole leaf tissue appeared yellow (chlorotic) where the infection was heavy, and the leaf had begun to senesce, but this could not be distinguished from the ordinary process of leaf senescence.

6.3.5. Comparison between components and NIAB field trials

Table 6.13 gives the mean ratings for each component for each cultivar, together with the overall mean of these, and the resistance ratings for the same cultivars from the field trials at NIAB (Anon., 1986).

Table 6.13
Mean cultivar ratings for latent period (LP), pustule density (PD), pustule length (PL), with the overall (mean) rating and the NIAB field trials ratings

9 = resistant, 1 = susceptible

Type	Cultivar	LP	PD	PL	Overall rating	NIAB rating
	Odin Longstanton	6	8	7	7	7
AM	Walton Mammoth	4	5	7	5	7
	Gennevilliers Splendid	6	8	6	7	6
	Winterreuzen	6	4	7	6	5
GW	Starina	6	6	6	6	5
AM	Goliath	4	4	7	5	5
SG	Albin Star	4	5	6	5	4
	Rolan	7	7	4	6	3
	Tivi	4	6	6	5	2
BW	Derrick	5	5	5	5	2
BW	Blizzard	5	6	2	(4)	2
BW	Durano	2	6	3	4	1
BW	Ludovicus	3	3	6	4	1
BW	Alaska	5	5	5	5	1
BR	Longa	(6)	(7)	(6)	(6)	1
	Musselburgh	4	5	6	5	N/A

( ) Not entered in all trials

Firstly, the range of the ratings from NIAB is clearly larger than those derived from the data here, since variation between the isolate trials here has tended to produce component means away from the extremes. This effect is most pronounced in the overall ratings due to low correlation between the components. The range of variation within the individual components is greater, though most of the cultivars still have middling values.

The correlations between the different component ratings were not strong. There was no significant correlation between latent period and pustule length ($r = 0.142$, $P > 0.05$) or between pustule density and pustule length ($r = 0.175$, $P > 0.05$). There was a correlation between latent period and pustule density ($r = 0.522$, $P < 0.05$). Also, the few extreme results would tend to influence the correlations strongly.

The correlations between latent period and pustule density and the NIAB ratings were not significant ($r = 0.329$ & $r = 0.228$, $P > 0.05$ respectively), but there was a correlation between pustule length ($r = 0.601$, $P < 0.05$) and a stronger correlation between the overall ratings and the NIAB ratings, ($r = .612$, $P < 0.05$), suggesting a 're-inforcing' factor between the individual components.

When looking at the types of cultivar, it is apparent that in terms of the trials here, and the NIAB ratings, the Blaugroene Winter type of leeks were rated as 'more susceptible' than the other cultivars. This

does not appear to be due to any single component measured, but to several components.

Details of the actual levels of replication remaining after loss of plants, leaves, etc. when measuring the components of resistance are given in appendices 4 to 6.

6.4. DISCUSSION

The results showed a generally high level of variation between isolates and cultivars. When considering the results and analyses it was not possible to distinguish between environmental effects that might have operated between the trials, given that each isolate was tested in a separate trial. Theoretically, a large two-way analysis of variance would have been the ideal solution to comparing cultivars within and between isolates, but such analyses are subject to error and misleading interpretation when there are significant gaps in the replication, as occured in certain trials here. Therefore, one-way analyses of variance, in conjunction with correlations were used to provide more accurate interpretations of the results.

6.4.1. Latent period

The high level of variation within the isolate-cultivar combinations is in part explained by the size of the divisions (days) compared with the length of the latent periods, reducing the sensitivity of the measurement. However a major factor must be the measurement itself. Most previous studies have measured latent period as the time from inoculation to eruption of 50% of the infection sites, which Parlevliet (1975) has rightly stated as being more accurate than actual rupture time, since the first few sites to rupture may not be representative of the majority. The major problem in this study was the length of this definition of latent period compared with the likely life-span of individual leaves. Thus improvements might be to

use a lower 'percentage eruption' figure such as 25% or so, and to measure individual leaves as additional levels of within-plant variation. Uma (1984) measured latent period in three of the cultivars used here; Gennevilliers Splendid (15.1 days), Winterreuzen (14.8 days) and Odin Longstanton (15.1 days). Although the actual figures are not comparable due to the different measuring criteria employed, it is interesting to note the actual differences between these cultivars were comparable in the two studies. However, only in the LUDD trial was the same ranking between these cultivars achieved.

Latent period is one of the most commonly measured components in plant pathology, due to its epidemiological importance and ease of measurement. Johnson (1986) working on Puccinia asparagi on asparagus found that latent period measured in the greenhouse correlated well with cultivar performance in the field, and therefore suggested that it was a major component contributing to resistance in the field. Shaner and Hess (1978) suggested that latent period as normally measured actually measures two components - time to onset of sporulation and rate of eruption thereafter. In this study latent period was effectively a measurement of the first character only. It may be desirable to measure these two sub-components separately to analyse their overall contribution to cultivar or plant disease performance. Parlevliet (1975) noted that latent period of Puccinia hordei on barley varied at the extremes of the leaf, but stated that it was immune from light and temperature effects. There were however differences between leaves and an interaction between leaf and plant ageing, with latent period increasing in older plants and decreasing on ageing leaves. McGregor

and Manners (1985) found that temperature did affect latent period in P. striiformis on wheat, with increasing temperature decreasing latent period as well as reducing the longevity of sporulating leaves. Parlevliet (1979) in a review of the components of quantitative resistance stated that latent period could be seen as a measure of growth of the pathogen in the host, although Sztejnberg and Wahl (1977) found no differences in latent period despite differences in colony size P. graminis f. sp. avenae on oats and Avenae sterilis. However, Parlevliet (1979) still maintains strongly that latent period is as good an overall measure of partial resistance as spore production, since the length of the latent period is of vital importance where a number of disease cycles are needed to complete an epidemic (Johnson & Taylor, 1976).

6.4.2. Pustule density

There were three main features of this component in the cultivar-isolate trials. Firstly, the high variation in the mean pustule density between trials, despite attempts to compensate for variations in inoculum viability is difficult to explain. One of the causes of variation, especially in the NIAB isolate, was a relatively large number of zero values on certain leaves; often pustules were present on the leaf, but not in the defined area. However, even accepting this there were still high levels of variation in the cultivar-isolate combinations with high pustule densities and no zero

values. Variation could have been due to experimental error, between the trials, (e.g. slight changes in technique or differences in the physiological state of the cultivars), or genuine differences in infectivity or germination between the isolates. It is possible that the isolates have differing environmental adaptations, but one would not expect these to be so large over such relatively small geographical differences. Secondly, the within-cultivars variation in each trial was very high, but despite this there were significant differences between cultivars in some of the trials. This suggests that there are large between-plant differences within cultivars (borne out by the hierarchical analysis) or that there were large experimental errors. It is impossible to distinguish the causes of this variation, but the lack of it in the other components suggests that it is related to this particular component, either genetically (variation) or experimentally (error). Thirdly, there were differences in relative cultivar performance between isolates. This could be due to pathogen specialisation or experimental error. It could also be a more general factor in the plants not necessarily related to the host-pathogen interaction, e.g. a leaf-surface effect like water retention or leaf surface microflora, or leaf shape affecting inoculum deposition. It is possible that such factors could have varied between trials, i.e. the variation was due to interactions between the experimental technique and the host material.

Uma (1984) noted that Gennevilliers Splendid had a higher pustule density than either Odin Lonstanton or Winterreuzen, but in this study Winterreu zen had consistently higher pustule densities than the other

two cultivars. However, this study agrees that all three moderate to low pustule densities compared with other cultivars.

Parlevliet and Kuiper (1977) noted very high levels of variation in infection frequency (equivalent to pustule density) of P. hordei on barley cultivars despite high levels of replication. Infection frequency varied between leaf parts and between plants - similar to the findings of this study. They stated that this character was difficult to measure in an accurate and representative way, and found that for adult plants at least, a spore settling tower did not reduce variation. Parlevliet (1979) echoed many of the earlier conclusions but added that this component was in fact the accumulation of many events, which could cause variation dependent on the developmental or physiological state of the host, the environment, as well as host genotype.

Nevertheless, Johnson (1986) found that infection frequency correlated with both latent period and the results of field trials of asparagus infected with P. asparagi. Ohm and Shaner (1976) using a spray-inoculation technique very similar to the one used in this study, used this character successfully on wheat infected with P. recondita by measuring it at a given area over several different parts of the leaf, i.e. tip, mid and basal portions. They found significant cultivar differences which also correlated with other components.

Therefore in this study, there appear to be several areas for improvement: Ensuring that the host material is as uniform as

possible; an increase in the number of replicates and possibly measuring a larger area to overcome uneven distribution of inoculum. Given the need to inoculate adult plants, a spore settling tower would need to be very large to accommodate leek plants with leaves up to 1 m long, but it might be possible to inoculate portions of attached leaves using a smaller tower.

6.4.3. Pustule Length

The variation in this component in cultivars and trials was considerably lower than for the other components. Of all the components this was the most accurately measurable, and it was possible to include much higher levels of replication. Furthermore, as a character it is more directly dependent on the host-pathogen interaction since it measures only successful intimate reactions. The hierarchical analysis showed the strong leaf-age component present, but the level of replication seemed adequate to show up significant cultivar effects. As in the other components, there was a considerable between-isolate variation in the cultivars. Despite this, there were some strong cultivar effects notably the pustule length in cv. Blizzard. Furthermore, the significant correlation with the NIAB field trials ratings suggests that this component is a good indicator of field resistance, and can be measured accurately enough for such comparisons. It is not necessarily the only useful component but would appear to be a valuable one in the study of the leek - leek rust interaction. Parlevliet (1979) points out that 'pustule size' characters are not actually true components of resistance themselves (i.e.

they do not actually affect epidemiology directly) but are usually strongly associated with spore production, which is. Inevitably there are exceptions - Habgood (1977) found no differences in pustule size in Rynchosporium secalis on barley, but there were significant differences in spore production between cultivars. However spore production is notoriously difficult to measure (Johnson & Taylor, 1976; Parlevliet, 1979) and especially difficult on intact plants as large as adult leek plants. It would also be possible to measure pustule diameter or area, but the 'longitudinal slit' nature of rust pustules on leeks means that length is probably the most indicative of 'pustule size'.

Uma (1984) found the pustules on Winterreuzen to be larger than those on Odin Longstanton and Gennevilliers Splendid. In this study, there were no large differences between these three cultivars, and this ranking was found only in the BIRM and NIAB isolate trials. Ohm and Shaner (1976) found pustule size to be affected by the growth stage of the host, but nevertheless found differences between cultivars of P. recondita on wheat, similar to the situation here.

6.4.4. Lesion Type

The main features in the results here were the lack of differences between cultivars in lesion type. The complete absence of any form of 'hypersensitive flecking' indicated that there does not appear to be any form of qualitative resistance in this pathogen-host system, and this also indicates the homogeneity of the pathogen population with regard to its performance on leeks.

This information agrees with that observed in the field, (Burchill, pers. comm.; personal observations). However Uma (1984) noted large differences in reaction type between seven leek cultivars and also described a hypersensitive response in several cultivars using detached leaf pieces. However, the scale used to assess reaction type had a large component of pustule size and density included in it which would account for a substantial proportion of the differences. Additionally, in this study leaf chlorosis and necrosis did occur on leaves where the infection was very intense, (i.e. with very high localised pustule densities) especially if the leaf was senescing. It could be that some of the reaction-type differences reported in Uma (1984) were due to either or both of these causes, which are indicative of stress and senescence, and did not occur on less densely inoculated areas of the same plant. Uma (1984) also pointed out that the hypersensitive response was not observed on intact plants of the same cultivars, and suggested that hypersensitive flecking on detached leaf pieces of an otherwise susceptible host was a general symptom of stress. This explanation agrees with the findings of Mayama et al. (1975) who found that normally susceptible lines of wheat produced hypersensitive reactions to infection by P. graminis when using detached leaf pieces.

Most authors have described 'slow rusting' characteristics on otherwise susceptible hosts. Johnson (1986) found a similar situation in the asparagus - P. asparagi host pathogen system to that described in this study, i.e. a lack of qualitative resistance but important levels of quantitative resistance. This is interesting because aspar-

agus is closely related to the genus Allium, especially compared with the cereal groups most commonly studied. Parlevliet (1979) noted that lesion type in practice tended to correlate closely with spore production, and to a lesser extent pustule size, since in many ways it includes a qualitative measure of both of these. However, for analysing and locating useful sources of quantitative resistance it may not be an accurate or reliable enough character compared with direct quantitative measurements.

6.4.5. Overall consideration of the results

The pustule length component would seem to be the most reliable character studied here and was certainly the best correlated with the NIAB field trial ratings. It was the component most independent from the technique, and most dependent on the intimate host-pathogen interaction.

Latent period is an important character but needsto be measured more accurately. Similarly, pustule density has been used often in other studies, but is susceptible to experimental error and high levels of variation from other sources, e.g. surface microflora. However, the overall ratings from latent period and pustule density were correlated indicating that the overall trends were linked. Also, the overall three-component rating was correlated with the NIAB ratings marginally more than the pustule length component alone, indicating that field resistance is a sum of these, and perhaps other components.

There also appeared to be a trend both in the results here and in the NIAB field trials for the Blaugroene-Winter (BW) types to be more susceptible than other types. In contrast, the Autumn Mammoth types (including Goliath and Walton Mammoth) in both trials had moderate or resistant ratings. Another feature of both rating systems was the preponderance of 'moderate' types with ratings of 4 to 6 - two-thirds of the cultivars tested at NIAB fell into this category. Only 13% of the cultivars in the NIAB trials were resistant (rating 7 - 9), the remainder being susceptible (1 - 3). Therefore there seems to be a need to breed cultivars with higher levels of resistance and there is some evidence that it might be found in Autumn-Mammoth types. Such resistance would be useful in leeks, if only to delay the autumnal build-up of disease until the colder winter weather arrived, since this has been shown to reduce the incidence of the disease (Doherty, 1981).

A refinement of the components studied here would seem to be a useful way of looking for resistance in glasshouse/laboratory trials. Other components could also be examined e.g. colony length or spore production. The former would hold fewer advantages over pustule length since the two were correlated (chapter 5) and colony length is more difficult to measure - due to the presence of runner hyphae and the need for very good staining techniques. Spore production is potentially very useful, but an easy and reliable way of measuring it needs to be developed on intact plants. Uma (1984) examined a wide range of characters including 'early' pre-colony formation characters, e.g. percentage appressorium formation, but generally found that the significant correlations were between the later components of

sporulation intensity, latent period, pustule density, infection and pustule length. Sporulation intensity correlated most with the other components, but might be expected to do so in a monocyclic test since it is a 'sum' of the other components.

Johnson (1986) working on asparagus and P. asparagi found that field resistance could not always be demonstrated in glasshouse trials, and suggested that other components might be involved, or that the cumulative or additive effects of apparently small components might be important in the field. Ohm and Shaner (1976) warned that the effectiveness of slow-rusting was affected by the environment as it was not an all or nothing effect. In weather conditions advantageous for the pathogen, the absolute level of the disease might still be sufficient to cause significant damage. In considering the use and study of components of resistance, Parlevliet and Ommeren (1975) noted that the selection for 'partial resistance' tended to select simultaneously for several components, leading to inflated correlations between components, which might in fact be independent. This might therefore be advantageous in selecting for 'polygenic resistance' (Neevoort and Parlevliet, 1978). There are in fact several examples of components varying together in a variety of different host-pathogen combinations (Umaerus, 1970; Ohm & Shaner, 1976; Umaerus & Lihnell, 1976; Parlevliet, 1979; Subrahmanyam et al. 1983) and there are examples of non-correlation (Sztejnberg & Wahl, 1977). However both Shaner & Hess (1978) and Subrahmanyam et al. (1983) pointed out that laboratory experiments could only measure individual components in monocyclic experiments, and it may be difficult to predict their

effect, cumulatively and additively in the field. Shaner (1973) identified two components of resistance in a wheat cultivar against Erysiphe graminis, the effect of which was to reduce the disease in the field to one third of that on a susceptible cultivar.

If the only immediately useful resistance in leek cultivars is quantitative, then it is important to know whether it is likely to be durable. Johnson (1984) stated that 'slow-rusting' is not diagnostic of any particular type of resistance, agreeing with Nelson (1978) that not all 'r-reducing resistances' were necessarily polygenic. Furthermore, even if the systems were polygenic, small race-specific effects have been shown in polygenic systems, in Phytophthora infestans on potato (Caten, 1974) and Puccinia hordei on barley (Clifford & Clothier, 1974). Slow rusting in P. graminis has been shown to be highly race-specific, (Wilcoxson, 1981) and race-specificity has also been found in the slow-rusting resistance in wheat against P. striiformis in the U.K. This resistance has been effective for some twenty years, and Johnson (1984) states that 'it cannot be assumed that P. striiformis will never be able to evolve greater pathogenicity towards it or that it consists of components that individually lack race-specificity.'

The variation in the components for many of the cultivars between isolates is difficult to interpret. Firstly, it was not possible to separate the isolate effects from any environmental factor which might have been present in any trial, and whilst one might expect such a factor to affect all the cultivars in the same way, an

environment-cultivar interaction is a possibility. The correlations for overall ratings for the components between each other and the field-trial NIAB ratings suggest an absence of physiologic specialisation. This agrees with Norwood (1984) who found no evidence for physiological specialisation in field trials at the NVRS). The relatively low levels of resistance in the leek cultivars might also work against the evolution of specialisation. However, the nature of correlations is to gain an overall picture of the comparison, and there may be very strong race-specific effects 'covered over' by more general correlation between cultivar-isolate comparisons. In the leek - leek rust system, the lack of a known sexual cycle is likely to reduce the variation in the pathogen, which further reduces the possibility of races of the pathogen arising to any quantitative resistance that might be developed or exploited in leeks. A proper investigation of specialisation would be difficult with the existing heterogenous cultivars. The development of male-sterile hybrids in leeks would therefore greatly enhance any study into the genetics of the resistance in leeks, a solution also suggested for work in P. asparagi, (Blanchette et al., 1982).

CHAPTER SEVEN

STUDIES ON THE GARLIC-RUST-VIRUS INTERACTION

7.1 INTRODUCTION

There are several hundred known viruses of plants, for which the chief route of infection is by a vector, usually aphids. If a plant becomes infected, then all vegetative parts of that plant will become infected, including any vegetative reproductive organs, e.g. bulbs or corms. As a result, vegetatively reproducing plants are often virus-infected and there are examples where every individual tested in a given cultivar has the virus (Matthews, 1981; Smith, 1974).

There are often interactions between viral and fungal pathogens where they infect the same host individual. Where fungi infect a host already carrying a virus, the most common reaction is an increased susceptibility to the fungus on the part of the host, though other reactions do occur (Matthews, 1981). For example, in beet, the yellows virus (BYV) has been shown to decrease host susceptibility to the mildew Erisyphe polygoni, whereas the mild yellows virus (BMYV) increases host susceptibility. Other cases where increased susceptibility due to a virus has been shown include Peronospora parasitica on Brassica juncea, (Bains & Jhooty, 1978) and Botrytis fabae on beans infected with either the yellow mosaic or leaf-roll virus (Omar et al, 1986). Cases of increased resistance include: Puccinia coronata on barley with ryegrass mosaic virus, (Latch & Potter, 1977); Puccinia

graminis on wheat with brome mosaic virus (Erasmus & Von Wechmar, 1983) and mildew (Uncinula necator) on vines with leaf-roll virus (Goheen and Schna thorst, 1961). There are also examples of variable responses, e.g. mildew on barley with yellow dwarf virus (BYDV), (Potter & Jones, 1981), and where no interaction was apparent e.g. Puccinia coronata on barley with BYDV (Latch & Potter, 1977).

Garlic is a purely vegetatively-propagated plant. Other members of the genus Allium also reproduce vegetatively e.g. A. babingtonii and A. ampeloprasum, by means of offset bubils and bulbils in the flower head. Field cultivars of garlic are known to contain many viruses , including onion yellow dwarf virus (OYDV) and leek yellow stripe virus (LYSV), as well as serologically- unidentified 600 nm and 750 nm rod viruses (Walkey, pers. comm.). All garlic cultivars so far tested have been found to contain virus, and the types of virus are usually consistent within a given cultivar. The interactions between rust and viral infected garlic are unknown but certain cultivars of garlic have shown differences in response to infection by the rust (see Chapter 3) which might be caused or affected by the presence of a virus.

In some systems the virus fails to infect the apical meristem , and virus-free plants can be obtained by meristem-tip culture with strict hygiene (Smith, 1974). This procedure has been used at the N.V.R.S. to produce virus-free material of certain garlic cultivars.

Therefore, the aim of this experiment was to :-

a) Compare the response of 'field' and 'virus-free' material of the same cultivars to infection by the BIRM isolate of leek rust, to examine the nature of the garlic-virus interaction (if any) and

b) examine the range of responses in certain field cultivars for which there was no viral-free material available, to look for signs of resistance which might warrant further investigation.

7.2 MATERIALS & METHODS

7.2.1 Materials

a) Inoculum

The inoculum used was a 12 week old sample of the BIRM isolate.

b) Host Plants

Details of the host cultivars are given in table 7.1. The plants were grown from cloves separated from the bulbs; the cloves of the field cultivars were numbered to distinguish the bulb of origin. The virus-free material was supplied ready separated.

Field Cultivars

These were supplied from Efford EHS. Separate supplies of the same cultivars had been tested at N.V.R.S. for the presence of virus (table 7.1) and those from Efford used in this experiment were therefore assumed to contain viruses too.

Virus-free Material

Virus-free cultivars were produced at the N.V.R.S. by meristem tissue culture and were raised subsequently in virus-free conditions. This material was therefore assumed to be virus-free.

Table 7.1
Details of the viral and virus-free material of garlic (A. sativum L.) inoculated with the BIRM isolate of leek rust. The viruses in the 'field' cultivars were identified at NVRS (Walkey, pers. comm.).

Cultivar	Identified Viruses					Origin
Field (viral)	OYDV	SLV	LYSV	UPV	UCV	
Fru tador	-	-	+			Efford EHS
Printanor	-	-	-	+	+	"
Rose du Var	+	+	+	+	+	"
Moulinin	+	-	-	+	-	"
Grulurose	-	-	+	+	+	"
Ail du Nord	-	-	+	+	+	"
Isles of Scilly	+	-	+	+	+	NVRS
Blanc de Dome*	-	-	-	+	+	NVRS
Virus-free						
Fructador	-	-	-	-	-	NVRS
Printanor	-	-	-	-	-	"
Moulinin	-	-	-	-	-	"
Isles of Scilly	-	-	-	-	-	"

Abbreviations

OYDV Onion yellow dwarf virus
SLV Shallot latent virus
LYSV Leek yellow stripe virus
UPV Unidentified potyvirus
UCV Unidentified carlavirus
+/- present/absent

* Not available for this trial

'Isles of Scilly' Cultivar

Both field and virus-free material of this cultivar were supplied by the N.V.R.S.

The virus-free cloves were planted in a separate greenhouse from the field cultivars to prevent cross-infection with the viruses. The plants were also covered with muslin sheeting supported on a metal frame to prevent aphids from reaching the plants and possibly infecting them.

7.2.2. Methods

7.2.2.1. Experimental Conditions

A ten centimetre long area was marked out with a non-phytotoxic waterproof pen on the adaxial surface of each of two leaves per plant, on two replicate plants per cultivar, either field or virus-free. The replicates of the field cultivars came from separate bulbs. The inoculum was applied using the brush-on technique (1.2.9.(a)). After inoculation the plants were placed in a randomised design in a growth room with a temperature of 19 +/- 2^{o}C and a 16-hour daylength. Lighting was from mercury-vapour lamps giving a photon-flux density in the photosynthetic range of 45 +/- 5 μmol m^{-2} s^{-2} at soil level.

7.2.2.2. Assessment Methods

The plants were examined daily after inoculation for the appearance of disease symptoms. For each plant, the incubation period (IP), and latent period (LP) were measured. After 20 days, assessments were made of lesion type (LT) and pustule quantity (PQ), (1.2.10).

7.3 RESULTS

The results are tabulated in table 7.2

The major feature of the results was the uniformity of the components in the non-viral (non-v) material compared with the field cultivars (v). IP and LT were the same in all the non-viral material with only minor variations in LP and PD. Even in the field cultivars, IP, LT, and PD were uniform, but there were larger differences between cultivars in LP. In cultivars Fructador and Ail du Nord, LP was 12 days compared with 14 days in cv. Printanor and cv. Isles of Scilly, with the other cultivars being intermediate. In the non-viral cultivars, the LP 's were the same as for the shorter LP 's in the field cultivars. The LP in cv. Fructador was the same in both field and virus-free material. The infection periods in all cases were the same, at 8 days. There were few differences between replicates of the same cultivars.

There were no noticeable differences in stature between the field cultivars and the virus-free material, and the field cultivars showed no symptoms of viral infection.

Table 7.2

Infection period (IP), latent period (LP), lesion type (LT) and pustule density (PD) of viral (V) and non-viral (non-V) material of garlic (*A. sativum*) infected with the BIRM isolate of leek rust. (IP and LP were means of two plants, LT and PD were from two leaves on each of two plants).

Cultivar	V/non-V	IP (days)	LP (days)	LT	PD
Fructador	non-V	8.0	12.0	3	5
Printanor	non-V	8.0	12.5	3	5
Moulinin	non-V	8.0	12.0	3	5
Isles of Scilly	non-V	8.0	12.0	3	4-5
Fructador	V	8.0	12.0	3	5
Printanor	V	8.0	14.0	3	4
Moulinin	V	8.0	13.0	3	5
Isles of Scilly	V	8.0	14.0	3	5
Rose du Var	V	8.0	13.0	3	5
Grulurose	V	8.0	13.5	3	5
Ail du Nord	V	8.0	12.0	3	5

7.4 DISCUSSION

Firstly it would appear that viral infection does not adversely affect the growth of garlic plants since there were no obvious visible differences between the virus-free or the field-grown material. All the field material tested at the N.V.R.S. has been found to contain at least one virus, and often several viruses , and these are generally consistent within a given cultivar (Walkey, pers. comm.). This is not surprising since garlic is vegetatively propagated, so that cloves will probably be infected before they are planted.

In terms of reaction to infection by the rust isolate, the great similarity between replicates within a given cultivar may be expected from cultivars which are effectively clones, all derived from the same source. The broad similarity between cultivars in reaction to the rust suggests that the only differences in resistance may be quantitative. However the cultivar Blanc de Dome has been found in other experiments here (Chapter 3) to be more resistant than any of the cultivars used in this experiment. Although not tested here, cv. Blanc de Dome is known to contain 600nm and 750nm rod viruses (Walkey, pers. comm.). However what differences there were between the cultivars, i.e. LP, were removed in the virus-free material to the level of the most susceptible field cultivar, cv. Fructador. This strongly suggests that any differences in latent period are due to an interaction between the host and the virus. However virus infection does not always appear to have resulted in 'resistance' since cv. Fructador also contains the LYSV.

The results here agree with most other studies on host-virus-fungal pathogen interactions that prior infection with a virus modifies the host-fungal pathogen interaction. However the direction of that modification varies according to the particular combination . Latch & Potter (1977) found that barley infected with ryegrass mosaic virus (RMV) showed a quantitative decrease in spore production by Puccinia coronata compared with the rust on virus-free plants, yet barley yellow dwarf virus (BYDV) did not induce such changes. Erasmus & von Wechmar (1983) found that Puccinia graminis f. sp. tritici produced smaller pustules and fewer spores on wheat infected with brome mosaic virus, and that there was a direct correlation between the quantity of virus and the level of induced resistance. Similarly, Goheen & Schnarthorst (1961) found leaf-roll virus infected grape vines supported fewer powdery mildew (Uncinula necator) lesions compared with virus-free plants. Latch & Potter (1977) found that both RMV and P. coronata decreased the level of water- soluble carbohydrate in the leaves and suggested that this could be the mechanism of induced resistance, i.e. that the two pathogens were in competition. Goheen & Schna thorst (1961) found that the virus increased carbohydrate levels and suggested that the subsequent increase in osmotic potential in the cells adversely affected the mildew. However, Potter & Jones (1981) found that BYDV in barley caused an initial decrease but subsequent increase in the levels of powdery mildew (Erysiphe graminis) and again suggested alterations in the carbohydrate levels as a mechanism, only this time as a cause of increased performance on the part of the mildew. They also reported a cultivar effect, in the level of subsequent increase in the mildew - in some cultivars it was

to levels comparable with the virus-free plants, but in others to a higher level. Omar *et al*. (1986) reported that both bean yellow mosaic virus and bean leaf-roll virus increased the susceptibility of *Vicia faba* plants to *Botrytis fabae*. They suggested that this was due to accelerated necrosis in virus-infected leaves, an effect they also showed to increase levels of *B. fabae* infection and development.

It would therefore appear that the host-virus-fungal pathogen complex is complicated and variable, and that there are other factors e.g. cultivar and timing of measurement, that effect this interaction. Most authors seem to suggest the effect of viruses on carbohydrate levels as an important factor in the mechanism of virus-induced resistance, at least in the case of biotrophic fungal pathogens. Both Erasmus & von Wechmar (1983) and Potter & Jones (1981) warn of the dangers of using 'resistance' which is in fact a virus-mediated effect. However, if infection with the virus does produce a form of quantitative resistance in garlic, then providing there are no major disadvantages to virus infection, it may be more useful to retain it in field cultivars as a form of rust resistance. It would be useful to expand the work begun here to investigate this interaction further in garlic, in particular to try virus-free material of other cultivars and to examine in more depth other quantitative characters. More information on the amount of variation between bulbs within a given cultivar would also be useful. It would be especially interesting to compare virus-free material of cv. Blanc de Dome with the ordinary field cultivar since it has shown the highest level of 'resistance'.

CHAPTER EIGHT

GENERAL DISCUSSION

8.1. General Discussion of the Taxonomy

Both morphological and infection studies support the concept of there being three distinct, but closely related 'biotypes', or species of rust on Allium spp. in the U.K., a pattern which conforms to the situation in Europe. These 'biotypes' have large and overlapping host ranges, but just how closely this resembles the field situation is difficult to predict. However, the evidence from the herbarium material suggests that the infection studies give a good indication of the field situation.

The morphological evidence suggests that the 'chive' and 'babingtonii' biotypes are more closely related to each other than to the 'leek' biotype, indicating a more recent evolutionary split. There would appear to be a trend in all three groups towards a simpler life-cycle, especially in the 'leek' biotype in the U.K. However the 'chive' and 'babingtonii' biotypes differed considerably in the infection studies in their performance on certain species, but this may indicate a host-pathogen interaction governed by a few genes compared with a more stable and fundamental similarity in morphological characters. The coevolution of the host-pathogen complex is not easy to elucidate from the results here, and more information is needed on the evolutionary relationships between the members of the section Allium. It is

difficult to explain why the morphologically 'intermediate' specimens of the 'chive-babingtonii' biotype on A. scorodoprasum should occur in Central Europe, when the most distinct form of the 'babingtonii' biotype is found in a small isolated enclave of host plants in Cornwall and W. Ireland. The evidence would suggest a greater affinity between A. scorodoprasum and A. babingtonii than is currently recognised, and certainly Gäumann (1959) regarded A. babingtonii as a synonym of A. scorodoprasum. An investigation into the diversity and interrelationship of plants currently recognised as A. babingtonii and A. scorodoprasum throughout Europe would therefore seem timely.

Nomenclature of the Species

Accepting that the three 'biotypes' merit specific rank, the question of the correct species names is a complicated one, because of the rather confused literature on the subject. This confusion arises from the inadequate descriptions of the rusts and their hosts by many early authors, and later, transferring specific epithets of the anamorph state based on host identity to a teleomorph state without actually describing the teleomorph on that host.

As mentioned in Chapter 2, there are essentially four names which have come into common usage for the rusts on Allium species in Europe:-

Puccinia allii (D C.) Rud.

Puccinia porri (Sow.) Wint.

Puccinia mixta Fuck.

Uromyces ambiguus (D.C.) Lev.

Other species mentioned by Laundon and Waterston (1965) included Puccinia blasdalei Diet. et Holw. and Uromyces durus Diet. P. blasdalei has normally been found on wild species in North America, but has been described in North Africa. Mayor and Viennot-Bourgin (1950) described a rust on A. sativum in Corsica as P. blasdalei, but it is difficult to distinguish between this species and P. allii. It may be that the P. allii in the Mediterranean are similar to P. blasdalei, or that they are the same species. Either way, P. blasdalei has never been reported in Central or Northern Europe and so is of no concern in this study. Uromyces durus has been seldom quoted in Europe, and was described by Sydow (1910) as having paraphyses in the telium (something never described in U. ambiguus), and occuring on A. nipponici in Japan. It thus appears sufficiently different from the the rusts on Allium spp. in Europe to be regarded as a separate species.

The 'genealogies' of the four species are rather complex and are summarised in figures 8.1 to 8.4. The first mention of any rust species on Allium spp. in Europe was by Sowerby in 1810 when he described the uredinial stage as a 'pretty parasite' on leeks, in England. The description is rather inadequate by modern standards, but the diagram of the pustule closely resmbles modern 'leek rust'. However this work remained unquoted for some time.

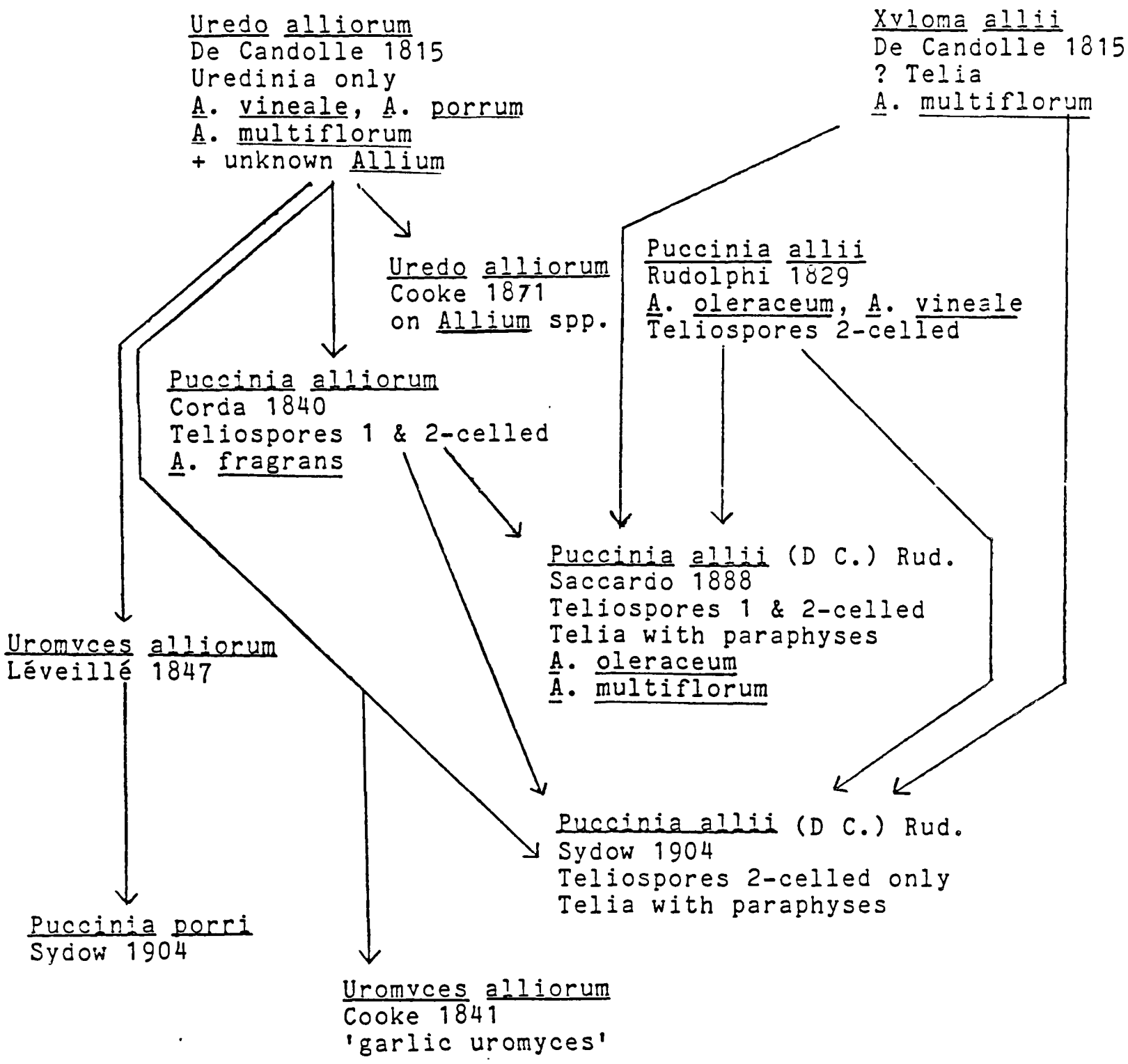


Figure 8.1
"Genealogy" of the nomenclatural literature of *Puccinia allii* (D C.) Rud., giving the main features of the descrptions

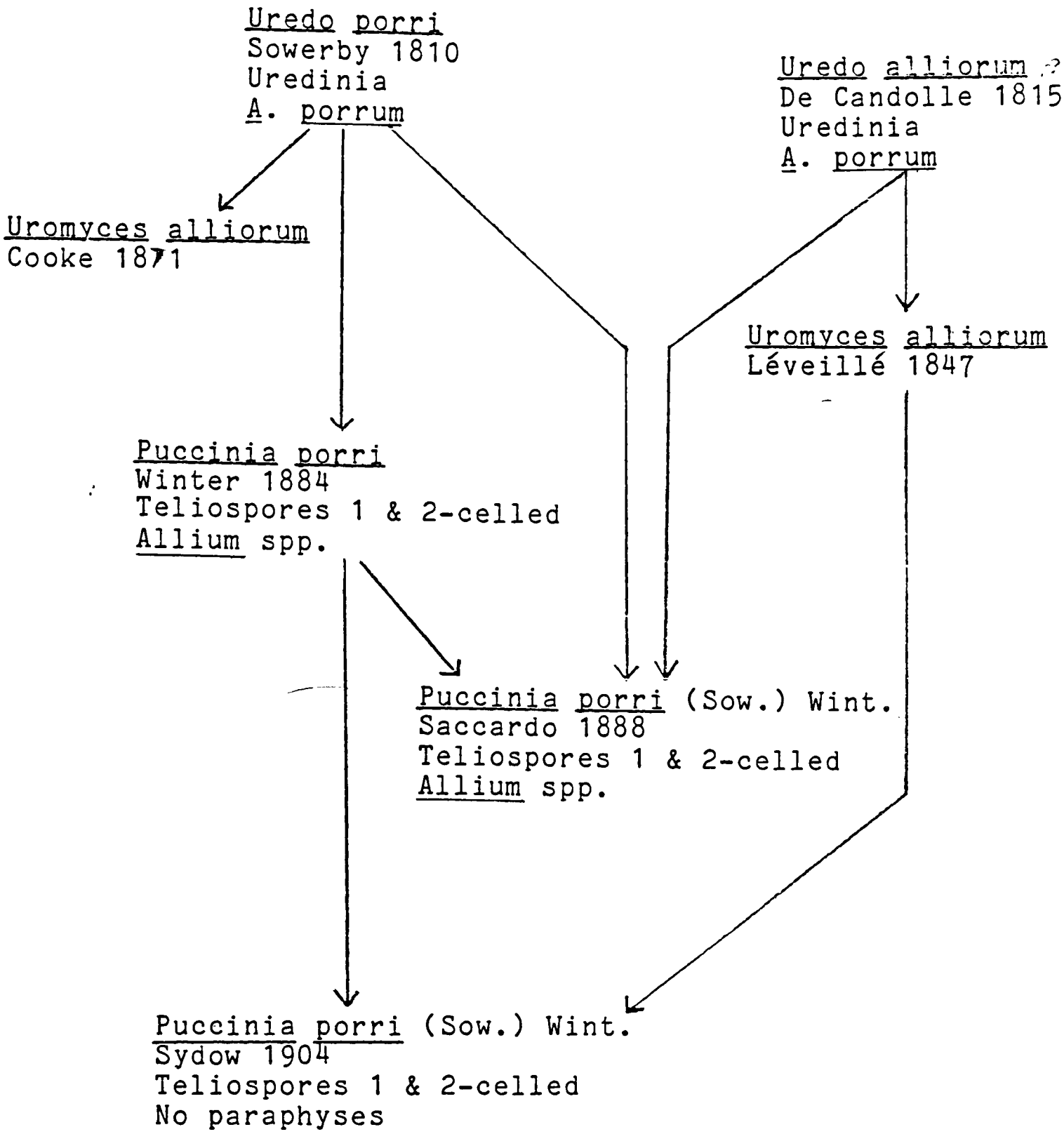


Figure 8.2
"Genealogy" of the nomenclatural literature of Puccinia porri (Sow.) Wint., giving the main features features of the descriptions

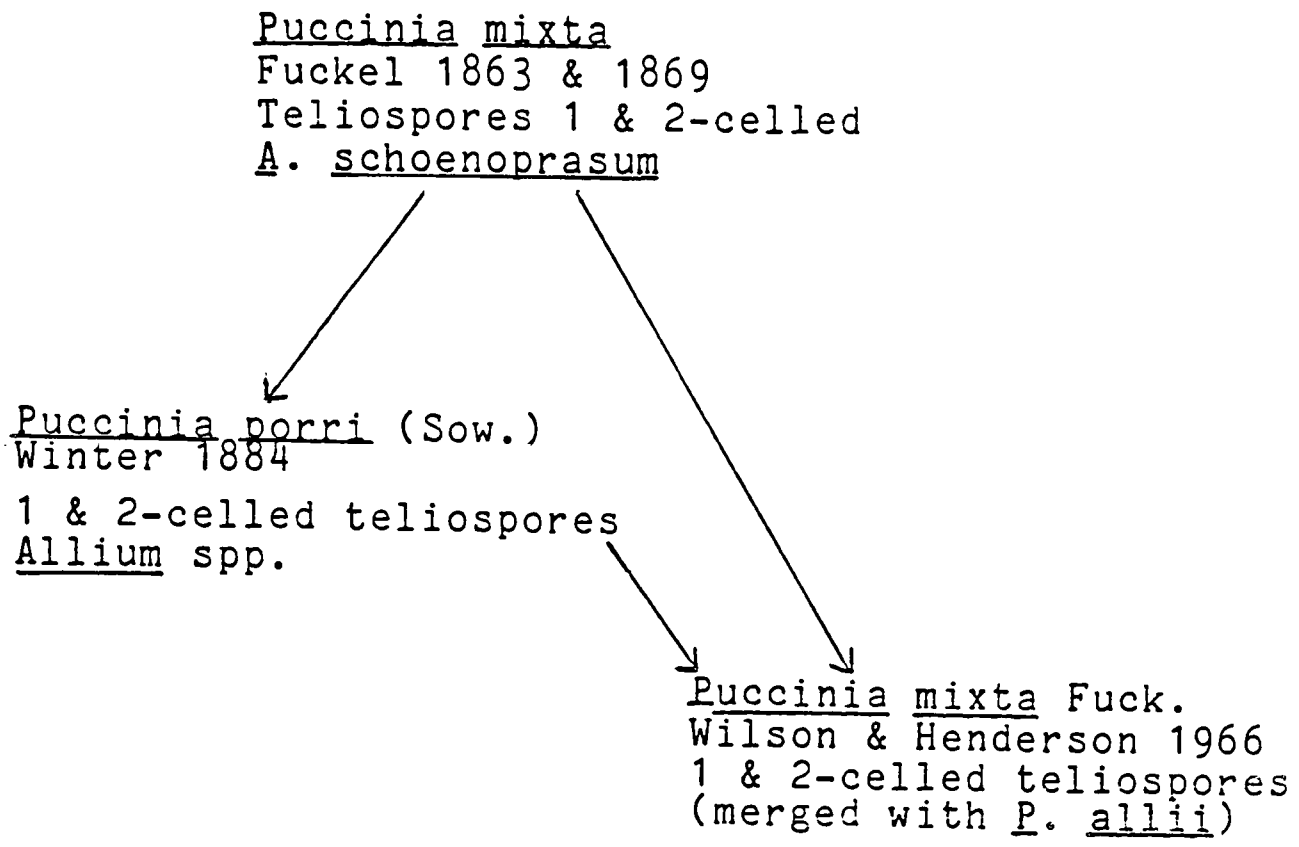


Figure 8.3
"Genealogy" of the nomenclatural literature of Puccinia mixta Fuckel, giving the main features of the decriptions

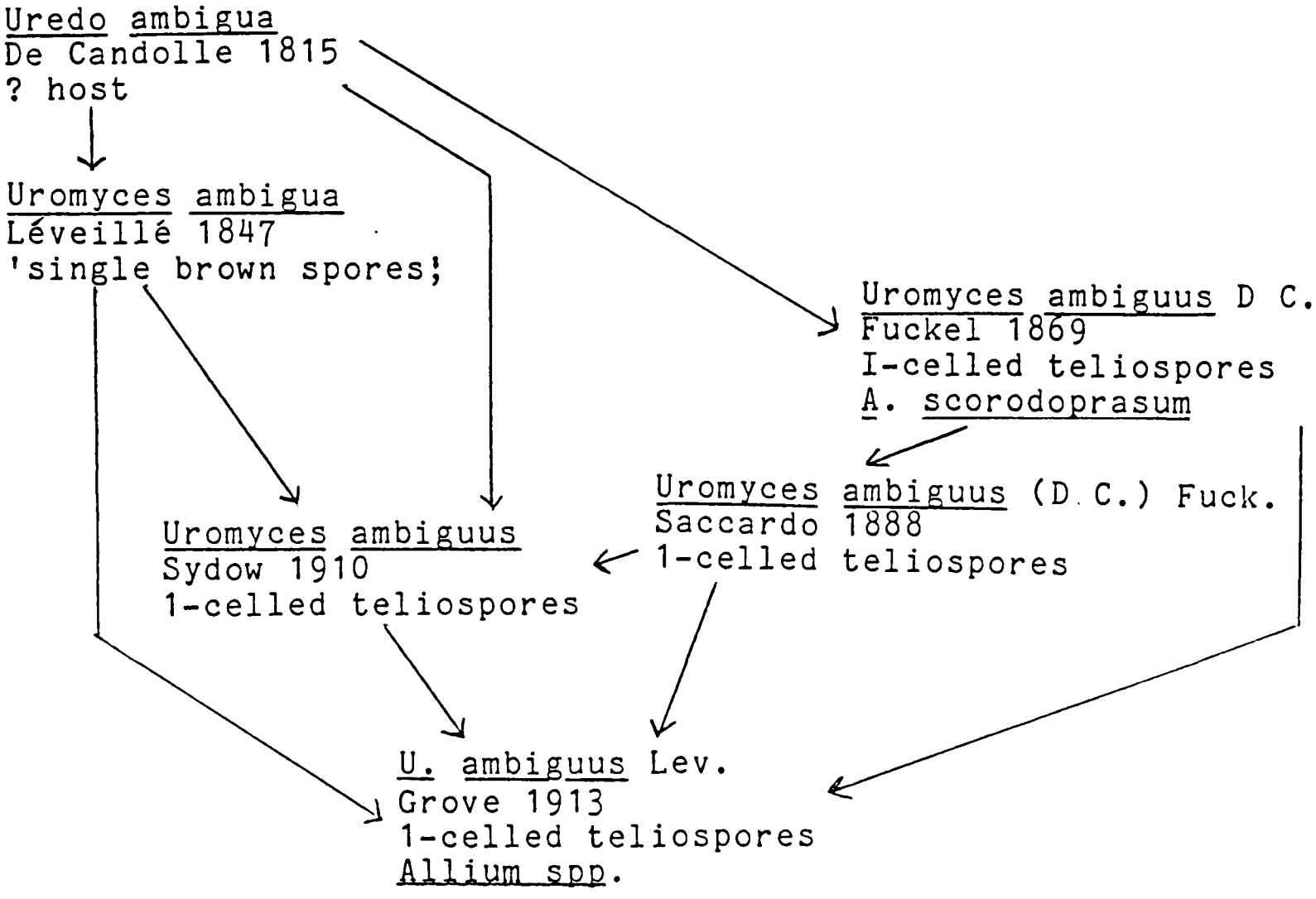


Figure 8.4
"Genealogy" of the nomenclatural literature of Uromyces ambiguus (D C.) Lév., giving the main features of the descriptions

In 1815 De Candolle described three 'species' of rust on various Allium spp. Firstly he described several 'forms' of Uredo alliorum: α on A. vineale, β on A. porrum, δ and ε on A. multiflorum (= A. polyanthum) and γ on an unknown Allium species. Secondly he described Xyloma allii forms α and β on A. multiflorum associated with the pustules of U. alliorum δ and ε. The 'dark interior' of Xyloma allii would therefore appear to be the telial stage of U. alliorum. The third species was Uredo ambigua, on an 'unknown Allium' with cylindrical leaves, together with the γ form of U. alliorum. In 1840 Corda described Puccinia alliorum on A. fragrans (=A. cyrilli), as having single and two-celled teliospores, but with no mention of paraphyses. Léveillé in 1847 reclassified Uredo alliorum as Uromyces alliorum but added no further description of the fungus or its host. Meanwhile Rudolphi (1829) had described Puccinia allii on A. oleraceum and A. vineale as having all two-celled teliospores, and often paraphyses forming a locular structure. Thus Saccardo (1888) referred to Puccinia allii (D C.) Rud. on A. oleraceum and A. sativum, which he also regarded as synonymous with Xyloma allii D.C. and Puccinia alliorum (D C.) Corda. Sydow (1904) enlarged further on the details of the spores and structure of the sori, and gave a large host species list. This description was followed by most authors, e.g. Schneider (1912), Klebahn (1914), Sawada (1929), von Tavel (1932) and Gäumann (1959).

In 1884 Winter described a rust with both single and two-celled teliospores, and no paraphyses, in large, grey telia on several species of Allium and named it Puccinia porri Sowerby. Allium porrum

was included among the host plants. This description was again followed by Saccardo (1888) and Sydow (1904) and seems to have come into common usage. One of the synonyms considered by Winter (1884) for P. porri was P. mixta, described on chives (A. schoenoprasum) by Fuckel (1863 & 1869). Winter's description of P. porri was very similar to the earlier description of P. mixta, but Winter chose to adopt Sowerby's earlier specific epithet of 'porri' (Sowerby, 1810) instead, i.e. uprating Uredo porri Sowerby to Puccinia porri (Sow.) Wint. It is unclear whether Winter actually found telia on leeks, and thus Wilson & Henderson (1966) concluded that the valid name for this species was indeed Puccinia mixta Fuck.

Léveillé (1847) reclassified Uredo ambigua D C. as Uromyces ambigua D C. (Lev.) but added no further description other than it having 'brown single spores'. Fuckel (1869) described Uromyces ambiguus on A. scorodoprasum and Saccardo described Uromyces ambiguus (D C.) Fuck. on the same host, as having single-celled teliospores and no paraphyses in the telium. Sydow (1910) described U. ambiguus (D C.) Lev. similarly, on several host species, noting how similar it was to a single-celled only version of Puccinia porri (Sow.) Wint., a comment echoed by Grove (1913).

Subsequent literature on these rusts has tended to use one or more of these names, but as Doherty (1981) noted, the descriptions - especially the size ranges of the spores often do not match up with other descriptions of the same species. The host ranges were also confused, especially by the use of the specific epithet of P. porri, the rust on

leeks being described on this species despite the rarity of telial material on cultivated leeks (Doherty 1981). This was particularly so in the U.K., (see Chapter 2).

The discovery of the teliospore stage on leeks, together with the results of this study gives an opportunity to establish the correct nomenclature for the various rusts.

Firstly, the correct name for the rust on A. babingtonii in Cornwall, and on certain specimens of A. scorodoprasum in Europe would appear to be Uromyces ambiguus (D C.) Lev. This has been in common usage, and although the earlier literature is confused (fig. 8.4), this would seem to present no real problem. However the findings of this study suggest that a new description of the urediniospores is required, especially the germ-pore number ranges, as this provides the strongest evidence for regarding this entity as a separate species.

Secondly, Wilson and Henderson's argument for regarding Puccinia mixta Fuck. as the correct name for P. porri would appear to be technically valid and pragmatic, as this species would rarely seem to attack leeks in the field going on herbarium material, since telial specimens on leeks are lacking. Furthermore, Fuckel's type host was A. schoenoprasum, which seems to be the major host species in the U.K.

Thirdly, the correct name of the 'leek-rust' species is more difficult. The description by Rudolphi (1829) seems to be adequate, and

the name Puccinia allii (D C.) Rud. is in common usage. However the descriptions of De Candolle (1815) for both Uredo alliorum and Xyloma allii are inadequate, and it is likely that the rust described by Sowerby (1810) as Uredo porri is in fact the same rust. The only way to establish the correct name would be to refer to the original type material. In all three species, such a reference would be desirable, and indeed necessary if any changes were to be made. In the meantime, there would appear to be adequate grounds for allocating the following names as both valid and sensible.

'Leek rust' Puccinia allii (D C.) Rud.

type host - A. oleraceum

(after Hylander, et al. 1950)

syn. Uredo porri Sowerby

Uredo alliorum D C.

Xyloma allii D C.

Puccinia alliorum Corda

'Chive rust' Puccinia mixta Fuck.

type host - A. schoenoprasum

syn. Puccinia porri (Sow.) Wint.

?Uromyces alliorum (D C.) Lév.

'Babingtonii rust' Uromyces ambiguus (D C.) Lév.

type host - ? A. babingtonii

? A. scorodoprasum

syn. Uredo ambigua D C.

Uromyces ambigua (D C.) Lév.

Uromuces ambiguus (D C.) Fuck.

8.2 General Discussion of the biology

One of the major features of this project has been the high level of apparently environmentally-dependent variation at all levels of the host-pathogen interaction e.g. the control of telial formation in U.K. leek rust isolates; urediniospore initiation and production from successful colony formation on A. cepa cultivars, and the ability of the leek rust isolates to infect A. ampeloprasum acc. 418 successfully. Harrison (1987) suggested that telial formation in the field might be induced by hot weather, and Benada (1966) suggested that telial formation was conditioned by internal leaf pH, itself partially light dependent. However Uma (1984) suggested that only certain strains of the rust could form telia, i.e. a genetic factor. Palti et al. (1958) noted telia and aecia of rust on onions in hot conditions in Israel. Norwood (1985), Burchill and Everitt (1985) and Burchill, Norwood and Everitt (1986) have demonstrated the environmental requirements for successful infection of leek rust on leeks with the aim of producing a leek-rust forecasting system. However, environ-

mental considerations remain a major feature of experimental work in this interaction and need to be elucidated more fully. Other non-environmental explanations might also be important in these studies. Norwood (1985) suggested that the water trapped in the leaf sheath enhanced infection of leeks by the rust. This anatomical feature may explain the higher incidence of the disease on leeks compared with other Allium crops in the field, despite its ability to infect them successfully in controlled environment tests. This 'avoidance' mechanism is probably of little use as far as leek breeding is concerned since it would require drastic changes in the type of leek currently grown. However it might be an important character to preserve or enhance in other Allium species, particularly in section Cepa. Despite this, occasional epidemics of rust have occured on A. fistulosum in the U.K. (Norman, 1978). Another factor applicable to all the infection studies is the difference in the fauna and flora of the leaf surfaces of host plants grown in glasshouses compared with field-grown plants. Both Doherty (1981) and Uma (1984) found bacteria and fungi on the surfaces of leeks to be inhibitory to the rust.

Another notable feature of the infection studies at all levels was the apparent absence of any form of hypersensitive resistance. There seems to be a continuum from purely quantitative levels of resistance in leek cultivars, with very uniform reaction types (Chapter 6), through much higher levels of quantitative resistance (Chapter 4) within the ampeloprasum complex, to qualitative resistance in several interactions in 'non-host sections' in Chapter 3, where the pathogen

was able to infect and form colonies of some size without sporulating. It would therefore be interesting to widen the infection studies to try to find out the 'limits' of successful infection/colony formation. More detailed studies need to be carried out on the early stages of infection to see what, if any, effect the very different leaf surfaces of different members of the genus Allium have on urediniospore germination, location of stomata, appressorium formation, etc. There is a large body of information on prepenetration studies in other host and non-host - pathogen interactions, (Heath, 1974 & 1977). Yarwood and Gardner (1941) indicated that rust on garlic could infect onions but not asparagus, suggesting that the host-range of the hosts might be within the genus Allium; Walker (1921) described telia and aecia of Puccinia asparagi on onions, so it is possible that the rusts on Allium spp. and P. asparagi may be closely related. The levels and type of resistance in the genus Asparagus (Blanchette et al. 1982; Johnson, 1986) certainly seem to be similar to those found in this study. It may be that the types of resistance and specificity in these host-pathogen systems are unlike those described in other host-pathogen interactions, but considerably further study needs to be done to elucidate this.

The levels of resistance found within leek cultivars and the greater levels found within the members of the ampeloprasum complex may well be useful in the field. However it is worth pointing out that while quantitative resistance has been useful in protecting cereals, and keeping yield losses of grain to a minimum, the need for resistance in leeks is rather different. That requirement is currently for

blemish-free leeks, implying absolute, not quantitative, levels of control. The objectives of resistance breeding in leeks therefore need to be considered before starting future breeding work. Quantitative resistance might still be useful in reducing the level of disease generally, and in reducing the need for chemical control, but is unlikely to be sufficient on its own.

Further studies into the genetics of the leek-leek rust interaction need to be carried out, especially to look for signs of physiological specialisation. If evidence for specialisation is 'hidden' by the genetic variability inherent in the leek cultivars currently available, then the best way to elucidate the genetics would be to derive single-spore isolates from the field isolates, and try these on studies on inbred lines of either garlic, or preferably male-sterile derived leeks. Work into the production of pure lines has only recently begun (Poths, pers. comm.).

If leek rust can be successfully controlled there remain the other two species of rust on Allium species which could pose a threat in the future. The possibility of the other rusts becoming more prevalent in agriculture depends to a large extent on the incorporation of susceptible host species or accessions into general breeding programmes. If A. babingtonii is used as a source of resistance to leek rust in leeks, then precautions should be taken not to introduce susceptibility to the 'babingtonii-type' rust in the hybrid. In this context there is a need to elucidate why the 'babingtonii-type' rust is prevalent on A. babingtonii instead of the 'leek-type' rust present

on adjacent leek crops. There also remains the problem of the susceptibility of A. fistulosum to all three species. As well as the problems already mentioned regarding the hybrids of A. fistulosum and A. cepa, there is the possibility of somatic hybridisation between the rust species on this host, which could provide variation in the rusts otherwise prohibited by their lack of a sexual life-cycle.

For future work therefore, an in-depth study screening of a wide range of germplasm from the A. ampeloprasum complex would seem to hold good potential for high levels of quantitative resistance to leek rust for use in leek breeding. Within leek cultivars, there seems to be useful levels of resistance within existing cultivars but greater levels may reside in individual plants given the high level of within-cultivar variation. To look for specialisation in the rust further work needs to be done, first to repeat the isolate trials using improved measures of the resistance components, ultimately aiming at single-spore isolates with pure-line host material. Further study of the rust-virus-host interaction in garlic is desirable as U.K. garlic production increases, and a study of resistance to all the rusts will be needed in the case of interspecific hybrids, especially those involving A. kurrat, A. babingtonii and A. fistulosum.

8.3 Conclusions

There are three distinct morphological species of rust on Allium spp. in the U.K., with wide and overlapping host ranges. The species should be described as Puccinia allii (D C.) Rud., Puccinia mixta Fuck. and Uromyces ambiguus (D C.) Lév.

In the Ampeloprasum complex, the kurrats appear uniformly susceptible to leek rust (P. allii), and the other species vary in quantitative characters of resistance. There would appear to be useful levels of resistance in certain A. babingtonii and A. ampeloprasum accessions. More material of A. ampeloprasum from the Mediterranean needs to be studied.

A. cepa appears to be fairly resistant to all the rusts; A. fistulosum was susceptible to all three rusts and this trait could present a problem in hybrid crops if it was carried into the hybrids.

Leek plants appear, in at least two components of resistance, to become more resistant with age to leek rust. Within leaves, the tissue appears to become more susceptible with age except in the tip regions which remain more or less unchanged in susceptibility. The level of variation in these tests was very high.

There appeared to be different levels of resistance between leek cultivars, with Autumn Mammoth selections being more resistant. The components used showed high levels of variation in some cases and

could be improved by more refined methods of measurement. There was no indication of a distinct level of specialisation between isolates of the pathogen, but the high degree of variation in both host and pathogen could have hidden low levels of physiological specialisation in the pathogen.

The virus content of several cultivars of garlic may be connected with resistance to leek rust, and warrants further study. Garlic would appear to be vulnerable to rust epidemics in the field. This questions the concept of reducing the virus load of garlic field cultivars, if evidence that viruses provide resistance against rust can be found.

General trends throughout the work included a high level of environmental variation, and a lack of 'hypersensitive-type' resistance, even in hosts quite distantly related from the normal host species.

Appendix 1

Composition of compost used throughout the project for growing *Allium* spp.

A mixture of Peat:Loam:Sand:Grit was prepared in proportions of 4:2:1:1 with the addition of 6.2 kgm^{-3} Vitax Q4 fertilizer and 3.1 kgm^{-3} Limestone flour (Streetly Minerals Ltd., Burscough, Lancs.)

Appendix 2

Bruzzese & Hasan Stain

For approximately 1.2 litres of stain, successively add:

95% Ethanol	300.0 cm^3
Chloroform	150.0 cm^3
90% Lactic Acid	125.0 cm^3
Phenol	150.0 g
Chloral Hydrate	450.0 g
Aniline Blue	0.6 g

Appendix 3

Note on the use of garlic cultivar names

The names of French garlic cultivars are frequently misspelled during the transfer of material. The use and spelling is consistent throughout this thesis, but may differ from other published work. Therefore, the alternative spellings of some of the garlic cultivar names are given below.

Name used here	Alternative name
Blanc de Dome	Blanc de Drome Blanc de la Drome
Fructador	Fructidor
Moulinin	Moulinon
Printanor	Printador

Appendix 4
Level of replication in the measurement of the Latent Period component in the Cultivar-Isolate trials (Chapter 6) after allowing for loss of plants, and plants remaining uninfected.

Possible maximum = 5 plants

Cultivar	BIRM	NIAB	LUDD	STOCK	WSCOT
Odin Longstanton	5	5	5	5	5
Gennevilliers Splendid	5	4	5	5	4
Winterreuzen	5	2	5	5	5
Starina	5	2	5	5	5
Blizzard	5	4	5	5	5
Longa	5	2			
Alaska	5	3	5	5	5
Derrick	5	3	5	5	5
Durano	5	2	5	5	5
Ludovicus	5	5	5	5	5
Rolan	5	2	5	5	5
Tivi	5	3	5	5	5
Walton Mammoth	5	4	5	5	5
Albin Star	5	5	5	5	5
Musselburgh	5	5	5	5	5
Goliath	5	5	5	5	5

Appendix 5
Level of replication in the measurement of the Pustule Density component in the Cultivar-Isolate trials (Chapter 6) after allowing for loss of plants.

Possible maximum level = 15 leaves (3 leaves on 5 plants)

Cultivar	BIRM	NIAB	LUDD	STOCK	WSCOT
Odin Longstanton	11	13	15	14	13
Gennevilliers Splendid	11	11	10	11	11
Winterreuzen	12	13	14	15	13
Starina	14	12	11	13	12
Blizzard	15	14	14	13	14
Longa	14	12			
Alaska	14	10	15	14	15
Derrick	15	10	15	14	15
Durano	14	14	15	14	15
Ludovicus	12	15	15	14	15
Rolan	11	10	12	15	12
Tivi	12	11	15	15	15
Walton Mammoth	14	15	14	14	15
Albin Star	12	12	14	15	14
Musselburgh	13	14	15	12	15
Goliath	15	15	14	14	12

Appendix 6
Level of replication in the measurement of the Pustule Length component in the Cultivar-Isolate trials (Chapter 6) after allowing for loss of plants and leaves, and pustules being too close together (colonies overlapping)

Possible maximum level = 150 pustules (10 pustules on 3 leaves on 5 plants)

Cultivar	BIRM	NIAB	LUDD	STOCK	WSCOT
Odin Longstanton	96	56	69	127	86
Gennevilliers Splendid	84	52	50	106	57
Winterreuzen	110	26	103	140	93
Starina	79	33	92	120	98
Blizzard	136	40	47	116	127
Longa	114	37			
Alaska	101	20	76	138	121
Derrick	128	31	86	137	123
Durano	136	47	68	136	130
Ludovicus	103	126	115	140	150
Rolan	68	39	33	130	104
Tivi	96	56	67	150	111
Walton Mammoth	128	92	60	131	105
Albin Star	97	87	51	150	128
Musselburgh	94	115	82	120	134
Goliath	140	97	80	131	100

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