LEAF SCALD OF BARLEY

by

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CONTENTS

SUMMARY

Barley (*Hordeum vulgare*) and barley grass (*Hordeum leporinum*) are the only known hosts of *Rhynchosporium secalis* in South Australia. Thirty-five isolates from the two hosts were separated into 20 races on the basis of pathogenicity to 12 barley cultivars. Each of 8 isolates from barley grass infected most barley cultivars but only 2 of 5 barley isolates attacked barley grass. An isolate of *Rhynchosporium orthosporum* from *Dactylis glomerata* did not infect barley or barley grass.

Cultural characteristics, morphology of leaf-borne conidia and growth rate of germ tubes were not related to pathogenicity but there were some similarities in pathogenicity between isolates from the same geographic area.

In some cases conidia germinated on barley leaves to produce short germ tubes and appressoria, and penetration of the cuticle was initiated from a proportion of these appressoria. However, more than 50% of conidia that effected penetration had not formed superficial germ tubes or appressoria. Penetration was aided by chemical modification of the cuticle.

Hyphae in the subcuticular position penetrated between collapsed epidermal cells to establish both intercellular and intracellular mycelia in the mesophyll which collapsed before being

reached by the hyphae.

Subcuticular stromata formed on inoculated sides of leaves and substomatal stromata formed on the opposite sides in substomatal cavities. Conidia, which were formed on both types of stromata, protruded through the cuticle above subcuticular stromata and were extruded through stomatal pores from substomatal stromata.

Symptoms similar to those shown by infected leaves occurred in leaves of barley seedlings whose cut stems had been immersed in cell-free culture filtrates of R. secalis. Barley leaves sprayed with culture filtrates developed dark brown spots similar to the dark brown margins of R. secalis lesions and the dark brown hypersensitive spots on barley leaves. Culture filtrates also induced some scald disease symptoms in wheat, oats and Dactylis glomerata which are not hosts to the pathogen. The toxicity of culture filtrates was not affected by dialysis or autoclaving.

Leaf-borne conidia smeared on leaves of susceptible Clipper barley adhered to the leaves for longer periods than those smeared on leaves of the more resistant barley cultivars, Atlas 46 and Osiris. Inoculum derived from potato-sucrose-peptone adhered to the leaves of the three cultivars longer than did leaf-borne inoculum. Germ tubes were produced at the same rate on leaves of the three cultivars, but frequency of penetration of the cuticle was

significantly higher on Clipper. Growth of subcuticular hyphae which formed in Atlas 46 and Osiris appeared to be inhibited by some fungitoxic material(s).

In the field, the disease progressed mainly between adjacent plants, but pockets of infection sometimes appeared at isolated positions several metres away from the nearest source of inoculum. Sporulation occurred when free water was available and conidia were caught in a spore trap during rainfall or irrigation. Some conidia were trapped under windy but rainless conditions. Conidia were trapped at any time of the day or night but few were obtained at any one time.

STATEMENT

This dissertation has not previously been submitted for a degree at this or any other University and is the original work of the author, except where due reference is made in the text.

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GENERAL INTRODUCTION

Rhynchosporium secalis (Oud.) Davis causes a disease described as leaf scald or leaf blotch of barley and some other grasses. Oudemans (1897) was the first to record the disease, having found it on rye in the Netherlands. Frank (1897) found it on barley and rye in Germany but his examination of herbarium specimens showed that the disease was present in Germany in 1894. It is now known to occur in many barley growing areas throughout the world (Sprague, 1950; Kajiwara and Iwata, 1963). Leaf scald thrives under conditions of low temperature and high rainfall (Skoropad, 1959 and 1963) and its incidence and economic importance appear to be increasing (Eugenio and Astolfo, 1961; Evans, 1969).

The ability of isolates of R. secalis from one host species to infect another host belonging to a different species is in doubt, but the existence of physiological races specialized to particular barley cultivars has been amply demonstrated (Caldwell, 1937; Sarasola and Campi, 1947; Owen, 1958). The identity of local races and the variation potential of the pathogen must be known before a breeding programme for the development of resistant cultivars can be undertaken.

A thorough knowledge of the germination processes of the conidia on the host surface, the mode of penetration and further development in host tissues is essential in investigations of

resistance mechanisms. However, very little is known about this aspect of the host-parasite interaction and reports of the manner in which the fungus enters its host, grows within the host and sporulates are few and contradictory in important respects (Bartels, 1928; Mackie, 1929; Caldwell, 1937).

Most of the generally accepted information on the epidemiology of the disease has been speculative and deficient in quantitative data. Comprehensive investigations must be carried out to obtain reliable information on the way in which the fungus is able to survive the summer and on the mode of dispersal of secondary inoculum. Modification of existing cultural practices based on results from such studies may reduce or prevent scald infection.

In South Australia, the only known host of the fungus before the present work started was barley (Talbot, 1964). Earlier records show that it had been recorded in New South Wales on barley in 1900 under the name Ramularia hordei McAlpine (McAlpine, 1902). The writer has also found that the disease commonly occurs on barley grass (Hordeum leporinum Link) which is widely distributed in the state. Boyd (personal communication) has mentioned the occurrence of the disease on barley grass in Western Australia.

The disease is becoming increasingly important in the state because of the increasing use of the new improved cultivar, Clipper. This cultivar has a number of advantages over the cultivars, mainly Prior, which it has replaced. Included among the advantages are

resistance to powdery mildew and net-blotch, two of the three most important fungal diseases of barley in the state. However, Clipper is particularly susceptible to the other important disease, leaf scald. It follows that this disease may become the most significant fungal disease of barley. Studies on the epidemiology of the disease were initiated in an attempt to determine the basis for practical control measures.

Considerable attention has also been given to the problem of controlling the disease by the development of resistant cultivars. The first part of the investigations reported in the thesis was therefore carried out to provide some information on the existence of physiological races of the fungus and the relative resistance of barley cultivars to them.

Processes in the infection of various cultivars of barley were also investigated to provide the basic knowledge necessary for future work on mechanisms of resistance.

LITERATURE REVIEW

1) Economic Importance

Frank (1897) and Heinsen (1901) described the disease as severe in Europe. In America, severe epidemics in the interior valley of California were recorded in 1921-22 when yields were reduced by as much as 20-30% (Calif. Agric. Sta. Rept, 1921-22). Schaller (1963) also estimated the yield loss in California and found a maximum reduction of 35%. Caldwell (1937) observed that the disease occurred in severe form on winter barley crops of the Pacific Coast and Idaho. Reed (1959) recorded that the fungus was widespread and destructive in a number of barley growing areas in North America. In East Tennessee the disease had wiped out whole crops of susceptible cultivars and yield losses were as high as 95% in other cultivars (Reed, 1959). Skoropad (1960) estimated that severe attacks reduced yield by 30% in Central and Northern Alberta and Northern Saskatchewan where the disease had reached its most damaging proportions. Ozoe (1956) reported from Japan that barley scald had been more severe than any other barley disease in 13% of the barley growing areas in Shimane Prefecture and was second or third in importance in 47% of those areas. Reduction in yield usually amounted to 30-45%.

A number of more recent reports provide evidence for the spread and increasing economic importance of the disease. Eugenio

and Astolfo (1961) stated that scald on barley was one of the factors limiting the establishment of barley plantings in the Highlands of Guatemala. Kajiwara and Iwata (1963), reporting from Japan, stated that since 1948, the disease had become widespread and caused severe damage especially on the coast of the sea of Japan and in the central mountainous districts. Increase in the frequency and intensity of scald disease in the south of England were mentioned in the Annual Report (1963-64) of the Plant Breeding Institute, Cambridge. Jenkins and Jemmett (1967) reported that by 1963, severe attacks in barley crops were fairly common in Devon and Cornwall and in 1965 the disease had reached a more serious level in most parts of England and Wales, especially in the South. The estimated yield loss in Devon and Cornwall in 1965 was as high as 35%. In a survey of eight crop regions in England and Wales, James (1967) found that 75% of the samples had scald and that the disease accounted for 2.3% of the loss in yield. Evans (1969) also reported that the disease was particularly severe in West Sussex in 1968. Estimated yield loss in spring barley was 11%. Manner (1968) mentioned that leaf scald was a new disease in Finland and that in wet years its importance was considerable.

There is no information in the literature on yearly damage from scald on barley in South Australia, but reports from district advisers of the Department of Agriculture indicate a decrease of up to about 25% in grain production. Trials in Victoria with fungicides (Jones, personal communication) showed that reduction of almost 18% of the average yield occurred in infected barley crops.

2) Distribution and Host Range

In addition to barley and rye, R. secalis has been reported on wheat (Heinsen, 1901), oats (Lind, 1913) and on a number of wild and cultivated grasses. Sprague (1950) listed the distribution and occurrence of the disease on twenty six species of non-cereal grasses belonging to ten genera of the Gramineae. Kajiwara and Iwata (1963) listed five genera of cereal and non-cereal grasses known to be hosts of R. secalis in Japan.

R. orthosporum was described by Caldwell (1937) as causing leaf scald of Dactylis glomerata L. and Sprague (1950) reported on a number of grasses naturally infected by this fungus. Owen (1952) also recorded the occurrence of R. orthosporum on D. glomerata in England. The symptoms produced by this fungus are similar to those caused by R. secalis but the fungi may be distinguished by their conidial morphology. The conidia of

R. secalis have been described as elongated, one-septate and beaked at the apical portion, whereas those of R. orthosporum are cylindrical and one-septate. But there are some collections of R. secalis with conidia intermediate in shape between those of the two species (Sprague, 1950).

3) Epidemiology

Several workers have reported that R. secalis persists from season to season as mycelium in barley debris (Caldwell, 1937; Ozoe, 1956; Skoropad, 1959; Evans, 1969). If the first leaf of an emerging seedling comes into contact with infected debris, under favourable conditions disease symptoms appear in a fortnight. Evans (1969) has shown that the extent and severity of the disease depends on the amount of stubble debris on the soil surface. Ozoe (1956) found that the fungus survived for about one year on infected straw kept in the laboratory. He noted that it usually failed to over-summer if the straw was left in the open field or buried in the soil. Skoropad (1966) reported that production of successive batches of conidia in the presence of free moisture, and invasion by microbial saprophytes, destroyed the ability of the pathogen to sporulate and that the most rapid deterioration of the stromata occurred when leaves in contact with the soil were continually moist at 18°C. He found that scald lesions retained their ability to

produce conidia for up to 340 days.

The fungus is also seed-borne and this is particularly important in long-range dissemination of the organism (Ozoe, 1956; Skoropad, 1959). Coleoptiles of seedlings from infected seeds are attacked when germination takes place under cool conditions (Skoropad, 1959).

Ozoe (1956) and Skoropad (1960) have suggested that after the development of the first lesions, secondary inoculum is dispersed by splashes of rain and by wind. Ozoe (1956) concluded that there were numerous conidia in the air between the rows of barley plants and that their dispersal had a close correlation with both rain and wind. He claimed that the number of conidia in the air appeared to be greater in the day than at night. Skoropad (1959) reported that conidia were most abundant during rainstorms and that they were usually trapped in clusters of three to ten, indicating that they were transported in droplets of water.

Laboratory studies by Caldwell (1937) and Ozoe (1956) have shown that the optimum temperature for germination of the conidia in distilled water is 18-21°C, the minimum being about 0°C and the maximum 25-30°C. Field observations have shown that a low level of primary infection can give rise to an epidemic if subsequent weather conditions encourage secondary infection (Jenkins and Jemmett, 1967). Skoropad (1960) has emphasized that cool, moist

conditions are necessary for the establishment of the disease in Canada. Ozoe (1956) reported from Japan that a warm winter with heavy rains caused more serious infection than occurred in normal years.

4) Physiological Specialization

Published work on physiological specialization of the fungus deals with two kinds of pathogenic races: (a) isolates distinguished by their ability or inability to attack a host of a genus other than that from which they were isolated; (b) isolates distinguished by their pathogenicity and virulence to different barley cultivars.

Caldwell (1937) in an extensive survey of the disease in North America, recognised six highly specialized races, each restricted to one of the following hosts:- Hordeum vulgare L. (barley), Apropyron repens (L.) Beauv, Bromus inermis Leyss., Elymus canadensis L., Hordeum jubatum L., and Secale cereale L. (rye). Caldwell also reported that isolates from barley infected H. murinum L.; those from H. jubatum L. were able to attack H. nodosum L. and H. pusillum Nutt. Two varieties each of rye, oats and wheat were immune to two isolates from barley. He also observed that each race showed constant and distinctive morphological characteristics in culture and that certain races differed

in the shape of their conidia.

Muller (1953) made cross-inoculations with R. secalis from barley and rye, and reported that each attacked only the host from which it was isolated.

In his studies in England, Owen (1958) inoculated barley, rye, Agropyron repens, Hordeum murinum L., Dactylis glomerata L, Bromus mollis L., B. sterilis L., Lolium perenne L. and Pheleum pratense L. with twenty two isolates from barley, two from rye, five from Agropyron repens and four from Hordeum murinum. The isolates infected only the hosts from which they had been isolated except those from H. murinum which also attacked barley. Two isolates from H. murinum and two from H. leporinum Link were tested on barley, H. jubatum, H. leporinum, H. murinum and H. secalinum Schreb. Both isolates from H. murinum infected this host and barley but not the others. One of the isolates from H. leporinum caused no infection but the other infected H. leporinum and H. murinum. Owen also reported that none of four isolates from barley infected any of the following:- wheat, oats, Agrostis stolonifera L., Bromus catharticus Vahl., Cynosurus cristatus L., Festuca elatior L., Holcus lanatus L. and Poa pratensis. Owen did not observe any features of spore shape which distinguished one race from another.

Kajiwara and Iwata (1963) inoculated wheat, rye, oats,

Agropyron semicostatum (Steud) Ness, Wash., A. ciliare (Trin.) Franch, Dactylis glomerata, Phalaris arundinacea L. and Arrhenatherum elatius (L.) Beauv. with twenty two isolates from barley collected from various parts of Japan. They recorded that none of the isolates infected the cereals and the grasses used except Agropyron ciliare, which was attacked by some of the isolates. Isolates from A. ciliare infected barley, but each of the isolates from the other grasses attacked only the host from which it was isolated.

On the other hand, there are several reports which conflict with those of Caldwell (1937), Muller (1953), Owen (1958) and Kajiwara and Iwata (1963). In 1937, Smith reported infection of Agropyron spp. with isolates from barley and vice versa. Sarasola and Campi (1947) were successful in infecting Elymus canadensis L., Agropyron smithii Rydb., Festuca elatior L. and Bromus unioloides H.B.K. with isolates from barley obtained from Argentina. Vienot-Bourgin (1949), working in France, reported that he found it easy to infect Bromus mollis, Hordeum murinum and H. secalinum with an isolate obtained from barley. In inoculation experiments on several species of Gramineae and Cypereceae with conidia of R. secalis obtained from barley, Ozoe (1956) infected barley, Agropyron ciliare and Festuca elatior var. arundinacea (Schreb.) Hack., while wheat, oats, rye and the Cyperaceae remained uninfected. He also found that an isolate

from A. ciliare could attack barley. Schein (1958) infected the following grasses with an isolate from barley: Agropyron intermedium (Host) Beauv., A. pugens (Pers.) Roem., A. riparium Scribn. and Smith, A. smithii, A. trichophorum (Lk.) Richt., Bromus arvensis L. and Festuca idahoensis Elmer. A culture he obtained from Europe infected Bromus inermis Leyss., Elymus triticoides Buckl. and Agropyron smithii.

It seems therefore that the ability of isolates of R. secalis from one host species to infect other hosts belonging to different species is in doubt.

Although differences in susceptibility of various barley varieties to R. secalis isolated from barley were known before 1920 (Johnson and Mackie, 1920), the existence of physiological races was not reported until 1947 by Sarasola and Campi (1947) in Argentina. Grouping of isolates into physiological races has been based on the range of barley cultivars attacked, and severity of attack. Sarasola and Campi (1947) used Wisconsin Winter x Glabron, West China and Nigrum as differential cultivars and distinguished four races among isolates from Argentina. Schein (1958) inoculated the cultivars used by Sarasola and Campi with isolates from the United States and found that although some differences existed they were not sufficient to separate the isolates into races. On using Wisconsin Winter, Glabron, Brier,

California 1311, Hudson, Atlas 46 and La Mesita, he identified seven physiological races. Kajiwara and Iwata (1963) used five differential cultivars to divide thirty nine Japanese isolates into ten races. They found that some of the races in Japan were similar to others from Argentina and the United States. Owen (1963) demonstrated differences among ten isolates from Britain and found considerable differences between United States and British isolates. He concluded, however, that differences between his isolates from Britain were not well marked, hence he could not designate them as physiological races. In tests involving twenty two cultivars and fifteen isolates, Skoropad (1960) found that differences existed in the time required for symptoms to appear but the end results were the same. He noted that under conditions very favourable to infection, the specificity of isolates to particular cultivars broke down. He also observed that an isolate from Ontario differed from Western isolates, and that different pathogenic races occurred in Argentina and Alberta.

Kajiwara and Iwata (1963) observed differences in the morphology of leaf-borne conidia among isolates from barley but they could not separate them into groups on this basis. However, they noted that some isolates differed significantly in their spore size and that those with smaller length/width ratio tended to be more virulent. They found considerable variation in the colour

and topography of cultures and the rate of growth of the isolates but there was no relationship between cultural characteristics or rate of growth of the isolates and pathogenicity.

5) Infection Processes

Mackie (1929) and Caldwell (1937) have reported that infection is preceded by the germination of the conidium and the formation of an appressorium at the tip of the germ tube. Bartels (1928) and Mackie (1929) claimed that germ tubes entered the mesophyll through stomata but Caldwell (1937) noted that direct penetration of the cuticle occurred from the appressorium, and the mycelium then developed, first between the cuticle and the outer epidermal cell wall. The subcuticular mycelium developed rapidly into a stroma on the infected surface of the leaf. As the subcuticular mycelium grew, the epidermal cells collapsed and hyphae penetrated the macerated cell walls of the collapsed epidermis to establish an intercellular mycelium in the mesophyll. Davis (1922) reported that conidia were produced on hyphae protruding from stomata whilst Caldwell (1937) claimed that conidia were formed only on subcuticular stromata developed at points of infection.

6) Symptoms

The fungus attacks plants at any stage from seedling to senescence (Caldwell, 1937; Skoropad, 1960; Eugenio and Astolfo, 1961). Scald lesions typically occur on both sides of leaf blades and on leaf sheaths, and to some extent on seeds and awns. Symptoms first appear as light-grey water-soaked patches, typically oval in shape and up to about 2 cm in length and 1 cm in width. The margins soon become dark brown as the centres dry and become pale brown or white in colour. The browning of the margin may extend well beyond the pale brown centre, covering areas on the leaf without hyphae. Caldwell (1937) observed that successive enlargements of the lesions might occur resulting in formation of concentric brown rings. When many lesions are formed on a leaf blade, they may coalesce forming irregular lesions. On leaves of seedlings, lesions may spread across the leaf, and as a result the mid-ribs collapse and the top halves of the leaves bend over. Such leaves are completely desiccated. Ozoe (1956) noted that infected plants showed stunted growth, retardation of kernel maturity and a decrease in the number of kernels.

MATERIALS AND GENERAL METHODS

1. MATERIALS

Isolates of R. secalis were obtained from lesions on barley and barley grass collected from various places in South Australia and Tasmania. Three isolates were received from Western Australia and one from New South Wales.

Seeds of barley cultivars [West China; Atlas 46 (CI. 7323); Tennessee Winter (CI. 6034); Nigrinudum (CI. 2222); La Mesita (CI. 7565); Osiris (CI. 1622); CI. 3576; Turk (CI. 5611); CPI. 18197*; Prior (CI. 13436); Bussell (CI. 13520); Clipper (CI. 14844)] used in the investigations were obtained from Mr. D.H.B. Sparrow, Department of Agronomy, Waite Institute. Barley grass seeds were collected from the campus of the Institute.

2. GENERAL METHODS

a) Isolation of the Fungus

The isolation of R. secalis is difficult because of its very slow rate of growth on agar, which enables other organisms present to take over, and its sensitivity to sterilizing chemicals. For a successful isolation, the time of exposure to the sterilizing

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*Commonwealth Plant Introduction.

liquid must therefore be as short as possible. At the same time the growth of other organisms must be eliminated. A thorough wash of diseased leaves in water removes most fungi growing on the leaf surface. Growth of fungi within the tissues cannot be avoided. R. secalis growing from such lesions is therefore likely to be overgrown. Organisms that contaminate the culture after a thorough wash in water are usually bacteria. During the present work it was observed that bacterial contamination was considerably reduced if lesions were transferred with the minimum of liquid onto nutrient agar. Soft agar kept the tissues sufficiently moist to enable the fungus to grow. A modification of the method described by Schein and Kerelo (1956) was subsequently used in order to reduce the period of immersion of lesions in the sterilizing liquid.

Diseased leaves were thoroughly washed in water using a camel hair brush. The lesions were then cut into small pieces (1-5 mm^2), immersed in 70% alcohol for 20 seconds and then transferred to sodium hypochlorite (commercial grade, 3.6% by weight) diluted 1:6 with water. The lesions were immersed in the hypochlorite solution for 45 to 60 sec., and then thoroughly washed in sterile water. After draining off the surplus liquid by means of a sterile filter paper, they were then placed on 1% PSP agar and incubated at 15°C. A single spore transfer was made from each mass isolate.

b) Media

i. 1% Potato-sucrose-peptone agar (1% PSP agar)

"Deb" dehydrated potato	22 g
Sucrose	10 g
Peptone	10 g
Agar (Davis)	10 g
Distilled water	1,000 ml

ii. 0.5% Potato-sucrose-peptone agar (0.5% PSP agar)

Composition as above except that 5 g agar was used.

iii. Potato-sucrose-peptone liquid medium (PSP liquid medium)

Composition as above but without agar.

iv. Malt-yeast-agar (MYA)

Malt extract (Difco)	10 g
Yeast extract (Difco)	10 g
Agar (Davis)	10 g
Distilled water	1,000 ml

v. Malt-yeast liquid medium (MY liquid medium)

Composition as above but without agar.

vi. Lima bean-peptone agar (LBP agar)

Ground Lima bean (without seed coat)	50 g
Agar (Difco)	10 g
Peptone	10 g
Distilled water	1,000 ml

vii. Water agar

Agar (Davis)	15 g
Distilled water	1,000 ml

The fungus sporulated abundantly on all the nutrient agar media but survived longer on PSP agar. Soft media promoted sporulation. 0.5% PSP agar was used in the preparation of inoculum in all pathogenicity tests. MY agar, LBP agar and 0.5% PSP agar were used as media in inoculations other than those for pathogenicity tests.

c) Storage of Cultures

Fourteen-day old single spore cultures on 1% PSP agar slants in McCartney tubes were stored at $-15^{\circ}C$.

d) Sterilization of Media and Culture Filtrates

Media were sterilized by autoclaving for 20 min at 15 lb/in^2 pressure.

Extracts for bioassay were sterilized under suction by passage through sterile membrane filters (cellulose ester membrane, pore size 0.5 μm).

e) Preparation of Inoculum

Stock cultures were macerated in sterile distilled water using the flattened end ("foot") of an L-shaped glass rod. 250 ml Erlenmeyer flasks containing a thin layer of nutrient agar were each inoculated with 5 ml of the fungal suspension. The mycelium of the fungus grows within and on the medium and the conidia become inter-mixed with mycelium and agar and hence are not easily washed off. A thin layer of nutrient agar makes it easier to separate the conidia by maceration and filtration. The fungal material was spread over the surface of the medium by tilting the flask. The flasks were incubated in the dark at 15°C for 14 days. Yeast-like growth was an indication that many conidia were being formed. Cultures with good mycelial growth produced very few conidia. A few drops of the corresponding liquid medium were spread over such cultures on agar media to stimulate sporulation.

f) Inoculation Methods

i. The spray method

Fourteen-day old agar cultures were used. Each culture was thoroughly macerated in its Erlenmeyer flask using a glass rod

with a "foot". 50 ml distilled water were added and the mixture thoroughly shaken. The suspension was strained through a single layer and then a double layer of muslin cloth. The conidial concentrations were determined by using a haemacytometer and adjusted to 1.5-2.0 x 10^5 conidia/ml (Schein, 1958). Seedlings were sprayed with conidial suspension until all leaves were covered by fine droplets. Spraying was stopped before run-off occurred.

ii. The smear method

Inoculum for the smear technique was prepared in petri-dishes. The agar medium was about 5 mm thick so that portions of the culture could be manipulated conveniently. Discs were cut from 14-day old cultures and their upper surfaces were placed on leaves and gently drawn over the area to be inoculated.

g) Preparation of Seedlings

Seedlings were grown in potting compost in 6-inch pots in a glasshouse at a temperature of 20 - 25°C. At the 3-leaf stage seedlings were sprayed with the systemic fungicide, Milstem, at a concentration of 250 ppm. Milstem is active against powdery mildew of barley but gives no control of R. secalis. It was kindly supplied by Dr. D.H. Brooks of Plant Protection Limited, Jealotts Hill Research Station, Bracknell, Berks.

h) Incubation of Inoculated Plants

Pots containing inoculated plants were stood in water in a metal tray in a growth cabinet maintained at 15°C and illuminated for 12 hr a day at 1,000 f.c. The plants were covered with a polyethylene hood to maintain a high humidity. Temperature in the polyethylene hood varied between 14 - 16°C. The plants were removed to the glasshouse (20 - 25°C) after 48 hr.

15°C was selected as the temperature for incubation because it is about the daily mean temperature of the period when scald epidemics occur in the field in South Australia.

i) Histological Methods

i. Staining with periodic acid-Schiff reagent

Inoculated leaf pieces (0.5 cm^2) were fixed in formalin-acetic-alcohol (90 : 5 : 5) for 24 hr. Dehydration, infiltration and embedding of the fixed material were carried out in the manner described by Johansen (1940). Sections, 20 μm thick, were stained with the periodic acid-Schiff reagent (Preece, 1959).

Also, inoculated and uninoculated leaf pieces were cleared in a mixture of equal volumes of glacial acetic acid and absolute alcohol for 24 hr. Cleared leaf pieces were stained with periodic acid-Schiff reagent with a slight modification of the method

described by Preece (1959). It was found that subcuticular hyphae stained well only if Preece's method was modified so that leaf pieces and sections were placed in 0.5% aqueous potassium metabisulphite solution rather than sulphurous acid, after being removed from basic fuchsin solution. Stained leaf pieces were mounted whole in dilute glycerine for studies on the development of the fungus on the leaf surface and within the tissues. Microscopic examinations were made immediately after staining but when this was not practicable the leaf pieces were stored in 70% alcohol. Transverse sections, 20 μm thick, were cut with the freezing microtome from stained pieces and mounted in dilute glycerine for examination.

ii. Staining with cotton-blue

Inoculated leaf pieces were immersed in F.A.A. for 24 hr. Transverse sections, 20 μm thick, were cut with the freezing microtome and the sections stained with cotton-blue lactophenol.

j) Statistical Analyses

Statistical analyses of data were carried out by courtesy of members of the Biometry Section of the Waite Agricultural Research Institute. Results quoted as statistically significant were at the 5% level of significance.

EXPERIMENTS AND RESULTS

1. PHYSIOLOGICAL RACES OF RHYNCHOSPORIUM SECALIS

a) Pathogenicity and Virulence

The twelve barley cultivars listed on p. 16 were used to differentiate the pathogenic forms of the fungus isolated from barley and barley grass. All isolates used and their sources are listed in Table 1. Barley grass seedlings were also inoculated with selected isolates. The inoculation tests were made over a period of 20 months starting in October 1968, and were carried out in two stages:-

i. Inoculation of barley cultivars with isolates from barley and barley grass

The spray method was used to inoculate 4-leaf stage seedlings of the twelve cultivars with each of the isolates listed in Table 1, except isolate SA-5b which did not produce conidia in culture. Ten seedlings of each cultivar were inoculated in each test.

ii. Inoculation of barley grass with isolates from barley and barley grass

Six seedlings, each of which grew into a tuft, were inoculated with each of the selected isolates by the spray method

Table 1

Sources of isolates of R. secalis

Isolate No.	Isolated from	Origin
SA-1	Barley	Two Wells, South Australia
" 2	"	Langhorne Creek, "
" 3	"	Waite Institute, "
" 4	"	Maitland, "
" 5	"	Aldinga, "
" 5b	"	Aldinga, "
" 6	"	Kadina, "
" 7	"	Crystal Brook, "
" 8	"	Pinery, "
" 9	"	Moana, "
" 10	"	Port Stanvac, "
" 11	"	Ashville, "
" 12	"	Balgowan, "
" 13	"	Ashbourne, "
" 14	"	Hope Forest, "
" 15	"	Woodchester, "
" 16	"	Coolnalpyn, "
" 17	"	Tintinara, "
" 18	"	Renmark, "
" 19	"	Moonta, "
" 20	"	Gawler, "
" 21	"	Noarlunga, "
" 22	"	Penneshaw, "
" 40	Barley grass	Waite Institute, "
" 41	"	Waite Institute, "
" 42	"	Agery, "
" 43	"	Antechamber, "
WA-308	Barley	Wongan Hills, Western Australia
" 309	"	Broomehill, "
" 310	Barley grass	Narrogin, "
N-1	Barley	Finely, New South Wales
TS-1	"	Richmond, Tasmania
" 2	"	Granton, "
" 10	Barley grass	Port Arthur, "
" 11	"	Lenah Valley, "
" 12	"	Barnes Bay, "

at the 6-leaf stage. 4-leaf stage seedlings of Clipper barley were also inoculated in each experiment to confirm the pathogenicity of each isolate on barley.

Most of the lesion types observed in the investigations are illustrated in Fig. 1. Disease assessment was based on the following scale:-

0 : No symptoms.

R : A narrow band (less than 0.5 mm wide) of lesion along the margin of the leaf.

1 : Up to three small discrete lesions [(diameter up to 2 mm) (Fig. 1, b)].

2 : More than three small discrete lesions (diameter up to 2 mm).

3 : Discrete lesion with diameter more than 2 mm (Fig. 1, a).

4 : Two or three lesions (diameter more than 2 mm) coalescing with evidence of discrete lesions (Fig. 1, c).

5 : Four or more lesions coalescing (Fig. 1, g).

6 : Large infected area occupying up to one quarter of the total leaf area (Fig. 1, i and j).

7 : Large infected area occupying up to half of the total leaf area.

8 : Large infected area occupying more than half the total leaf area.

Figure 1.

Naturally and artificially infected leaves of barley showing typical scald lesions.

FIG. 1

The following rating of virulence based on symptom production, was also used:

Low virulence = R, 1 and 2

Moderate virulence = 3, 4 and 5

High virulence = 6, 7 and 8

Distinction is made between pathogenicity and virulence of an isolate. As used here, pathogenicity refers to the ability of an isolate to infect specific barley cultivars and the term virulence to denote aggressiveness. Criteria for the degree of virulence are:-

1. Mean lesion size.
2. $\frac{\text{Number of leaves infected}}{\text{Number of leaves inoculated}}$.
3. Rapidity of appearance of symptoms.

Lesion types on each of seven leaves and the period for the first leaf to show symptoms were recorded for each barley cultivar and for barley grass. Mature lesions did not increase in size, therefore symptoms were scored as soon as lesions were fully formed on a leaf and before the infected leaf dried. Scoring was completed within twenty five days after inoculation. The incubation period, the number of leaves infected and mean lesion type produced by R. secalis isolates on each host are recorded in Tables 2, 3, 4, 5 and 11.

Results

i. Pathogenicity and virulence of R. secalis isolates on barley cultivars

Cultivars which were susceptible to some isolates and immune to others were considered to be suitable as differential cultivars. The more susceptible they were to isolates which could attack them, the more reliable they were considered as differentials.

SA-7, SA-22 and SA-41 were distinguished from all other isolates by their inability to infect West China, a cultivar on which most of the remaining isolates gave a mean lesion type of 6 (Tables 2, 3, 4 and 5). SA-22 and SA-41 resembled each other in their inability to infect Atlas 46, Tennessee Winter, Nigrinudum, La Mesita and CI.3576 but were separated by the susceptibility of Turk, Prior and Clipper to SA-41. SA-22 was the only isolate which did not infect Prior and Clipper. SA-7 was differentiated from SA-22 and SA-41 by its ability to infect Atlas 46 and Tennessee Winter.

CI.3576, Prior, Bussell and Clipper were very susceptible to all the remaining isolates and were therefore of no value in the differentiation of pathogenic forms among them. The rest of the test cultivars were arranged in descending order of reliability as differential cultivars as follows:- Turk, Tennessee Winter,

Table 2: The incubation period, the number of leaves infected and mean lesion type produced by *R. secalis* isolates on barley cultivars, West China, Atlas 46 and Tennessee Winter.

Isolate No.	West China			Atlas 46			Tennessee Winter		
	Mean lesion type	Number of leaves infected	Incubation period (days)	Mean lesion type	Number of leaves infected	Incubation period (days)	Mean lesion type	Number of leaves infected	Incubation period (days)
SA - 1	5-6	*	5	R	1	7	6-7	*	5
" 2	7-8	*	10	7	4	10	7	4	10
" 3	6	*	9	0	-	-	3	2	11
" 4	7-8	*	7	R	3	9	6	*	7
" 5	7-8	*	8	3	4	10	6-7	6	8
" 6	3-6	6	10	6	2	10	4	6	11
" 7	0	-	-	5	3	13	7	1	10
" 8	R-3	4	9	0	-	-	0	-	-
" 9	6	6	13	0	-	-	0	-	-
" 10	R	2	11	6	*	7	3	5	9
" 11	7	*	7	0	-	-	6-7	*	8
" 12	7	*	8	6	2	10	7-8	*	8
" 13	7	*	10	0	-	-	6-7	*	10
" 14	6-7	*	8	6	*	8	4	*	8
" 15	6-7	*	7	R-3	4	10	4	*	8
" 16	3-7	6	10	0	-	-	3-6	4	11
" 17	4	*	9	3	*	11	3	*	9
" 18	4-6	*	8	3	3	10	6-7	*	7
" 19	7-8	*	9	R-1	5	11	6-7	*	9
" 20	6	*	11	0	-	-	R	*	10
" 21	R-1	*	11	0	-	-	3	*	9
" 22	0	-	-	0	-	-	0	-	-
" 40	R	1	12	0	-	-	0	-	-
" 41	0	-	-	0	-	-	0	-	-
" 42	R-3	*	9	0	-	-	4-6	*	9
" 43	R-3	*	12	0	-	-	R-3	*	11
WA-308	6	4	8	6	5	7	6	6	8
" 309	5-6	*	9	R-3	2	15	0	-	-
" 310	6	*	7	3	*	7	8	*	7
N - 1	3	*	14	0	-	-	R-1	2	14
TS - 1	6	*	8	0	-	-	6	*	8
" 2	5	*	10	0	-	-	5-6	*	11
" 10	R	*	8	3	4	11	6	*	11
" 11	5	*	14	0	-	-	0	-	-
" 12	3	*	10	0	-	-	3	*	10

* 7 or more leaves

Table 3: The incubation period, the number of leaves infected and mean lesion type produced by *R. secalis* isolates on barley cultivars Nigrinudum, La Mesita and Osiris.

Isolate No.	Nigrinudum			La Mesita			Osiris		
	Mean lesion type	Number of leaves infected	Incubation period (days)	Mean lesion type	Number of leaves infected	Incubation period (days)	Mean lesion type	Number of leaves infected	Incubation period (days)
SA - 1	R	1	6	R	3	7	0	-	-
" 2	0	-	-	R	5	13	R	1	13
" 3	R	2	13	0	-	-	0	-	-
" 4	R	2	10	R	1	11	0	-	-
" 5	6	3	11	R	5	11	R	3	12
" 6	0	-	-	0	-	-	0	-	-
" 7	0	-	-	0	-	-	0	-	-
" 8	0	-	-	0	-	-	0	-	-
" 9	0	-	-	3	*	13	0	-	-
" 10	0	-	-	R	1	11	0	-	-
" 11	0	-	-	R	2	13	0	-	-
" 12	0	-	-	0	-	-	0	-	-
" 13	R	3	10	0	-	-	R	1	10
" 14	3	1	12	0	-	-	0	-	-
" 15	R-1	2	11	0	-	-	0	-	-
" 16	0	-	-	0	-	-	0	-	-
" 17	0	-	-	3	3	12	0	-	-
" 18	R	2	10	R-3	*	8	0	-	-
" 19	R	2	13	R	3	13	0	-	-
" 20	0	-	-	0	-	-	0	-	-
" 21	R	2	11	0	-	-	0	-	-
" 22	0	-	-	0	-	-	3	3	12
" 40	0	-	-	0	-	-	0	-	-
" 41	0	-	-	0	-	-	0	-	-
" 42	0	-	-	R	*	11	0	-	-
" 43	R	4	12	0	-	-	0	-	-
WA-308	R	2	9	R	4	9	0	-	-
" 309	0	-	-	R	2	15	R-3	2	15
" 310	0	-	-	R	1	9	0	-	-
N - 1	0	-	-	0	-	-	0	-	-
TS - 1	0	-	-	0	-	-	0	-	-
" 2	3	3	13	0	-	-	0	-	-
" 10	0	-	-	0	-	-	0	-	-
" 11	0	-	-	0	-	-	0	-	-
" 12	0	-	-	0	-	-	0	-	-

* 7 or more leaves

Table 4: The incubation period, the number of leaves infected and mean lesion type produced by R. secalis isolates on barley cultivars, CI 3576, Turk and CPI 18197.

Isolate No.	CI 3576			Turk			CPI 18197		
	Mean lesion type	Number of leaves infected	Incubation period (days)	Mean lesion type	Number of leaves infected	Incubation period (days)	Mean lesion type	Number of leaves infected	Incubation period (days)
SA - 1	6-8	*	6	5-6	6	6	0	-	-
" 2	6-7	6	11	7	6	10	0	-	-
" 3	6-8	*	10	7-8	*	9	0	-	-
" 4	6-7	*	8	7	5	7	0	-	-
" 5	8	*	10	6	4	11	R	5	10
" 6	6	*	10	8	5	11	6	4	10
" 7	6	*	13	6	6	10	6-7	4	10
" 8	3	*	9	0	-	-	R	1	9
" 9	6-8	*	9	0	-	-	0	-	-
" 10	6-8	*	8	0	-	-	0	-	-
" 11	7-8	*	8	0	-	-	0	-	-
" 12	8	*	8	0	-	-	R	3	10
" 13	7	*	9	0	-	-	R	2	10
" 14	7-8	*	8	0	-	-	0	-	-
" 15	7	*	9	0	-	-	0	-	-
" 16	3-6	*	10	0	-	-	3	3	12
" 17	6-7	*	9	0	-	-	R	3	11
" 18	6-7	*	7	6	*	7	6	*	8
" 19	6	*	9	7	*	9	7	*	9
" 20	6	*	11	6	3	8	0	-	-
" 21	R	*	10	0	-	-	4	2	9
" 22	0	-	-	0	-	-	0	-	-
" 40	6-8	*	12	R	*	13	R	*	12
" 41	0	-	-	3	1	16	3-6	3	16
" 42	6-8	*	9	4	*	11	0	-	-
" 43	6-8	*	10	6	*	10	R-3	5	11
WA-308	5-7	*	7	R	3	8	6	4	8
" 309	3-5	*	9	5	4	14	-	-	-
" 310	5	5	7	6	3	7	6	1	7
N - 1	R	3	13	2	1	13	-	-	-
TS - 1	6	*	8	7-8	*	8	6	4	10
" 2	R	*	8	5-6	*	9	6	2	9
" 10	R	*	10	6-7	*	8	6	4	10
" 11	3	5	14	7	*	12	3-6	4	14
" 12	R	*	10	2	2	10	6-7	*	10

* 7 or more leaves

Table 5: The incubation period, the number of leaves infected and mean lesion type produced by *R. secalis* isolates on barley cultivars, Prior, Bussell and Clipper.

Isolate No.	Prior			Bussell			Clipper		
	Mean lesion type	Number of leaves infected	Incubation period (days)	Mean lesion type	Number of leaves infected	Incubation period (days)	Mean lesion type	Number of leaves infected	Incubation period (days)
SA - 1	7-8	*	5	6-8	*	5	7-8	*	5
" 2	6-8	*	9	7-8	*	8	6-8	*	10
" 3	7-8	*	9	8	*	9	6-8	*	10
" 4	7-8	*	7	7-8	*	7	5-7	*	7
" 5	8	*	9	8	*	10	8	*	9
" 6	6	*	11	5-7	*	11	6-8	*	10
" 7	7-8	*	8	4-6	*	10	3	*	13
" 8	3	*	10	R-3	4	9	3	*	8
" 9	6-8	*	10	6	6	12	6	*	11
" 10	7-8	*	7	7-8	*	7	7-8	*	7
" 11	7-8	*	8	7-8	*	8	8	*	7
" 12	6-8	*	9	7	*	9	6-7	*	7
" 13	6-7	*	8	7	*	9	7-8	*	8
" 14	7	*	8	5-6	*	8	5-6	*	8
" 15	6-7	*	8	6-8	*	9	8	*	8
" 16	7	*	10	7	*	10	7	*	10
" 17	6	*	8	6-7	*	8	7-8	*	8
" 18	6-8	*	7	7-8	*	7	6	*	5
" 19	6-7	*	9	7-8	*	9	5-8	*	8
" 20	6-7	*	9	6-7	*	8	6-7	*	8
" 21	4	*	9	4	*	9	5-7	*	9
" 22	0	-	-	1	1	9	0	-	-
" 40	8	*	12	6-8	*	12	6-8	*	12
" 41	3-6	*	15	3	2	20	3-5	*	15
" 42	6-7	*	8	6-7	*	8	8	*	8
" 43	7	*	8	4-6	*	8	7-8	*	7
WA-308	6	*	8	6	*	8	6-8	*	8
" 309	3	*	9	5-7	*	9	4-5	*	9
" 310	6-7	*	7	6-8	*	7	6	*	7
N - 1	3-4	*	11	2-4	*	12	R-3	*	11
TS - 1	6-8	*	7	3-6	4	12	7-8	*	8
" 2	3-6	*	8	6	*	8	6-7	*	8
" 10	5-7	*	8	5-6	*	8	5-6	*	8
" 11	3-5	*	12	3-5	*	12	5	*	12
" 12	6-7	*	10	7-8	*	10	6-8	*	10

* 7 or more leaves

Table 7.

Pathogenic sub-groups of R. secalis group A isolates based on pathogenicity of 7 cultivars of barley

GROUP A		a		b		c		d		e
SA-1	1	SA-40	1	SA-40	1	SA-3	1	SA-20	1	SA-20
SA-2		WA-309		TS-11		SA-20		SA-42		N-1
SA-3		TS-11	2	WA-309		SA-42	2	N-1	2	SA-42
SA-4		SA-1	3	SA-3	1	N-1		SA-3		
SA-5		SA-2		SA-20		SA-43	3	TS-1		
SA-6		SA-3		SA-42		TS-1		TS-12		
SA-18		SA-4		SA-43		TS-2	4	SA-43		
SA-19		SA-5		N-1		TS-12		TS-2		
SA-20		SA-6		TS-1	2	SA-1	5	SA-2		
SA-40		SA-18		TS-2		SA-2	6	SA-1		
SA-42		SA-19		TS-12	3	SA-4		SA-4		
SA-43	2	SA-20		SA-1		SA-5	7	SA-6	3	SA-6
WA-308		SA-42		SA-2		SA-6		WA-310		TS-10
WA-309		SA-43		SA-4	4	SA-18		TS-10	4	WA-310
WA-310		WA-308		SA-5		SA-19	8	SA-5		SA-18
N-1		WA-310	4	SA-6		WA-308		SA-18	5	SA-19
TS-1		N-1		SA-18		WA-310		SA-19		WA-308
TS-2		TS-1		SA-19		TS-10		WA-308	6	SA-5
TS-10		TS-2		WA-308						
TS-11		TS-10		WA-310						
TS-12		TS-12		TS-10						

Table 8.

Pathogenic sub-groups of *R. secalis* group B isolates based on pathogenicity on 7 cultivars of barley

GROUP B		f		g		h		i
SA-8	1	SA-8	1	SA-9				
SA-9		SA-9	2	SA-8				
SA-10		SA-10		SA-11	1	SA-11		
SA-11		SA-11	3	SA-13		SA-13	1	SA-16
SA-12		SA-12		SA-16	2	SA-16		SA-21
SA-13	2	SA-13		SA-21		SA-21	2	SA-13
SA-14		SA-14		SA-10		SA-10	3	SA-14
SA-15		SA-15		SA-12	3	SA-14		SA-15
SA-16		SA-16	4	SA-14		SA-15	4	SA-10
SA-17		SA-17		SA-15	4	SA-12	5	SA-12
SA-21		SA-21		SA-17		SA-17	6	SA-17

Table 9.

Key to the identification of pathogenic sub-groups (Table 7) of *Rhynchosporium secalis* group A isolates

A. Not pathogenic to Tennessee Winter (a1)

B. Not pathogenic to Atlas 46 (b1)

- pathogenic to CPI.18197 but not pathogenic to Nigrinudum, La Mesita and Osiris

BB. Pathogenic to Atlas 46 (b2)

- not pathogenic to CPI.18197 but pathogenic to La Mesita and Osiris

AA. Pathogenic to Tennessee Winter (a2)

C. Not pathogenic to Atlas 46 (b3)

D. Not pathogenic to CPI.18197 (c1)

E. Not pathogenic to Nigrinudum (d1)

F. Not pathogenic to La Mesita (e1)

- not pathogenic to Osiris

FF. Pathogenic to La Mesita (e2)

- not pathogenic to Osiris

EE. Pathogenic to Nigrinudum (d2)

- not pathogenic to both La Mesita and Osiris

DD. Pathogenic to CPI.18197 (c2)

G. Not pathogenic to Nigrinudum (d3)

- not pathogenic to La Mesita and Osiris

GG. Pathogenic to Nigrinudum (d4)

- not pathogenic to both La Mesita and Osiris

CC. Pathogenic to Atlas 46 (b4)

H. Not pathogenic to CPI.18197 (c3)

I. Not pathogenic to Nigrinudum (d5)

- pathogenic to both La Mesita and Osiris

Table 9 (continued)

Key to the identification of pathogenic sub-groups (Table 7) of *Rhynchosporium secalis* group A isolates

II. Pathogenic to Nigrinudum (d6)

- pathogenic to La Mesita but not pathogenic to Osiris

HH. Pathogenic to CPI.18197 (c4)

J. Not pathogenic to Nigrinudum (d7)

K. Not pathogenic to La Mesita (e3)

- not pathogenic to Osiris

KK. Pathogenic to La Mesita (e4)

- not pathogenic to Osiris

JJ. Pathogenic to Nigrinudum (d8)

L. Not pathogenic to Osiris (e5)

- pathogenic to La Mesita

LL. Pathogenic to Osiris (e6)

- pathogenic to La Mesita

Table 10.

Key to the identification of pathogenic sub-groups (Table 8) of *Rhynchosporium secalis* group B isolates

A. Not pathogenic to Tennessee Winter (f1)

B. Not pathogenic to CPI.18197 (g1)

- not pathogenic to Atlas 46, Nigrinudum and Osiris but pathogenic to La Mesita

BB. Pathogenic to CPI.18197 (g2).

- not pathogenic to Atlas 46, Nigrinudum, La Mesita and Osiris

AA. Pathogenic to Tennessee Winter (f2)

C. Not pathogenic to Atlas 46 (g3)

D. Not pathogenic to CPI.18197 (h1)

- pathogenic to La Mesita but not pathogenic to Osiris

DD. Pathogenic to CPI.18197 (h2)

E. Not pathogenic to La Mesita (i1)

- not pathogenic to Osiris

EE. Pathogenic to La Mesita (i2)

- pathogenic to Osiris

CC. Pathogenic to Atlas 46 (g4)

F. Not pathogenic to CPI.18197 (h3)

G. Not pathogenic to La Mesita (i3)

- not pathogenic to Osiris

GG. Pathogenic to La Mesita (i4)

- not pathogenic to Osiris

FF. Pathogenic to CPI.18197 (h4)

H. Not pathogenic to La Mesita (i5)

- not pathogenic to Osiris

HH. Pathogenic to La Mesita (i6)

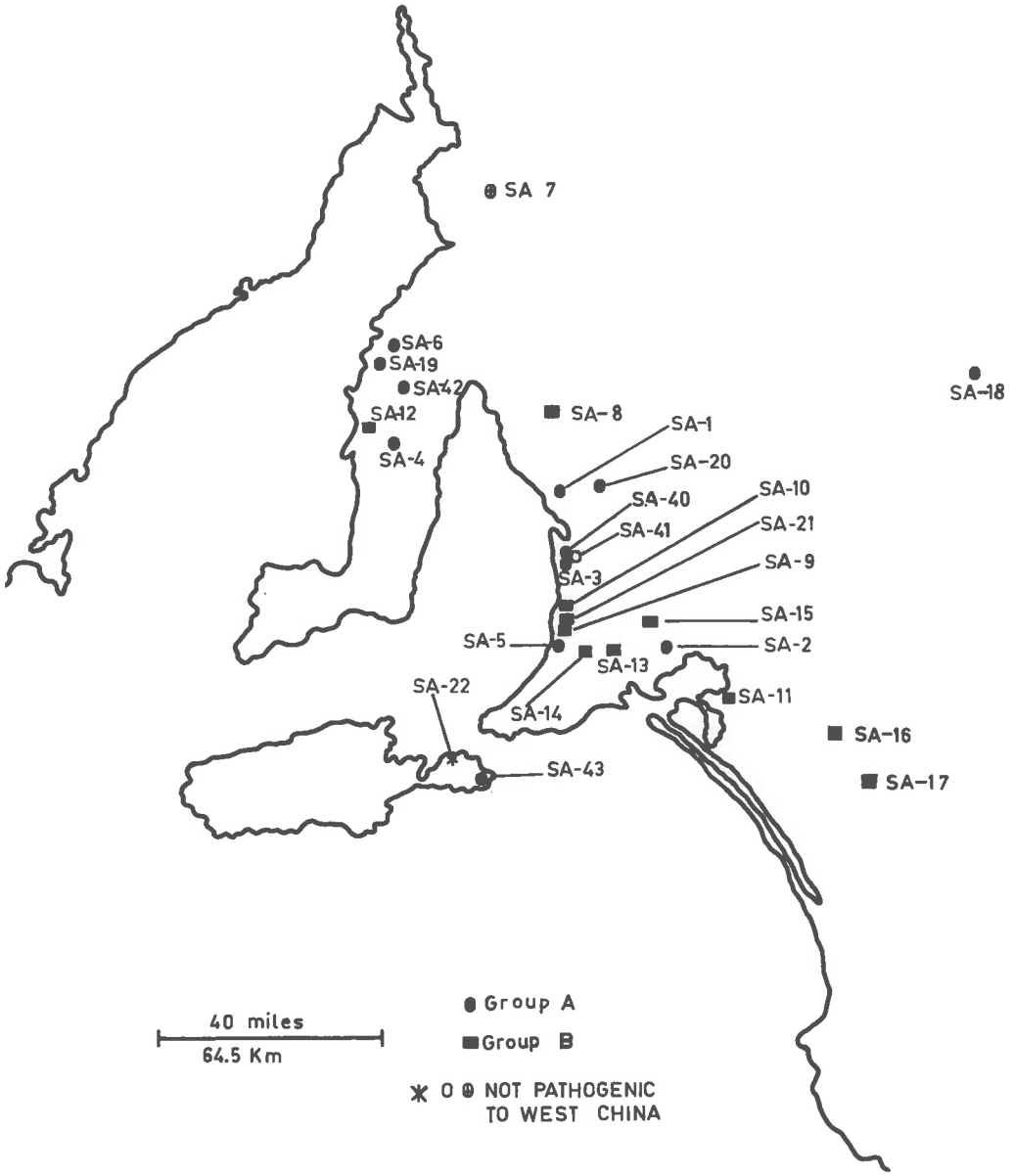
- pathogenic to Osiris

Atlas 46, CPI.18197, Nigrinudum, La Mesita and Osiris. Turk, which was very susceptible to about half of the South Australian isolates and immune to the other half, was used to separate the isolates into two main pathogenic types: a) isolates pathogenic to both West China and Turk (Table 7, Group A), b) isolates pathogenic to West China but not pathogenic to Turk (Table 8, Group B). The remaining cultivars were used progressively to subdivide each group. Groupings at the various levels are shown in Tables 7 and 8 and keys to them are provided in Tables 9 and 10.

Isolates from barley grass could infect barley and by using the twelve barley cultivars as differential varieties the thirty five isolates of R. secalis from barley and barley grass could be divided into pathogenic types on the basis of cultivars each isolate attacked. The number of pathogenic types depended on the number of cultivars used to identify them.

Generally, isolates from any one area tended to be related in pathogenicity as shown by the following examples: a) SA-6, SA-19, SA-42 and SA-4 (Fig. 2), four of the five isolates collected within a radius of about thirty miles on Yorke Peninsula belonged to a2 (Table 7 and Fig. 2). SA-4, SA-6 and SA-19 are grouped together in b4 (Table 7). b) SA-10, SA-21, SA-14, SA-13, SA-15, SA-11, SA-16 and SA-17 (Fig. 2), eight of eleven isolates obtained

FIG. 2



DISTRIBUTION OF RACES OF R. SECALIS IN SOUTH AUSTRALIA

from areas south of Adelaide (on the mainland) belonged to f2 (Table 8 and Fig. 2). c) Three of the five isolates from Tasmania, i.e. TS-1, TS-2 and TS-12, are grouped together in c2 (Table 7).

On the other hand, a few isolates from widely separated areas were found to have the same pathogenicity or were closely related, e.g. SA-43 from South Australia and TS-2 from Tasmania (Table 7, d4) and SA-6 from South Australia, WA-310 from Western Australia and TS-10 from Tasmania (Table 7, d7).

SA-8, SA-22, SA-41 and N-1 showed low virulence on the cultivars they attacked. The mean lesion type incited by them ranged from type R to type 3. Generally they attacked fewer leaves than other isolates. SA-41 and N-1 were consistently slow in causing symptom expression. SA-22 attacked only two cultivars and showed very low virulence. The remaining isolates showed differences in virulence but these were not well marked. SA-5 had high virulence giving a mean lesion type 7 to 8 on Tennessee Winter, West China, CI.3576, Prior, Bussell and Clipper.

ii. Pathogenicity of selected isolates to barley grass

Results of inoculation of barley grass with selected isolates (Table 11) showed that all four isolates from barley grass infected this host but only two of the five isolates from barley caused infection. All the barley grass isolates and one

Table 11.

The incubation period, the number of leaves infected and mean lesion type produced by selected *R. secalis* isolates on barley grass

Isolate No.	Mean lesion type	Number of leaves infected	Incubation period (days)
SA-1	R-3	3	10
SA-2	0	-	-
SA-5	0	-	-
SA-42	3-6	*	8
SA-43	3-6	*	9
N-1	0	-	-
TS-2	4-6	*	8
TS-10	3	2	11
TS-11	3	2	16

* 7 or more leaves.

of the isolates from barley showed moderate to high virulence while the other isolate (SA-1) from barley showed low virulence.

b) Cultural Characteristics of Selected Isolates

A selection of isolates was studied on 1% PSP agar in petri dishes 4.5 cms in diameter. Each plate was inoculated with a single spore of one of the isolates and incubated at 15°C in the dark.

Colonies consisted mainly of submerged mycelium with very little aerial hyphae (Figs. 3 and 4). They were slow growing and hyphae frequently anastomosed, forming compact hyphal mats. Their growth form varied considerably. They grew flat throughout or were raised in the centre with flat peripheral zones. Some were smooth while others were variously wrinkled or had radial or concentric furrows. Colony margins were mostly smooth but were lobed in a few cases. Colonies were white initially but developed pigments after two weeks. Pigment colour varied greatly from one isolate to another and the pigmentation commonly occurred in concentric zones. Growth forms and colours of 6-week old colonies of some isolates are shown in Table 12.

Under uniform conditions, colony characteristics were fairly

Figure 3.

Six-week old colonies of R. secalis each grown from a single conidium on 1% PSP agar at 15°C in the dark.

FIG. 3

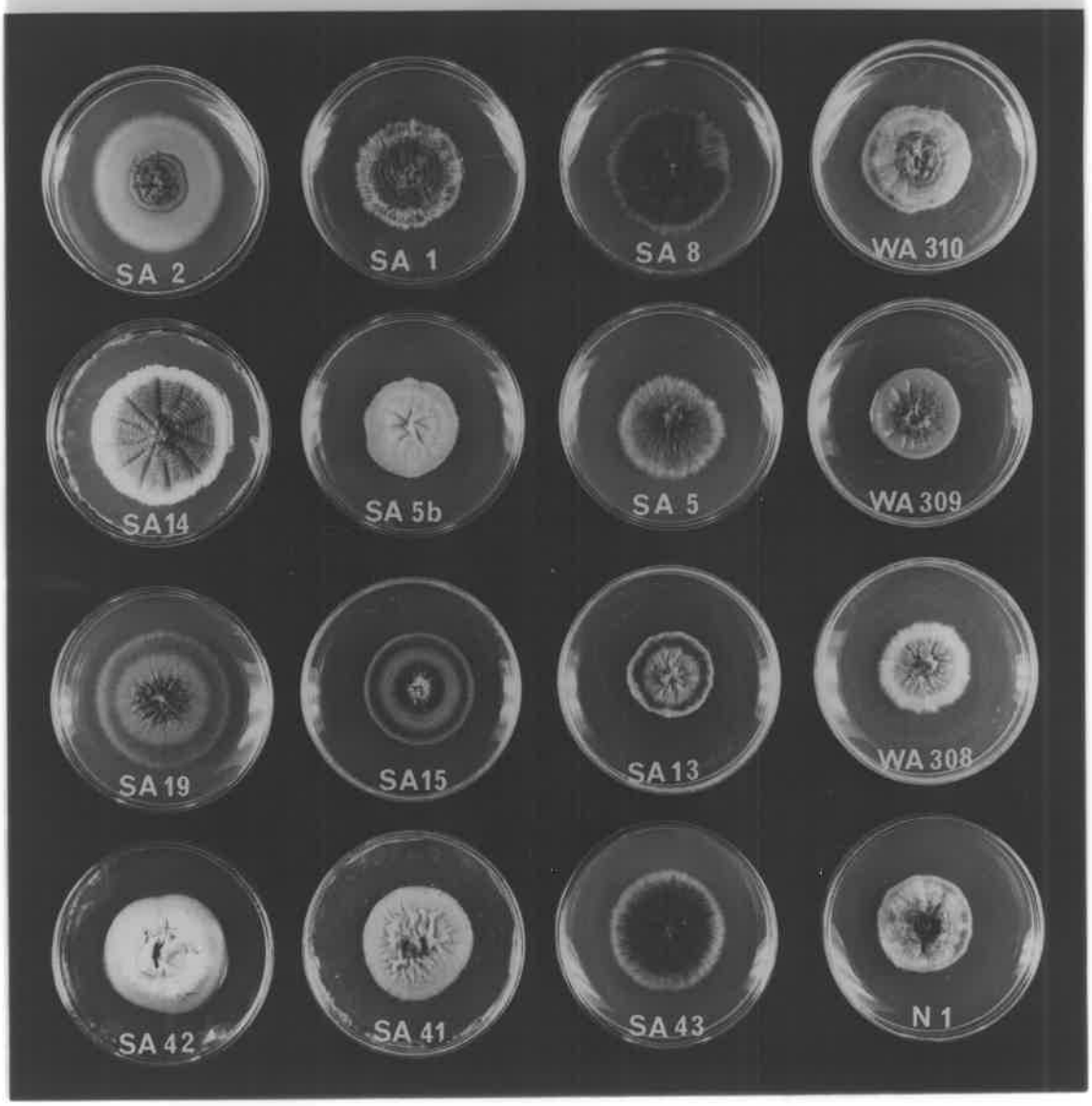

SA 2
SA 1
SA 8
WA 310
SA14
SA 5b
SA 5
WA 309
SA 19
SA15
SA 13
WA 308
SA 42
SA 41
SA 43
N 1

Figure 4.

Six-week old colonies of *R. secalis* each grown from a single conidium on 1% PSP agar at 15°C in the dark.

FIG- 4

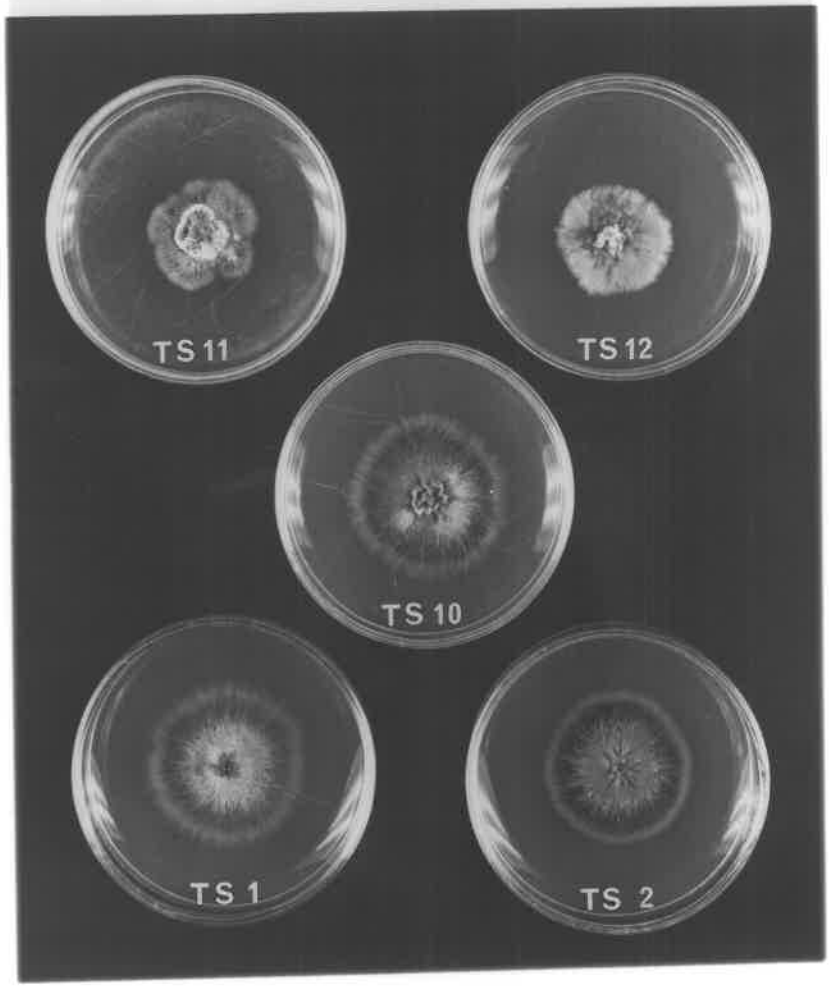


Table 12.

Growth form and colour of some isolates of R. secalis

Isolate No.	Colour	Growth form
SA-2	Yellow	Flat, with smooth outer zone and wrinkled centre
SA-5	Greyish-black	Flat and smooth
SA-5b	Pink	Raised at centre with radial furrows
SA-8	Black	Flat and smooth
SA-14	Yellow	Slightly raised at centre with radial and concentric furrows
SA-19	Shades of olive green in concentric zones	Flat and smooth, slightly wrinkled at the centre
SA-40	Cream	Flat and smooth
SA-41	Pink	Flat and wrinkled
SA-43	Black	Flat and smooth
TS-11	Cream	Heaped up at centre with lobed margin

stable for each isolate and some could be identified by their growth form and colour. However it was not possible to estimate pathogenicity or geographical origin of isolates on the basis of cultural characteristics. Isolates from barley grass did not show any characteristics which differentiated them from those from barley.

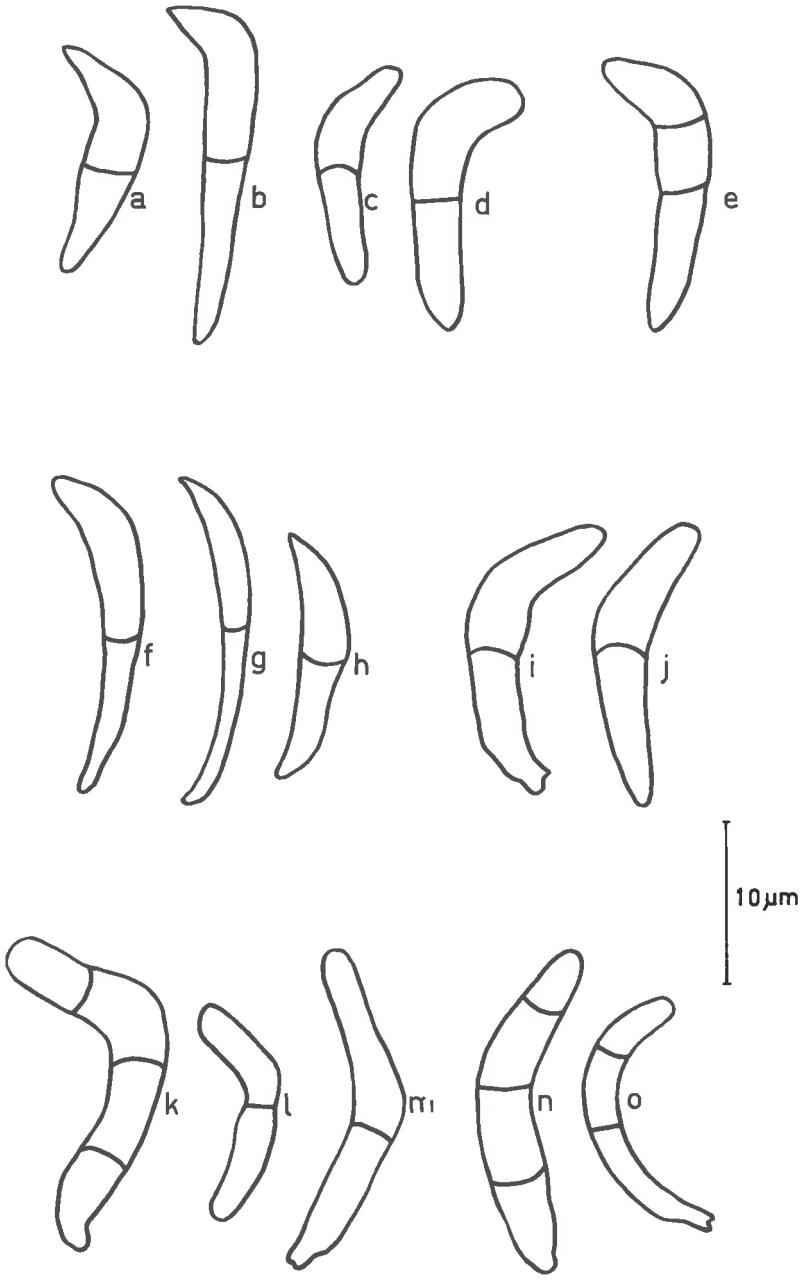
c) Morphology of Leaf-borne Conidia

The shape and size of conidia formed in lesions on Clipper barley plants inoculated with selected isolates were studied. Lesions were thoroughly washed with tap water and floated on water in petri dishes at 10°C for 72 hours. Clean slides were then brought into contact with sporulating lesions so that some conidia adhered to them. After drying, the conidia were stained with cotton blue lactophenol.

i. Conidial shape

The conidium of *R. secalis* has been described as elongated, one-septate and beaked at the apical portion (Fig. 5, a-e). This basic form was found to be very variable in the present work. The angle of the beak varied considerably, the transition between beak and conidial body was abrupt (Fig. 5, a and b) or gradual (Fig. 5, f)

FIG. 5



TYPES OF R. SECALIS CONIDIA PRODUCED ON INFECTED CLIPPER BARLEY LEAVES

or beak and conidial body merged without demarcation (Fig. 5, g and h). In some forms, the apical end was rounded (Fig. 5, d) rather than pointed though still inclined with respect to the conidial body. Some conidia, while retaining the typical form, showed a greater degree of overall curvature (Fig. 5, i-k). Others had lost the typical form and were boomerang-shaped (Fig. 5, m-o).

Typically the conidium is one-septate but forms with more than one septum occurred. The maximum number of septa observed in the present work was four. Septation appeared to depend on the size of the conidium, large conidia tending to have two or more septa.

Beaked conidia were the most common in all isolates, but there were marked variations in shape both within and between isolates (Figs. 6 and 7). In some isolates many forms were represented while in others variation was less pronounced. For example, conidia of TS-10 (Fig. 6) were very variable. Multi-septate conidia and large boomerang-shaped forms appeared more frequently in this isolate than in the others. TS-12 (Fig. 6) differed from most isolates in having many conidia with rounded apical ends. SA-13 (Fig. 6) had many long and slender conidia while many of the forms encountered in SA-19 (Fig. 6) were distinctly

Figure 6.

Conidia of selected isolates of *R. secalis*.

FIG. 6

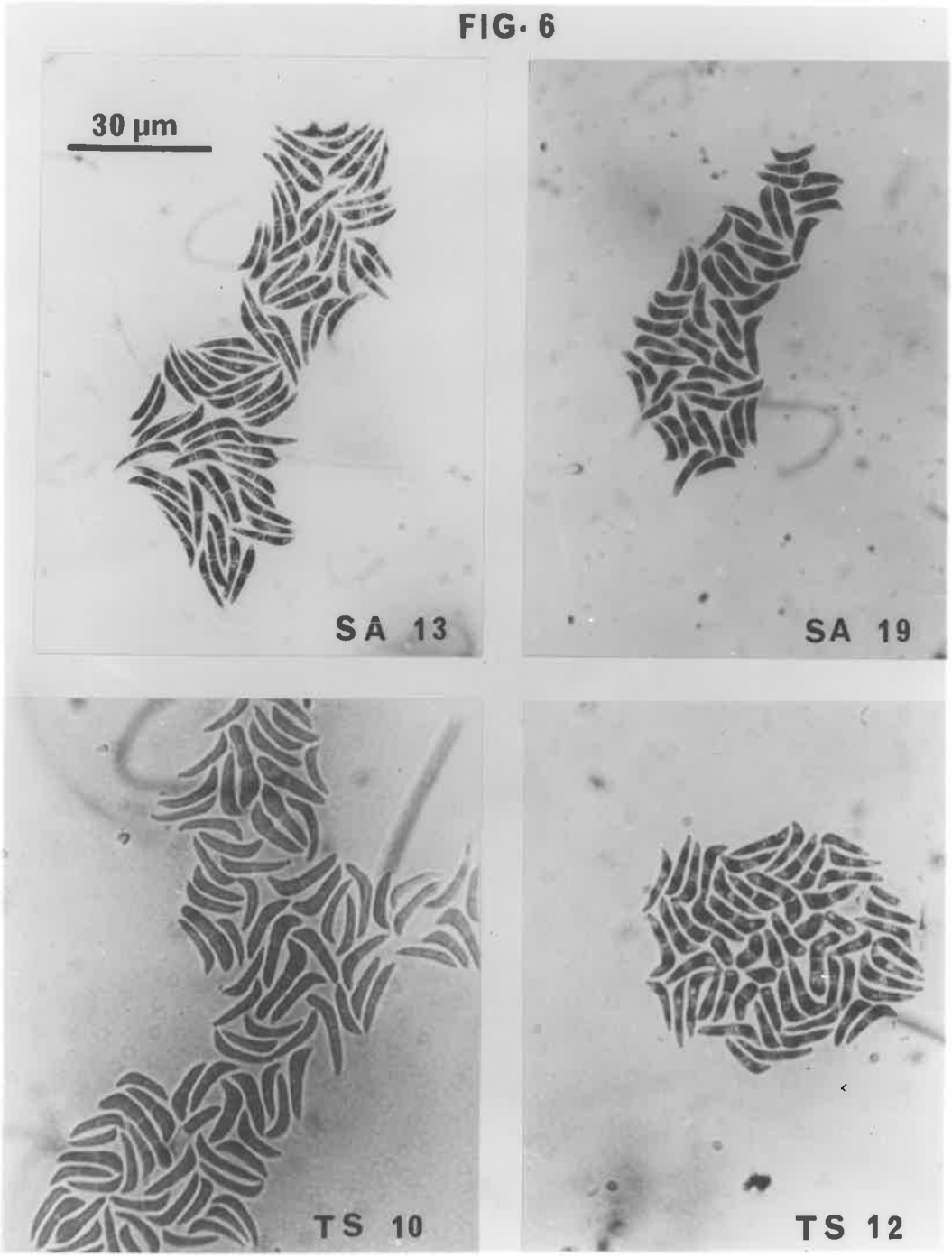
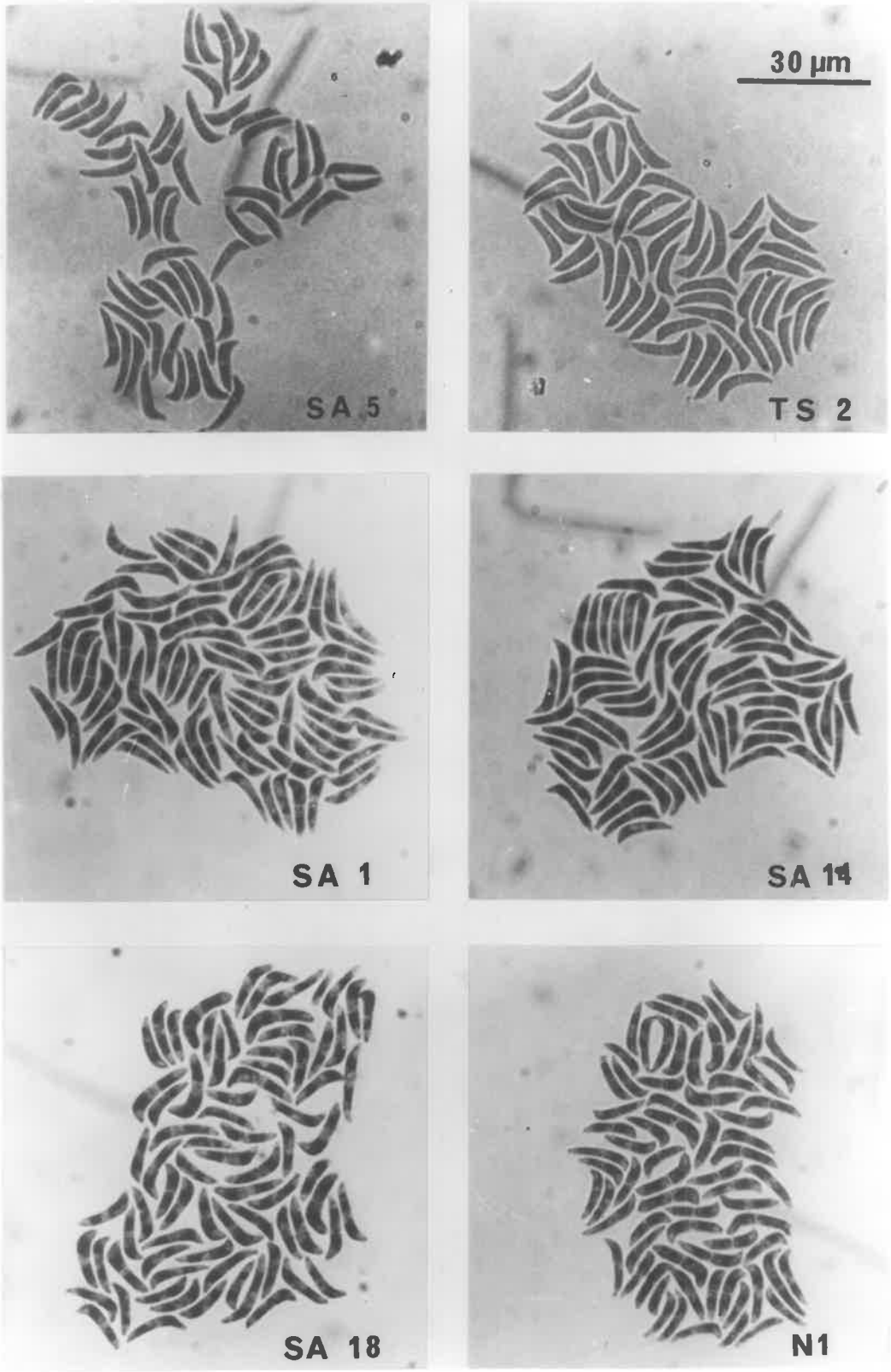


Figure 7.

Conidia of selected isolates of R. secalis.

FIG-7



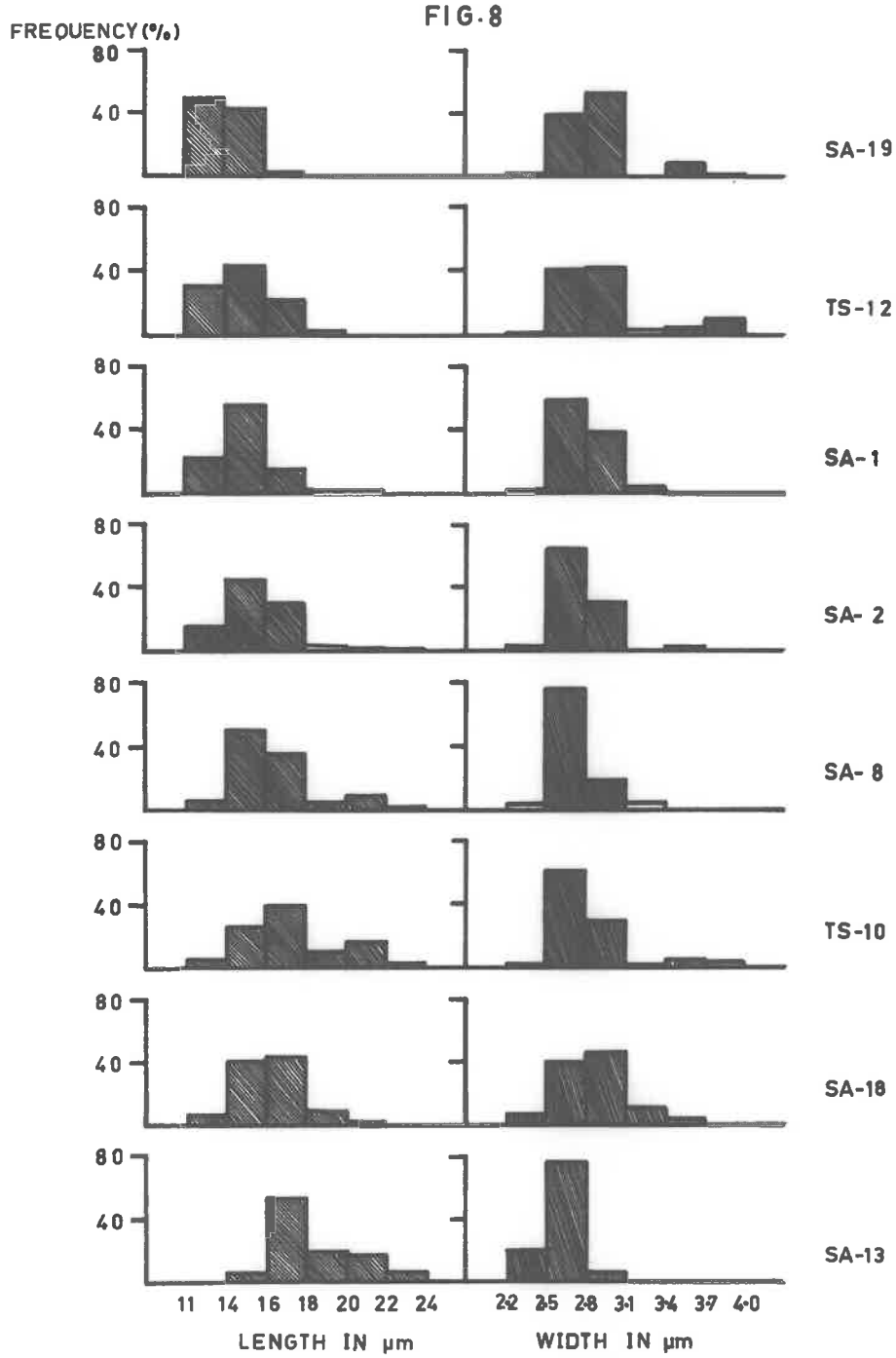
shorter than those of other isolates. Thus some isolates, e.g. SA-13, TS-10, TS-12 and SA-19 could be identified on the basis of their conidial morphology, whereas others (Fig. 7) could not.

Conidial variation did not follow any clear pattern. Isolates in the same pathogenic group or those collected from the same area did not necessarily show morphological features in common.

ii. Conidial size

The lengths and widths of 110 conidia of each isolate were measured and variation in them analysed. Width and length measurements were considered together adopting a stepwise discriminant analysis. The isolates were grouped as follows:- 1) SA-19 and SA-42. 2) SA-42 and TS-12. 3) SA-1 and SA-5. 4) N-1, TS-11 and TS-12. 5) SA-18 and TS-2. 6) SA-2. 7) SA-8. 8) TS-10. 9) SA-13. Conidial dimensions of isolates in each group differed significantly from those of the remaining isolates, but the variation shown was not correlated with pathogenicity or source. Fig. 8 shows the frequency distribution of length and width measurements of representative isolates from the groups.

FIG.8



FREQUENCY DISTRIBUTION OF LENGTHS AND WIDTHS OF CONIDIA OF ISOLATES OF R. SECALIS

d) Comparative Studies of Growth Rate of Germ Tubes

Rate of germ tube growth was studied at 10°C, 15°C and 20°C using conidia obtained from 14-day old cultures of selected isolates grown on 0.5% PSP agar. Media used in all experiments were prepared in the same batch.

For each isolate, two 1.5 cm discs cut from fresh cultures were thoroughly macerated in 20 ml sterile distilled water and strained through a 4-layer muslin cloth. Four drops of the spore suspension were spread on each of three water agar plates. Each was then incubated in the dark at one of the selected temperatures. The petri dishes were examined at 24-hour intervals and the germ tubes measured on twenty randomly-selected conidia.

Results

Ten isolates were investigated and in all cases germ tubes grew fastest at 20°C and slowest at 10°C. SA-8 differed from the other isolates in that most of the conidia which showed signs of viability budded off more conidia instead of producing germ tubes. The data for this isolate was therefore excluded from the analysis.

Mean germ tube lengths at the end of 5 days were compared at each temperature. Statistically significant differences were

Table 13.

Isolates of R. secalis grouped on the basis of growth rate of germ tubes on water agar at 10°C.

Group 1 and Group 2 (Fast growing^{+})

SA-1 SA-13

SA-14 Group 3 (Moderate growing^{++})

TS-11

SA-18 Group 4 and Group 5 (Slow growing^{+++})

SA-19 N-1

SA-5

SA-42

+	Germ tube length attained in 5 days;	280 - 395 µm
++	" " " " " " " ;	150 - 180 "
+++	" " " " " " " ;	60 - 110 "

Table 14.

Isolates of *R. secalis* grouped on the basis of growth rate of germ tubes on water agar at 15°C.

Group 1 (Fast growing^{+})

SA-1	**Group 2**			
SA-13	SA-13	**Group 3** (Moderate growing^{++})		
	SA-14	SA-14		
		SA-19	**Group 4**	
		N-1	4a	
		SA-18	SA-18	
		TS-11	TS-11	
			4b	(Slow growing^{+++})
			SA-5	
			SA-42	

+	Germ tube length attained in 5 days;	1000 - 1200 µm
++	" " " " " " " ;	580 - 730 "
+++	" " " " " " " ;	250 - 340 "

Table 15.

Isolates of *R. secalis* grouped on the basis of growth rate of germ tubes on water agar at 20°C

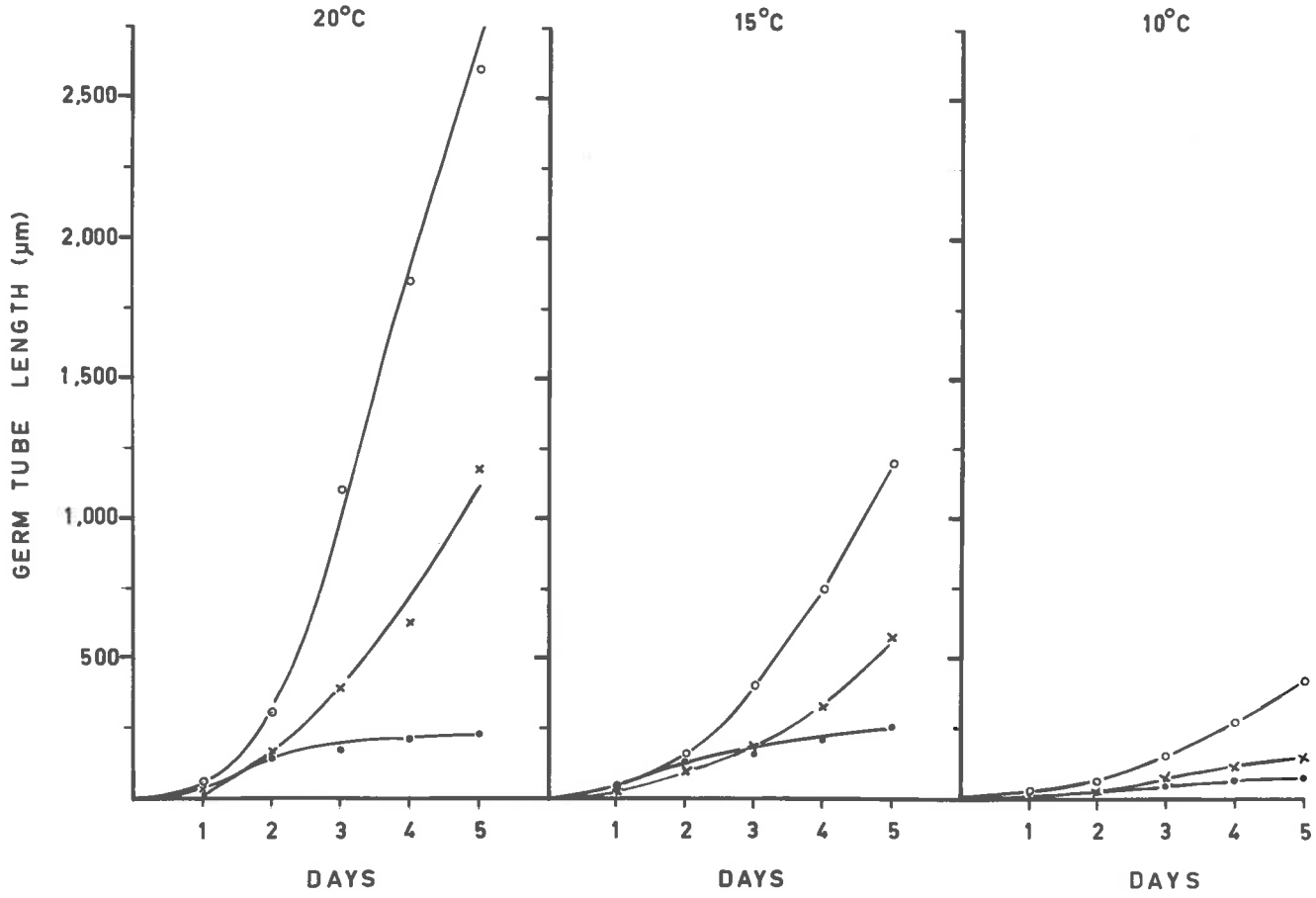
Group 1 and Group 2 (Fast growing^{+})	Group 3 and Group 4 (Moderate growing^{++})		Group 5 and Group 6 (Slow +++ growing)	
SA-1	TS-11			
SA-13	SA-19	SA-19		
		N-1	SA-14	SA-42
		SA-18	SA-5	

+	Germ	tube	length	attained	in	5	days;	2450 - 2600	µm
++	"	"	"	"	"	"	";	1550 - 2070	"
+++	"	"	"	"	"	"	";	600 - 1050	"

found between the isolates and they were grouped on this basis (Tables 13, 14 and 15). By comparison with the growth rate of isolate SA-1, which grew fastest at each of the three temperatures, the groups of isolates were classed as fast, moderate or slow growing (Tables 13, 14 and 15).

The growth curves of representative isolates in each class are shown in Fig. 9. A study of these results and those summarised in Tables 2, 3, 4 and 5 shows that rapidity of germ tube growth was not correlated with pathogenicity, virulence or sources of isolates.

FIG.9



GROWTH OF GERM TUBES OF ISOLATES OF R.SECALIS ON WATER AGAR AT 20, 15 AND 10°C. —o— SA-1; —x— SA-18; —•— SA-42.

2. INFECTION OF BARLEY LEAVES BY R. SECALIS

a) Materials and Methods

In these studies, the development of R. secalis on both surfaces of Clipper barley leaves and subsequent development within the leaf tissues were followed using detached leaf pieces and intact leaves on potted plants. Three isolates, SA-1, SA-5 and SA-13 were used. The inoculum used was obtained from 14-day old cultures grown on 0.5% PSP agar.

Leaf pieces (0.5 cm^2) were placed on drops of spore suspension on (a) slides contained in petri dishes with moist filter paper at the bottom to maintain a moist atmosphere, (b) 1.5% water agar in petri-dishes. They were incubated at 15°C in the dark. Marked areas on leaves of potted plants were also inoculated by the smear method and the plants placed in a humid chamber at 15°C and illuminated for 12 hr a day at 1,000 f.c. Inoculated plants used for studies on the development of stromata and conidia were transferred to the glasshouse after 48 hr.

Inoculated leaf portions were cleared at 48 hr intervals in a mixture of equal volumes of glacial acetic acid and 95% ethyl alcohol. They were stained with the periodic acid-Schiff reagent (Preece, 1959), and mounted in dilute glycerine for microscopic examination. Transverse sections, 25 μm thick, were cut with a freezing microtome from stained leaf pieces. Other leaf pieces were

fixed in F.A.A. in preparation for embedding and sectioning.

b) Results

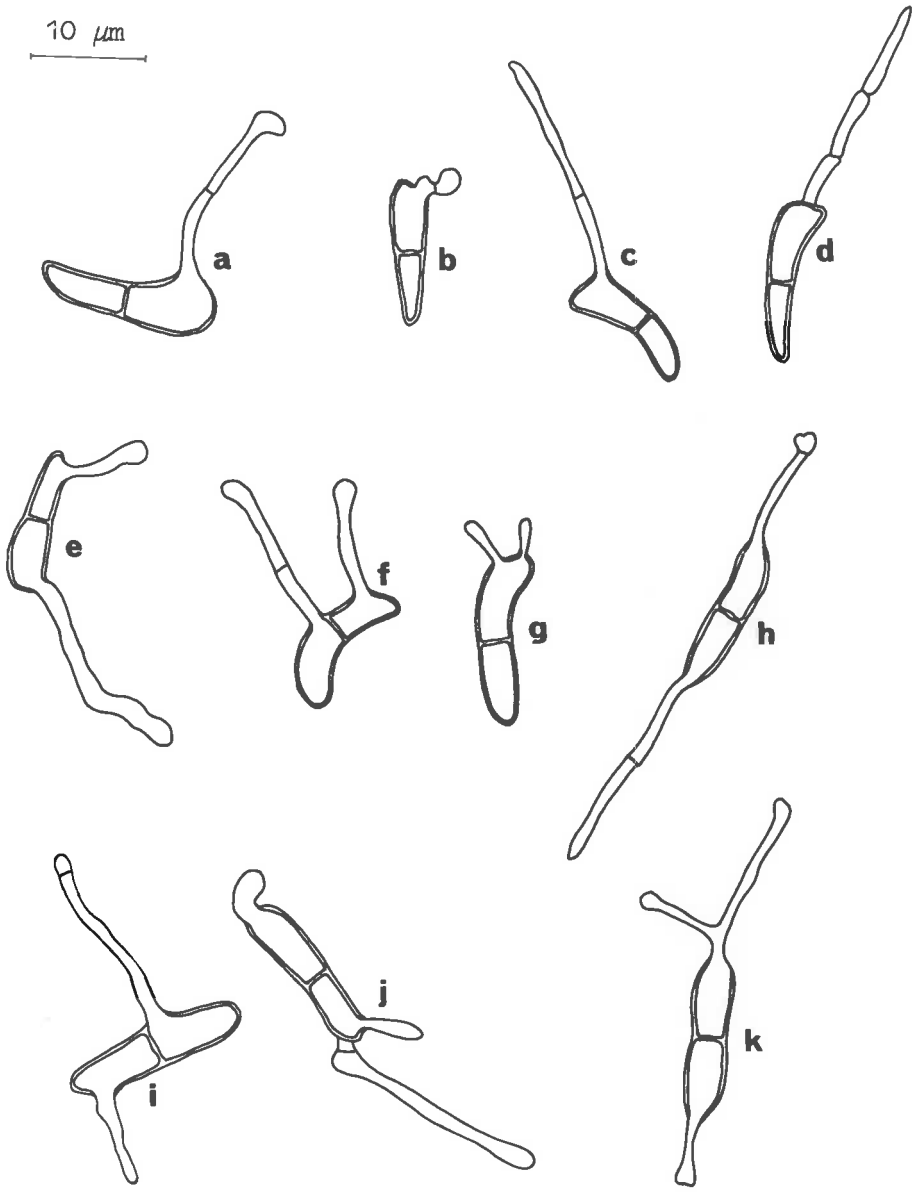
Conidial germination, penetration and development within leaf tissues were similar in all cases investigated. Percentage germination, percentage of conidia forming appressoria, and rapidity of penetration were greater on leaf pieces placed on spore suspension on water agar and least on leaves inoculated by the smear method.

i. Conidial germination patterns

At 15°C in the presence of liquid water, most germinable conidia produced germ tubes within 12 hr. One or two germ tubes were produced by most conidia (Fig. 10, a-i) but a few produced three germ tubes (Fig. 10, j). Germ tubes emerged from any free surface of the two cells of the conidium. The first germ tube usually arose from the larger cell; where two germ tubes were formed the second usually came from the other cell, but in a few cases both developed from a single cell (Fig. 10, g). Branching of germ tubes on leaves was infrequent (Fig. 10, k) and never elaborate. The germ tubes were septate, usually about 0.8 μm in diameter, and reached a maximum length of 20-30 μm in 24 hr.

Soon after their formation, some germ tubes enlarged slightly at the apices to form appressoria (Fig. 10, a) which in

FIG. 10



GERMINATING CONIDIA AFTER INCUBATION
FOR 24HR IN LIQUID WATER ON LEAF AT 15°C

a few cases were cut off by septa (Fig. 10, h and i); a few appressoria were sessile on the conidia (Fig. 10, b).

ii. Modification of the cuticle

Often, haloes which stained deep red with the periodic acid-Schiff reagent appeared in the cuticle at points of contact with the germinated or ungerminated conidia, and were generally formed at the following positions:- a) at the tip of an ungerminated conidium (Fig. 11 A, h_2) or rarely at the middle portion of the conidium (Fig. 11 A, h_1), b) at the tips of germ tubes without appressoria (Fig. 11 A, h_4), c) around sessile appressoria (Fig. 11 A, h_3), d) around appressoria at the tips of germ tubes (Fig. 11 B, h).

iii. Penetration by hyphae

A) Penetration of the cuticle

Subcuticular hyphae formed beneath the tips of conidia without the formation of superficial germ tubes (Fig. 11 C and D) or beneath appressoria (Fig. 12) within 24 hr after inoculation. The dark staining haloes mentioned above, were frequently found around points of penetration (Fig. 11 C and Fig. 12, h) though some conidia which successfully effected penetration were not associated with haloes (Fig. 11 D). On the other hand some conidia associated with haloes failed to effect penetration.

Figure 11.

A and B.- Haloes (h_1, h_2, h_3, h_4 and h) in the cuticle of a leaf inoculated with conidia and stained with periodic acid-Schiff reagent, 24 hr after inoculation.

C.- Conidium without superficial germ tube penetrating cuticle of leaf after incubation for 48 hr at 15°C. Note dark staining halo (h) around point of penetration; c, conidium; s, subcuticular hypha.

D.- Conidium without superficial germ tube penetrating cuticle of leaf after incubation for 48 hr at 15°C. Note absence of a halo around point of penetration; c, conidium; s, subcuticular hypha.

FIG·11

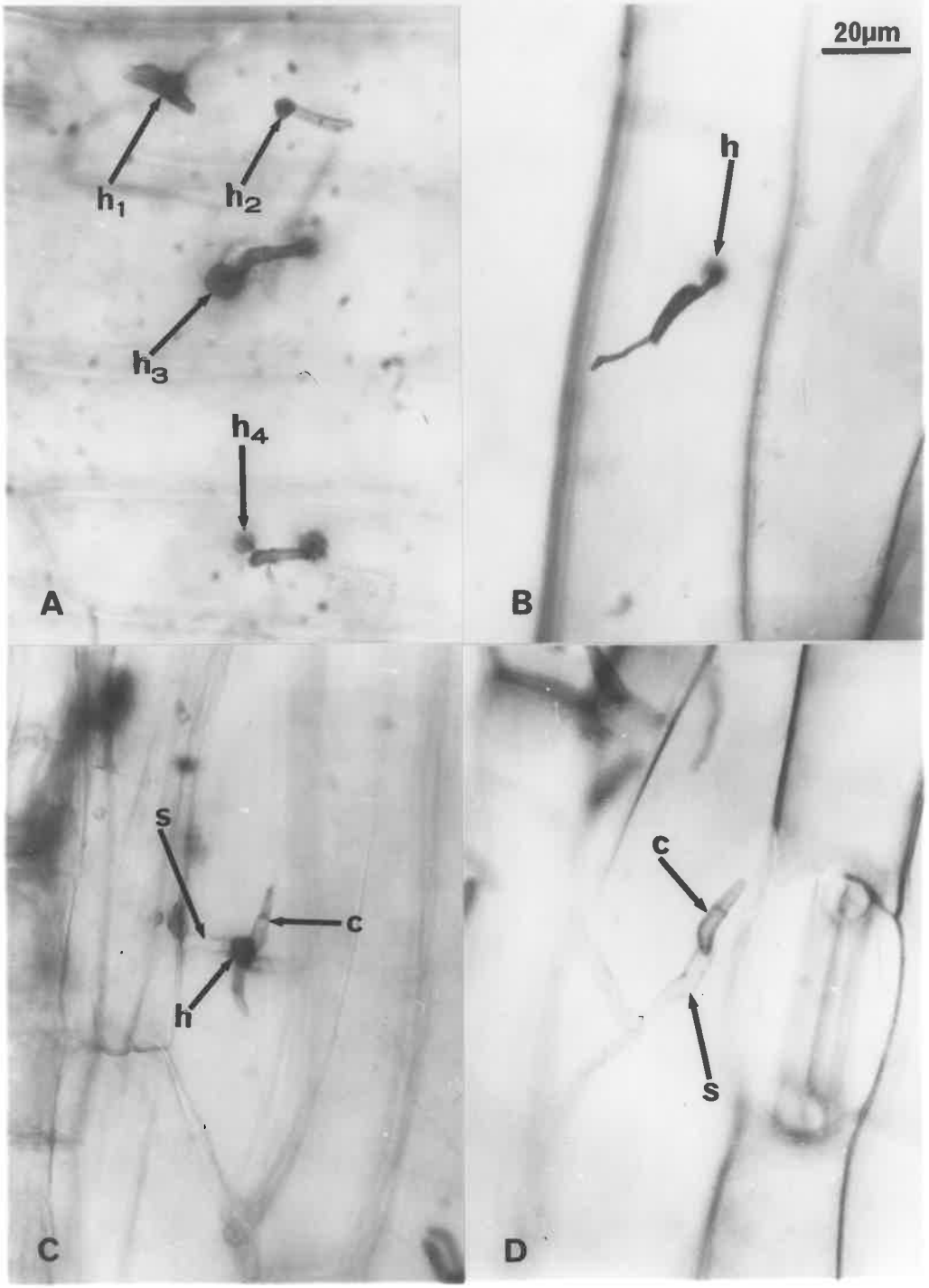
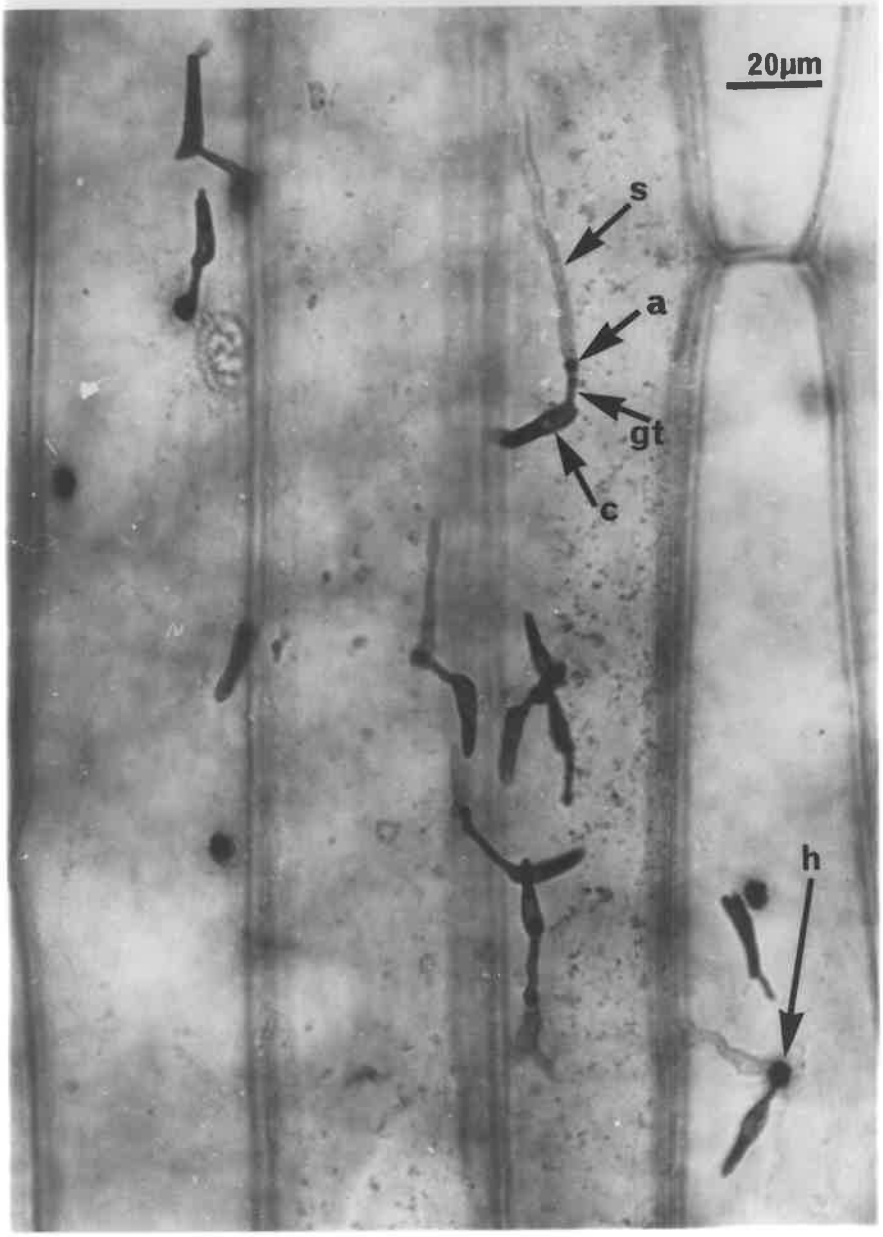


Figure 12.

Conidia germinating and penetrating the leaf cuticle from appressoria after incubation for 24 hr at 15°C. a, appressorium; c, conidium; gt, superficial germ tube; s, subcuticular hypha; h, halo.

FIG.12



After penetrating the cuticle, the infection hyphae enlarged in width and grew between the cuticle and the epidermis (Fig. 13, s). The first cell of a subcuticular hypha was commonly either rounded or consisted of a short rectangular segment with a rounded free end (Fig. 13, fc). The subcuticular hyphae grew along the grooves between the epidermal cells and branched profusely (Fig. 13 and 14). They were broad with short segments about 10 μm long and 1.5 - 3.5 μm in diameter.

The periclinal walls of the epidermis swelled and became lamellate in the vicinity of the subcuticular hyphae (Fig. 15 A and B); the anticlinal walls collapsed and, consequently, the outer periclinal walls fell against the inner walls (Fig. 15 B) forming a composite layer which persisted beneath the subcuticular mycelium (Fig. 16). The collapse of the mesophyll cells just beneath the epidermis followed; mesophyll cell walls retracted and adjacent walls remained in contact only at the positions of the plasmodesmata (Fig. 18) and chloroplasts aggregated in each of the affected cells (Fig. 16). All these changes took place while the fungus was confined between the cuticle and the epidermis.

B) Penetration of the epidermis

Ten days after inoculation the subcuticular mycelium was extensive. Hyphae encircled stomatal pores, anastomosed, and

Figure 13.

Penetration of leaf cuticle by germinating conidium 72 hr after inoculation. s, subcuticular hypha; fc, first cell of subcuticular mycelium; a, appressorium; c, conidium; gt, germ tube.

FIG-13

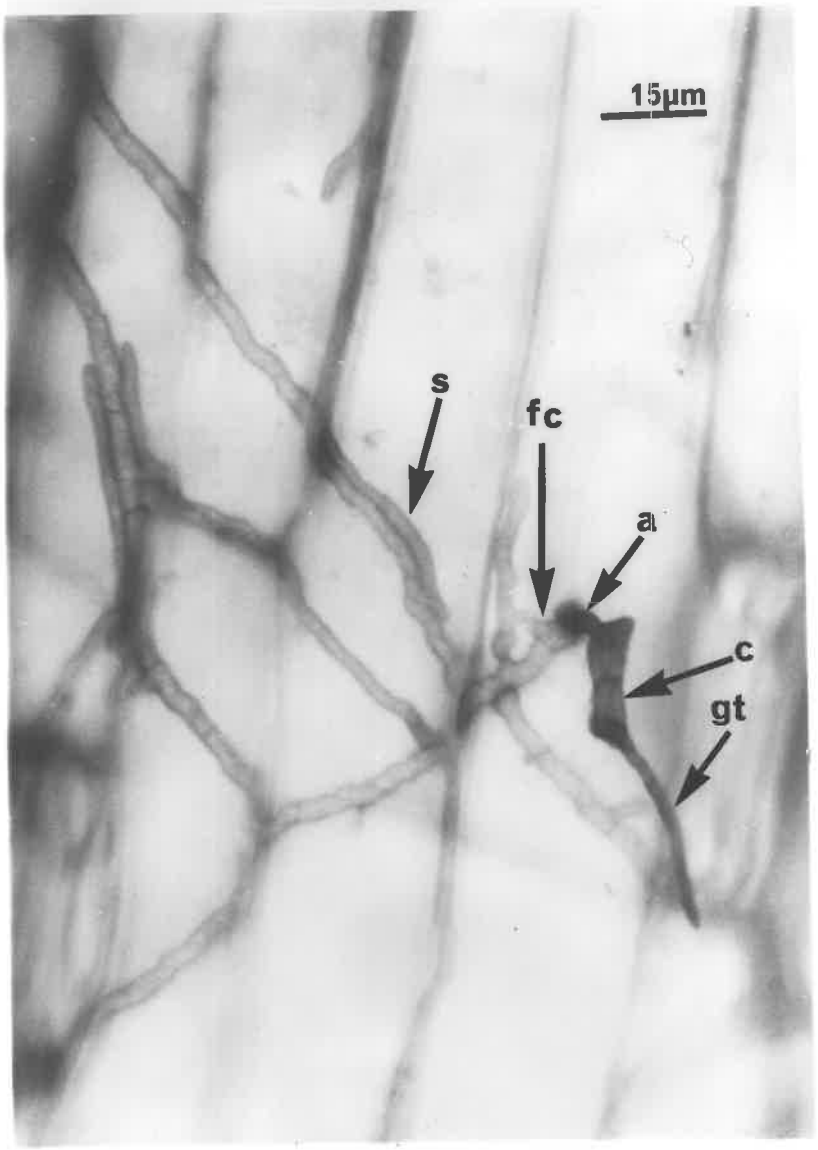


Figure 14.

Subcuticular hyphae in a portion of a leaf inoculated with conidia, incubated for 6 days at 15°C, and stained with the periodic acid-Schiff reagent; p, junction between the ends of a guard cell and a contiguous epidermal cell. Penetration of the epidermis was most frequent at this position, where subcuticular hyphae also anastomosed.

FIG-14

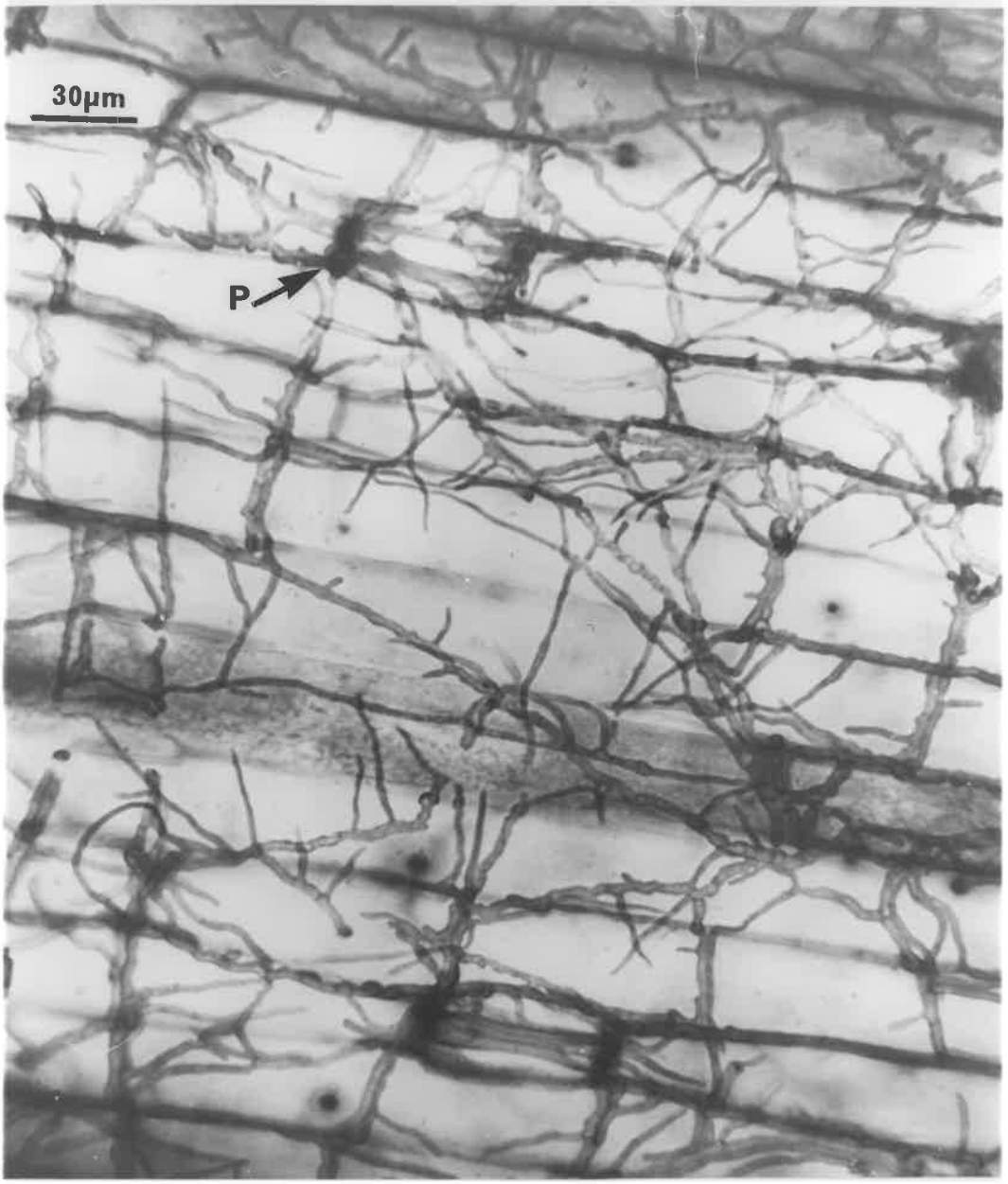


Figure 15.

A.- Transverse section of infected leaf showing swollen, lamellate epidermal cell walls (ew) and subcuticular hyphae (s).

B.- Transverse section of infected leaf showing stages in the collapse of anticlinal cell walls of the epidermis. Note the swollen lamellated cell walls; aw, collapsing anticlinal cell wall; opw, outer periclinal cell wall collapsed against inner wall; pw, periclinal cell wall; s, subcuticular hyphae; m, collapsed mesophyll.

FIG-15

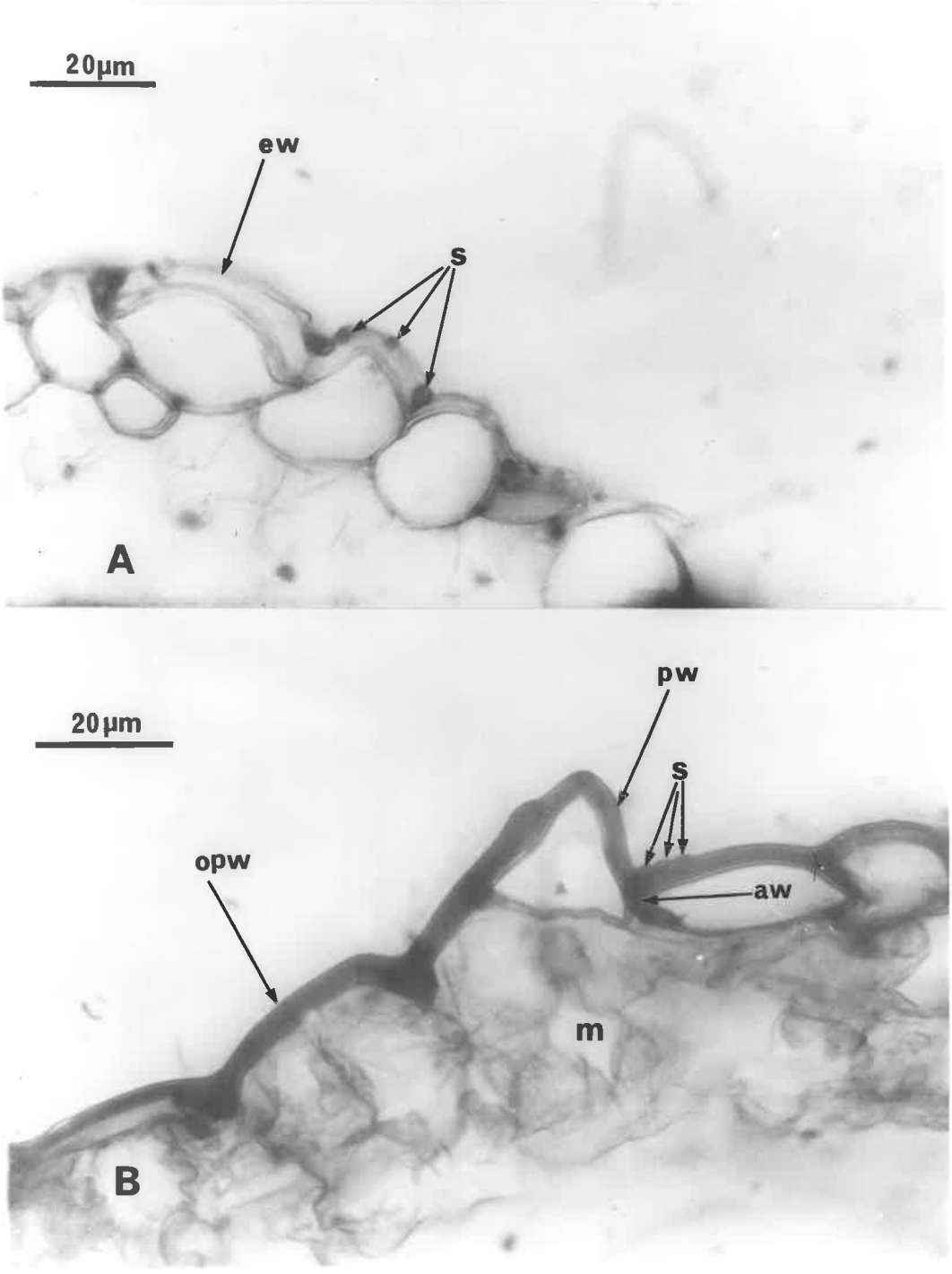


FIG.16

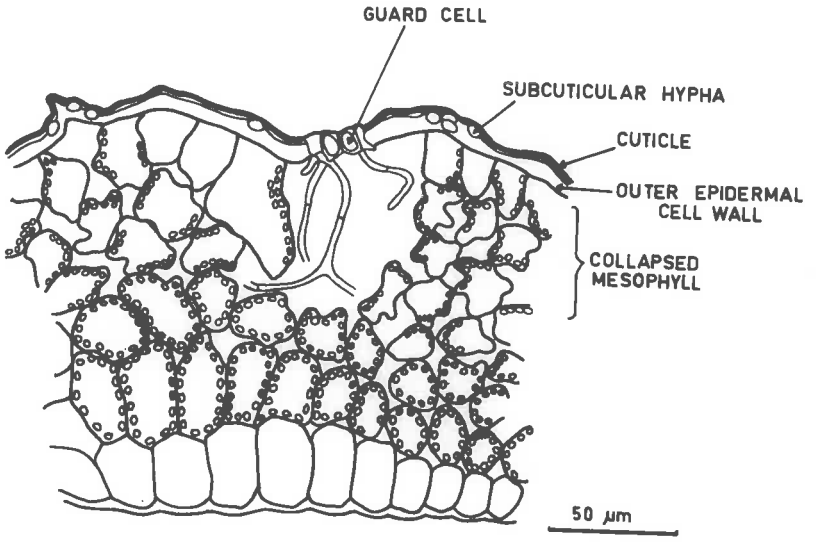


FIG.17

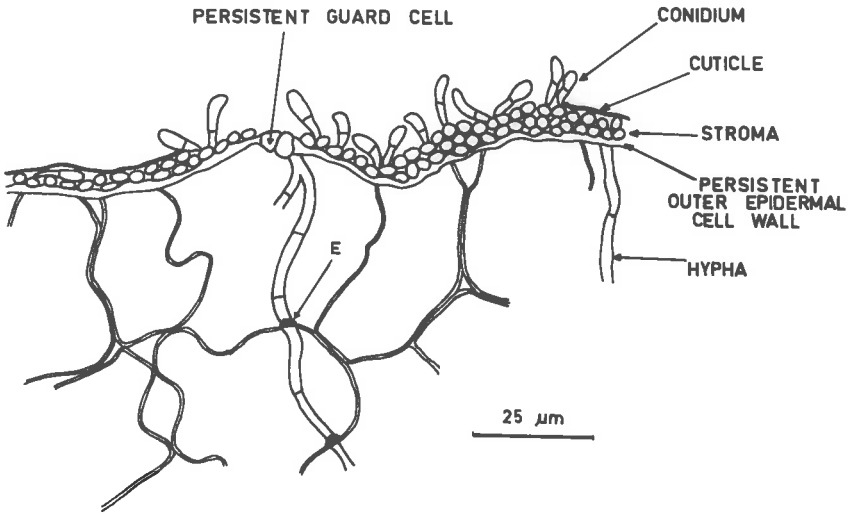


FIG. 16.- TRANSVERSE SECTION OF INFECTED LEAF SHOWING INVASION OF THE MESOPHYLL. THE INVADED EPIDERMIS AND MESOPHYLL HAVE COLLAPSED AND CHLOROPLASTS IN EACH INVADED MESOPHYLL CELL HAVE CLUSTERED TOGETHER.

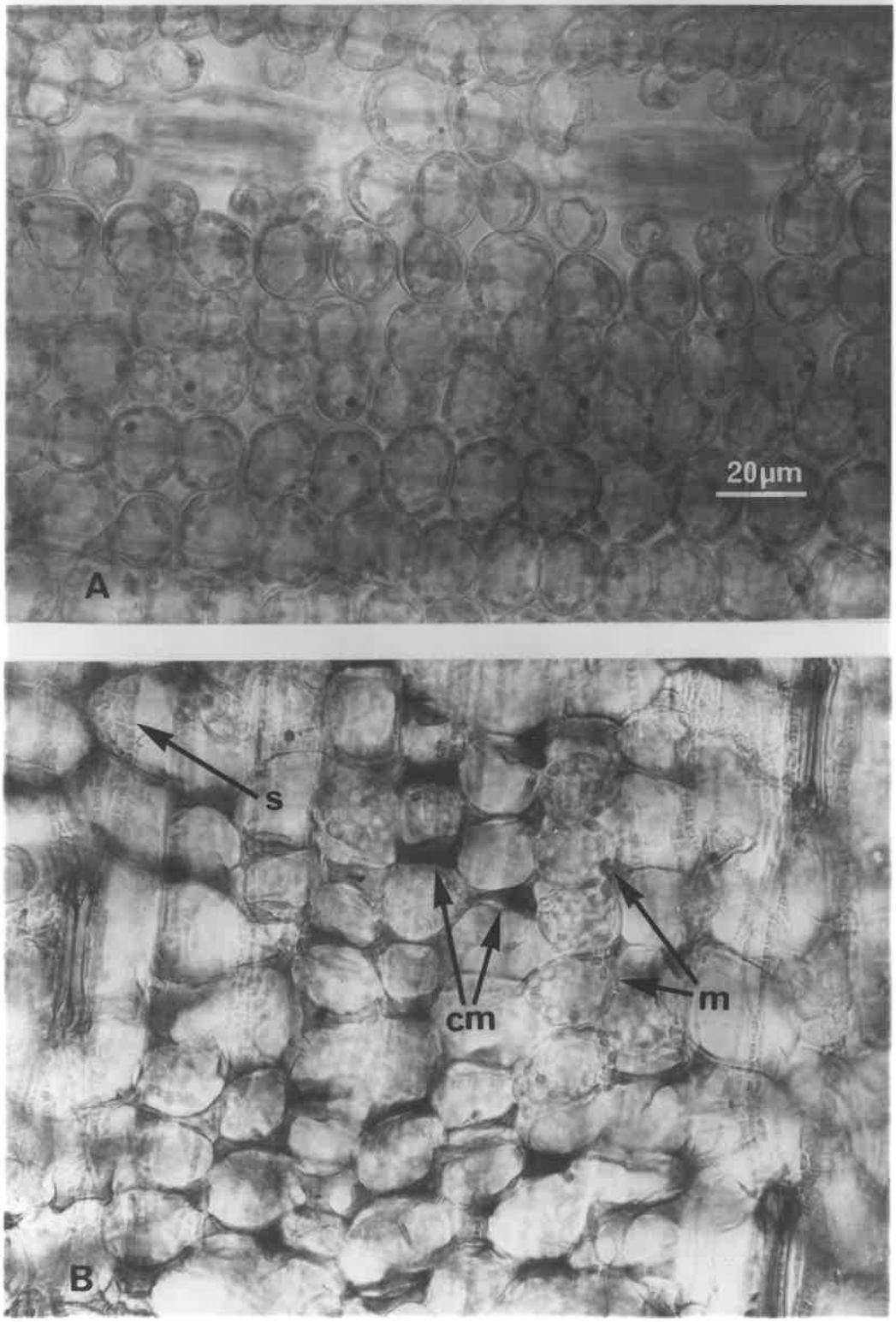
FIG. 17.- TRANSVERSE SECTION OF INFECTED LEAF SHOWING STRUCTURE OF SUBCUTICULAR STROMA. E, POINT OF ENTRY OF HYPHA INTO CELL WALL IS MARKED BY A DEEP STAINING REACTION WITH THE PERIODIC ACID-SCHIFF REAGENT.

Figure 18.

A.- Surface view of cleared healthy leaf showing mesophyll cells.

B.- Surface view of cleared infected leaf showing collapsed mesophyll cells (cm) and subcuticular hyphae (s); m, normal mesophyll cells.

FIG-18



covered guard cells (Fig. 14). Branches grew from the hyphae in the depressions between epidermal cells, penetrated between the side junctions of these cells and entered the mesophyll. Penetration was most frequent at the end junctions between guard cells and contiguous epidermal cells (Figs. 14 and 16). At this position penetration was commonly initiated by hyphal tips.

Intercellular mycelium grew between collapsing mesophyll cells and the protoplasts and chloroplasts of the cells disintegrated leaving shrunken cell walls. Following the death of the mesophyll cells, the hyphae penetrated the mesophyll cell walls and grew intracellularly (Figs. 17 and 19).

iv. Formation of stromata

Subcuticular mycelia continued to grow, forming a closely packed hyphal mat, the subcuticular stroma, on the inoculated side of the leaf. The stroma varied in thickness from one to several layers of cells in any one infected area and it gradually separated the cuticle from the epidermis (Fig. 17).

Hyphae grew from the inoculated sides of leaves, through the mesophyll, and developed substomatal stromata in the cavities beneath stomata.

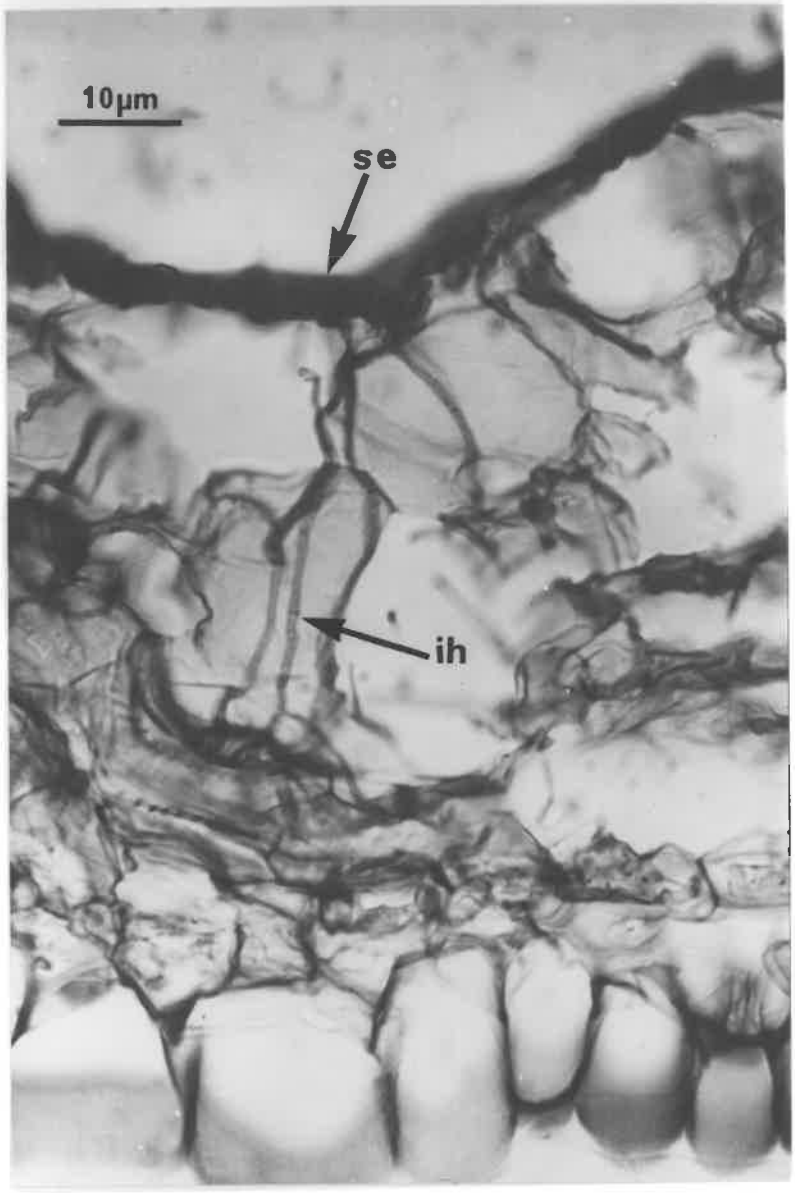
v. Sporulation

Production of conidia began while the mycelium was still

Figure 19.

Transverse section of infected leaf showing intracellular hyphae. ih, intracellular hypha; se, stroma on collapsed epidermis.

FIG·19



confined to the subcutivular zone. Sessile conidia were formed on short hyphal cells, each cell producing one or several conidia (Fig. 20 A and B). Mature conidia became erect and protruded through the cuticle (Fig. 20B). After a build-up of conidia, the cuticle cracked in various places while remaining intact in others (Fig. 20 B). In the presence of free water, each lesion produced a large number of conidia (Fig. 20 C). The substomatal stromata also produced conidia, which were extruded through the stomatal pores (Fig. 21 A and B).

Figure 20.

A.- Sessile conidia (c) on subcuticular hyphae (s) produced 10 days after inoculation.

B.- Subcuticular stroma (ss) producing conidia. Subcuticular conidia (sc) stain lightly; protruding conidia (pc) stain heavily.

C.- Portion of subcuticular stroma producing many conidia in the presence of free water.

FIG-20

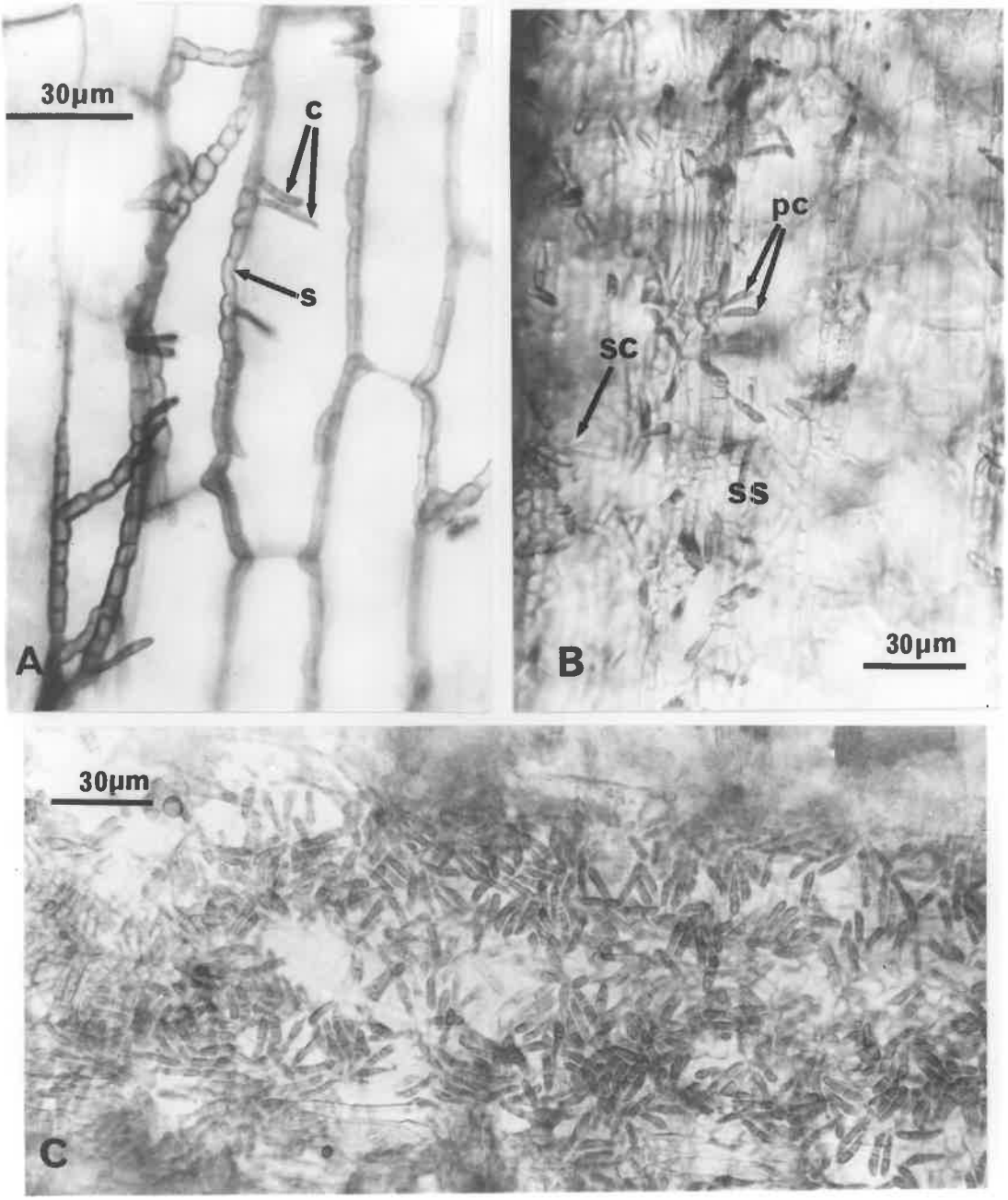
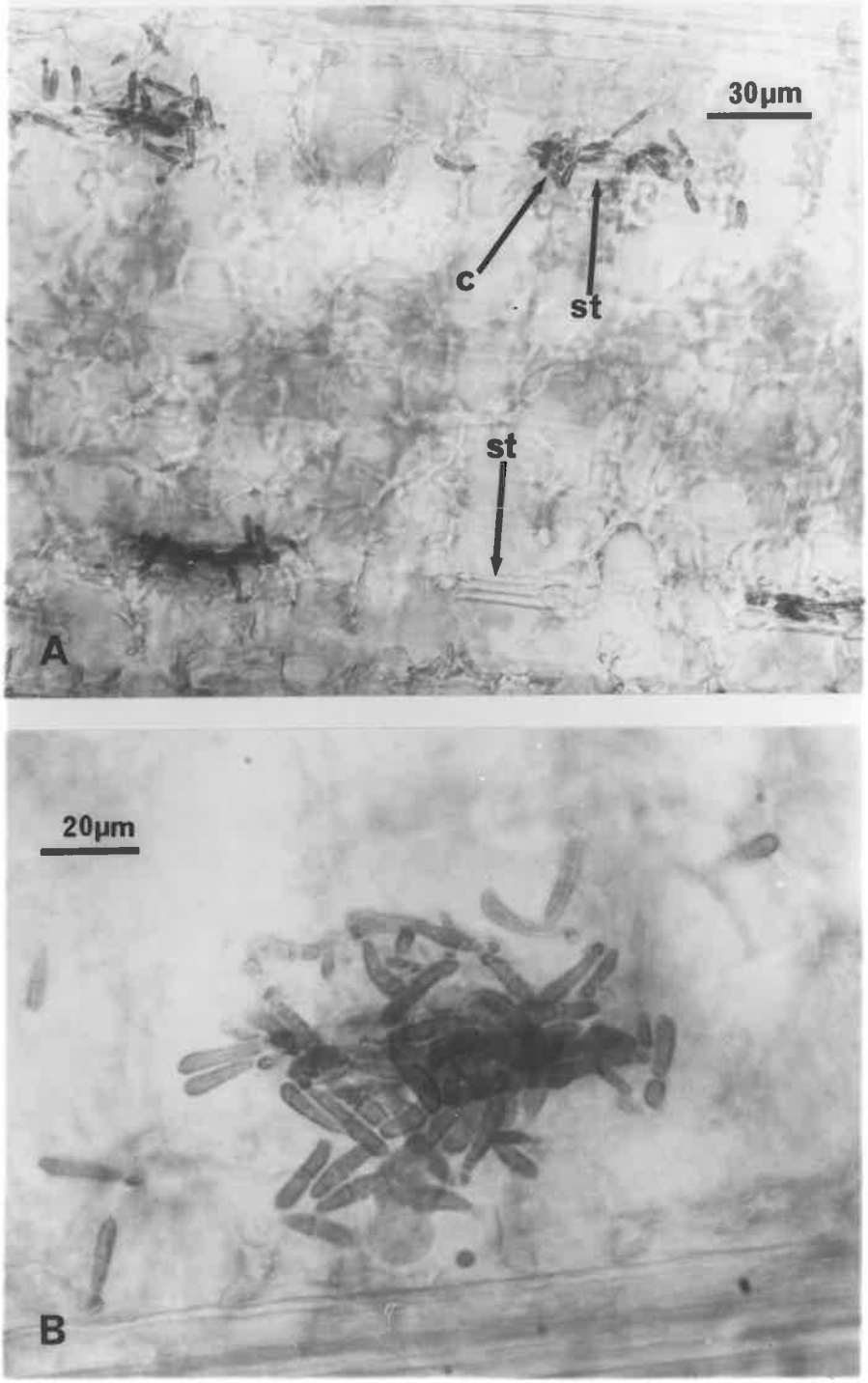


Figure 21.

A.- Conidia (c) produced by the substomatal stromata, being extruded through stomata (st).

B.- Many conidia produced by the substomatal stroma being extruded through a stoma.

FIG. 21



3. R. SECALIS TOXIN(S) AND THEIR ROLE IN SYMPTOM EXPRESSION

a) Introduction

Scald lesions on naturally or artificially infected leaves of barley begin as grey water-soaked patches. Some lesions become dry at this stage and retain a grey-green colour which persists even when the uninfected parts of the leaf senesce and become straw coloured (Fig. 22 A). More usually, the water-soaked patches develop into typical lesions with pale brown or white centres surrounded by dark brown margins. Also, small dark brown hypersensitive patches frequently appear on barley leaves where infection has been unsuccessful (Fig. 23 A). Preliminary experiments showed that lesion development is associated with increased respiration and decreased water content of leaves.

In lesions showing the initial grey, water-soaked symptoms, mesophyll and epidermal cells had collapsed (Fig. 16, p. 70). At this stage, hyphal growth was scanty and confined between the cuticle and the outer cell walls of the epidermis. It appeared that toxic substance(s) from the fungal hyphae were responsible for the collapse of the host cells.

The experiments described in this section report the

Figure 22.

A.- Artificially inoculated leaves of barley cultivar, Clipper, showing grey-green colouration (t), 10 days after inoculation.

B.- Leaves of Clipper, 24 hours after seedlings had been inserted into tubes containing sterile culture filtrate; t, affected part of the leaf.

C.- Leaves of wheat, 24 hours after seedlings had been inserted into tubes containing sterile culture filtrate; t_1 and t_2, affected parts of the leaf.

FIG·22

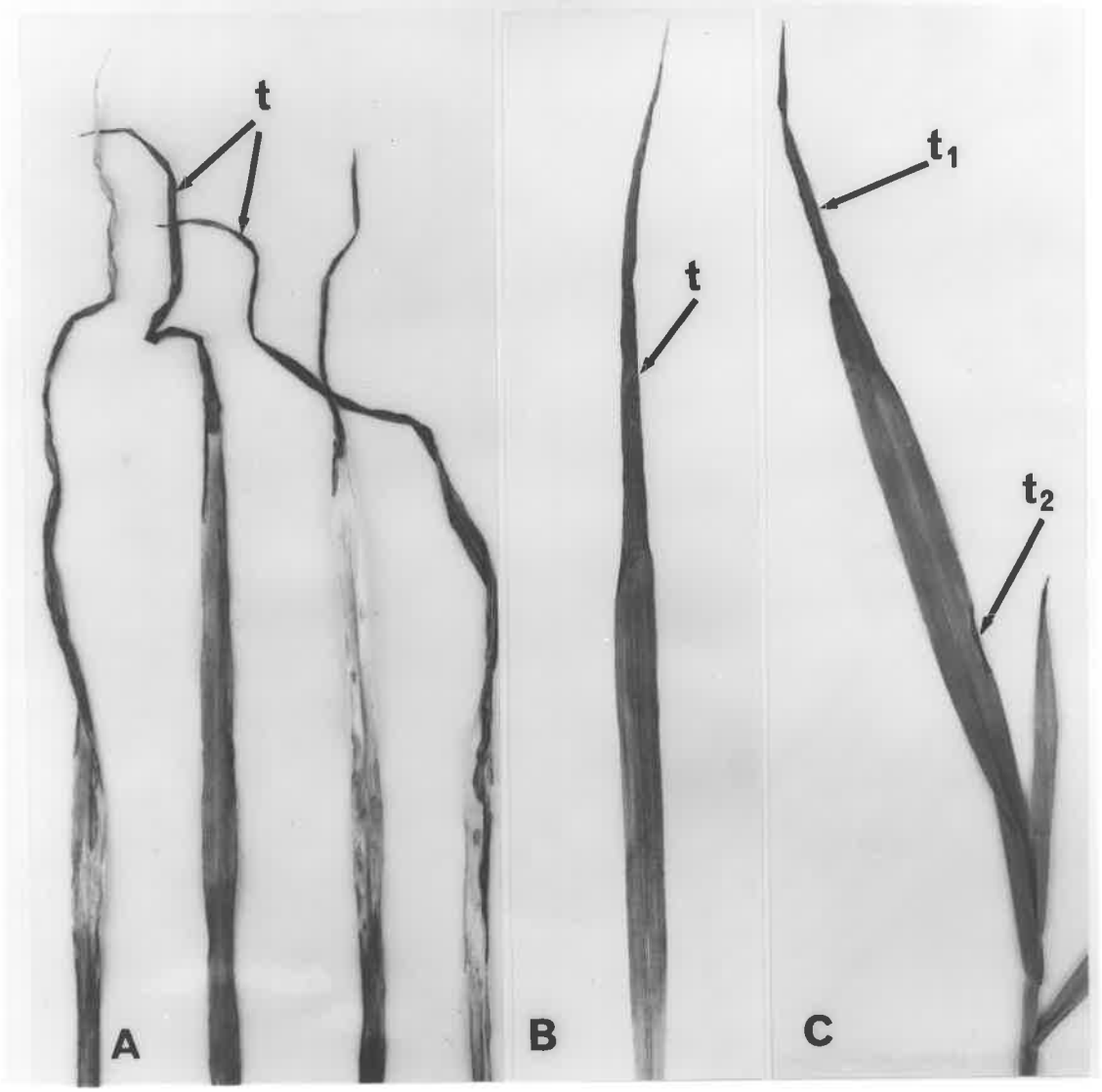


Figure 23.

A.- Naturally infected leaf of barley. Arrow points to brown hypersensitive spots.

B.- Leaves of barley cultivar, Clipper, showing brown spotting 5 days after being sprayed with sterile culture filtrate.

FIG-23

production of scald disease symptoms on leaves of barley, oats, wheat and Dactylis glomerata when they were treated with culture filtrates of R. secalis.

b) Materials and Methods

i. Preparation of culture filtrates

Clipper barley was inoculated with R. secalis isolate SA-1 and the fungus was re-isolated and grown on 1% PSP agar at 15°C. Each colony of about 3 cm diameter was macerated and used to inoculate 50 ml MY or PSP liquid medium and incubated without agitation, at 15°C, in the dark for 14 days. The cultures were then thoroughly strained through a double layer of muslin, and centrifuged at 110,000 g for 1 hour at 15°C. The supernatant was sterilized by passage through a membrane filter at 0.5 μm pore size. Uninoculated MY liquid media were sterilized in the same way.

ii. Treatment of seedlings with culture filtrates

Test plants were barley cultivars (West China; Atlas 46; Tennessee Winter; Nigrinudum; La Mesita; Osiris; CI. 3576; Turk; CPI. 18197; Prior; Bussell; Clipper), wheat (Heron), oats (Scotch Grey) and D. glomerata L. (Currie). Of these, only barley is susceptible to infection by R. secalis.

Clipper barley seeds were surface sterilized with 5.5% sodium hypochlorite solution and placed aseptically on 1% PSP agar in large test tubes (20 x 5 cm) which were placed in a growth cabinet at 25°C and 12 hr daily illumination at 1,000 f.c. The other test plants were grown in pots in a glasshouse at 20-25°C. When seedlings had reached the 3-leaf stage, the stems were cut 1 cm below the lower-most leaf and were immersed in 20 ml portions of either sterile culture filtrate or sterile MY or PSP liquid medium. In addition, Clipper barley seedlings were cut and immersed in autoclaved (20 min at 15 lb/in^2) culture filtrate or dialysed (in distilled water for 16 hours at 5°C) culture filtrate.

Clipper barley seedlings were grown in pots in a glasshouse until they reached the 3-leaf stage and were then sprayed with culture filtrate or liquid MY medium. The sprayed plants were placed in a humid chamber for 48 hr at 15°C and illuminated for 12 hr a day at 1,000 f.c. They were then placed in a glasshouse at 20-25°C.

iii. Sectioning of leaves

Sections were prepared from inoculated Clipper barley leaves which had dark brown hypersensitive spots, from those which had lesions with dark brown margins and from uninoculated leaves which had developed dark brown spots after being sprayed with culture

filtrates. Sections were also made from leaves showing scald symptoms and from seedlings whose stems had been immersed in culture filtrates.

Pieces 0.5 cm^2 were cut from the leaves, cleared in formalin-acetic-alcohol, sectioned with a freezing microtome and stained with cotton-blue lactophenol.

iv. Measurement of respiration

Respiration measurements were made with (i) healthy leaves, (ii) leaves inoculated by spraying with spore suspensions (1.5-2.0 x 10^5/ml) or the smear method, (iii) leaves from seedlings whose stems had been immersed in either sterile culture filtrate or sterile MY liquid medium for up to 26 hours as described above in (ii).

Clipper barley seedlings at the 3-6 leaf stage grown at 20-25°C, were used in these experiments. Inoculated and uninoculated plants were placed in a humid chamber for 48 hours and then in a glasshouse, as described above in (ii). Samples were taken from the distal halves of the 2nd leaves between 7 and 13 days after inoculation.

Measurements of oxygen uptake were made at 30°C in a Warburg respirometer using 300 mg (fresh weight) of leaf pieces (0.5 cm^2) and 3 ml of 0.1 M KH_2PO_4 in each flask. The centre well contained

0.2 ml of 10% NaOH. The flasks were covered with black cloth to prevent photosynthesis and, after one hour's equilibration, measurements were made at 5 min intervals for 50 min on triplicate samples of leaf tissue. The leaf tissue was then removed and dry weights of the samples were determined. Respiration rates were calculated on a dry weight basis.

c) Results

When cut stems of Clipper barley seedlings were immersed in sterile culture filtrates, the leaves developed grey water-soaked patches similar to those produced on infected leaves. These patches which often appeared within one hour of treatment with culture filtrate, gradually coalesced to form large, dry, grey-green areas, usually towards the leaf tips (Fig. 22 B), which retained their colour when the rest of the leaf had dried to a straw-colour. Similar lesions also developed on infected plants. These effects were not produced in seedlings treated with sterile, uninoculated MY or PSP liquid medium. The effectiveness of sterile culture filtrates was not reduced by dialysis or by aut claving. The other test plants reacted in a manner similar to that of Clipper barley when treated with sterile culture filtrates and sterile culture media (Fig. 22 C).

When the affected parts of leaves treated with culture

filtrates were examined microscopically it was found that the mesophyll and the anticlinal walls of the epidermis had both collapsed so that the outer and inner periclinal walls were in contact (Fig. 24). This damage is similar to that observed in infected leaves in the initial stages of infection (Fig. 15 B, p. 69).

When Clipper barley was sprayed with a sterile culture filtrate, brown spots appeared on the leaves three days later (Fig. 23 B). Plants sprayed with uninoculated culture medium did not react in this way. The intensity of the colour gradually increased and the margins of the spots became necrotic. When these spots were examined microscopically it was found that the dark pigment was confined to epidermal cells which had collapsed (Fig. 25 A). This was also true of the darkened margins of lesions on infected plants (Fig. 25 B) and the dark brown patches on leaves which reacted hypersensitively when inoculated.

The respiration rates of Clipper barley leaves varied markedly depending upon the stage of seedling development. However, there was a consistent increase in the rate of respiration of Clipper barley leaves, showing symptoms of infection, until the lesions began to bleach and lose appreciable amounts of water at which stage respiration rates fell below those of healthy leaves. Table 16 shows the water content and respiration rates of healthy

Figure 24.

Transverse section of leaf of barley cultivar, Clipper, 24 hr after treatment with toxin, showing stages in the collapse of mesophyll cells and anticlinal cell walls of the epidermis; aw, collapsing anticlinal cell wall; pw, periclinal cell wall; opw, outer periclinal cell wall; m, collapsed mesophyll cells.

FIG·24

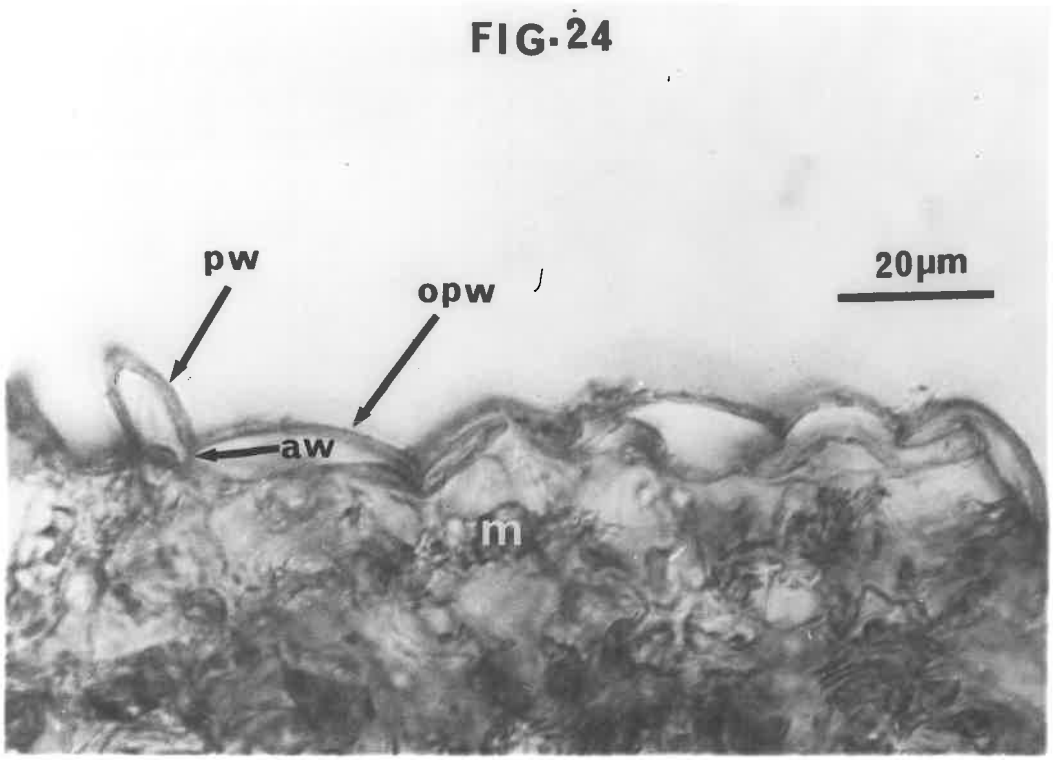
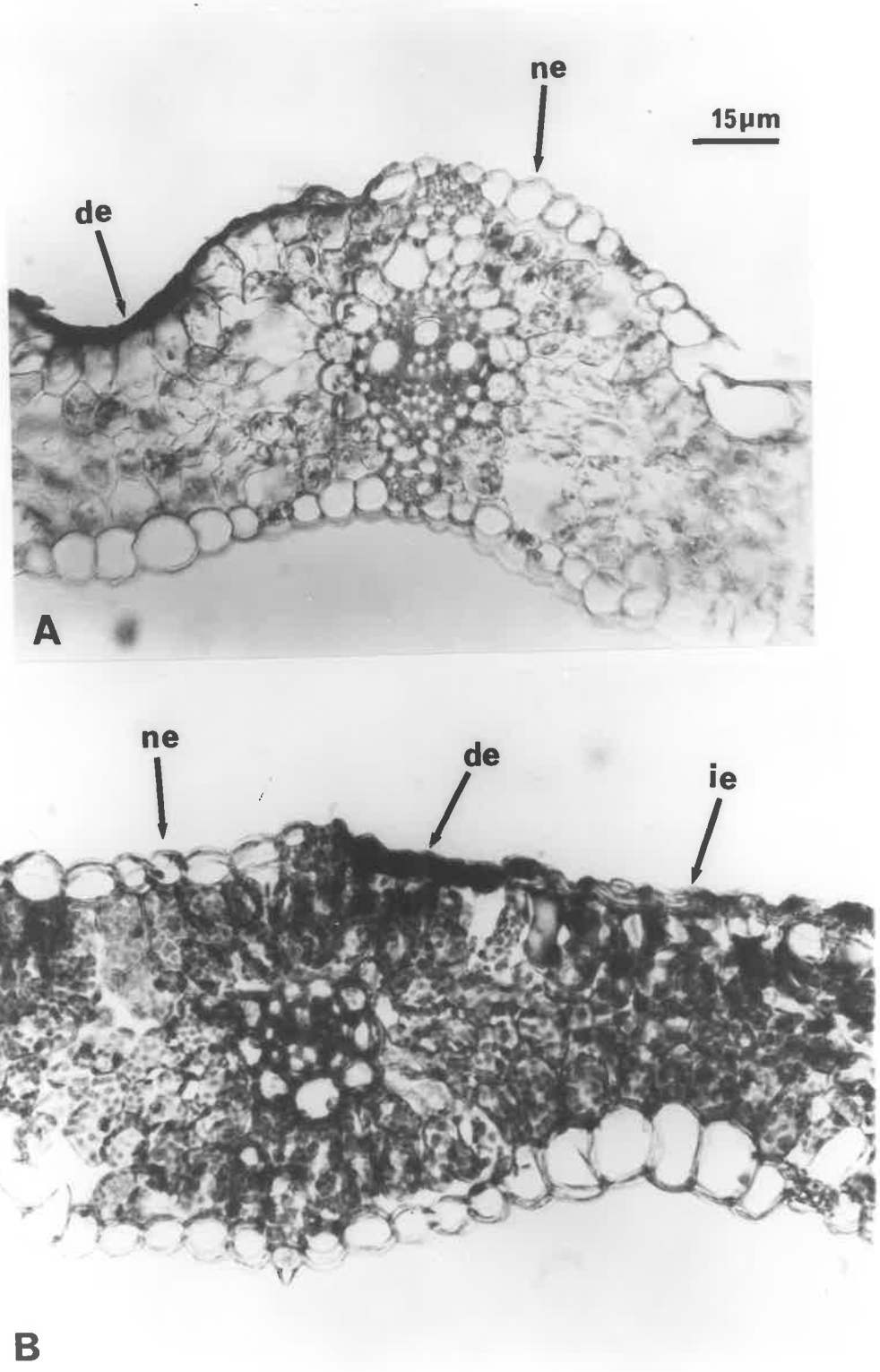


Figure 25.

A.- Transverse section of leaf of barley cultivar, Clipper, cleared with F.A.A. and stained with cotton blue lactophenol, showing dark brown pigments in the epidermis of a leaf which had been sprayed with sterile culture filtrate 5 days previously; de, dark brown and collapsed epidermis; ne, normal epidermis.

B.- Transverse section of R. secalis lesion on leaf of barley cultivar, Clipper, cleared in F.A.A. and stained with cotton blue lactophenol; ie, infected and collapsed epidermis; de, dark brown and collapsed epidermis; ne, normal epidermis.

FIG-25



and inoculated leaves.

The respiration rates of leaves treated with sterile culture filtrates were also higher than those of untreated leaves or of leaves treated with sterile, uninoculated MY liquid medium. These increases began between the second and fifty hours of the treatment period and were maintained until wilting became severe (Table 17). The respiratory increases were not as great as those in infected leaves. This was probably due to the fact that symptoms were not apparent in the early stages of treatment, and the samples used would have contained a mixture of affected and unaffected tissue. In addition, the metabolic effects of culture filtrates on leaf tissue are so rapid that it is probable that respiratory stimulation would soon be lost as cells became metabolically disorganised.

Table 16

Respiration of leaves* of barley cultivar, Clipper, infected by R. secalis

Time after inoculation (days)	Symptoms	Water content (expressed as % of that of healthy leaves)	Respiration rate (expressed as % of that of healthy leaves)
7.0	Slight greying	100	158
7.5	Slight wilting	100	174
8.0	Lesions yellowing	96	168
13.0	Lesions dry and bleached	68	15

* Plant inoculated at the 3-leaf stage.

Table 17

Respiration of leaves* of barley cultivar, Clipper, treated with *R. secalis* culture filtrate/ or with uninoculated MY liquid medium/

Treatment	Time of treatment (hr)	Symptoms	Water content (expressed as % of that of healthy leaves)	Respiration rate (expressed as % of that of healthy leaves)
Culture filtrate	2	0	100	110
Medium	2	0	100	111
Culture filtrate	5	0	98	132
Medium	5	0	100	112
Culture filtrate	7	Slight greying	97	121
Medium	7	0	100	109
Culture filtrate	26	Grey and wilting	90	103
Medium	26	0	100	92

* Seedling at 4-leaf stage.

/ Malt-yeast liquid medium in both cases.

4. EPIDEMIOLOGY OF LEAF SCALD OF BARLEY

a) Introduction

Leaf scald of barley is widespread throughout the barley growing areas of South Australia. The disease first appears on self-sown barley, which is often severely infected, in late June. Barley grass, a possible source of primary inoculum for infection of barley is widely distributed in the state. It grows on way-sides, on the margins of cultivated land, on waste land, in gardens, around houses and also along some of the coasts. Barley grass growing in all these places is almost always infected.

Cultivated barley is generally sown in July and August, by which time the disease is established on self-sown barley and barley grass. Very few barley fields seem to escape scald infection despite the practice of crop rotation. The disease persists throughout the growing season.

Quantitative studies were made of the pattern of spread of the disease and of the abundance of air-borne conidia under field conditions.

b) Materials and Methods

i. Pattern of scald development

Clipper barley was sown in rows running north and south in a field plot measuring 18 m. x 38 m. at the Waite Institute on

June 12, 1969. Barley had not been grown previously in this field and the nearest barley crops were about 900 metres away. Plants from a random sample of the seed, sown in pots and kept at 10°, 15° and 20°C in growth cabinets, were free from scald infection.

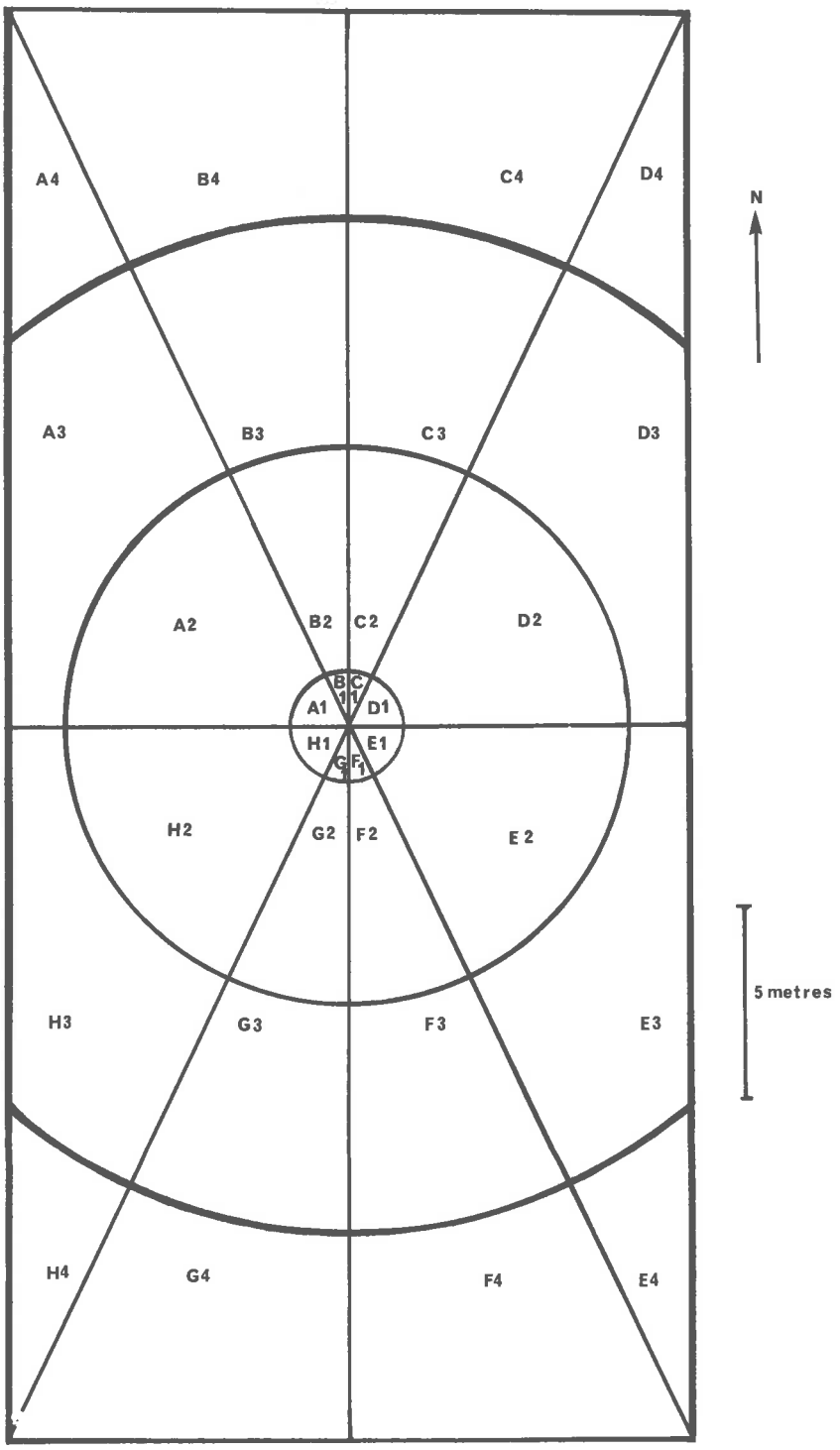
A day after the seed was sown, barley straw naturally infected with R. secalis in the previous year and stored dry in the laboratory, was scattered over a circular area, 3 m. in diameter, at the centre of the plot. A thin layer of soil was spread over the straw to prevent it from being blown away.

In October and November, a sprinkler mounted 30 cm. above crop level near the centre of the plot, was operated intermittently. This scattered water drops over an area about 12 m. in diameter.

For the purpose of disease assessment, the plot was divided into eight sectors each of which was subdivided into four subsectors by a series of concentric circles marked by stakes (Fig. 26). The innermost circle marked the area into which the pathogen was introduced.

After seedling emergence, plants were examined weekly for scald and the position of the infected plant most distant from the centre of the plot was recorded in each sector. Between July 28 and September 8, the percentage of plants infected in each subsector was estimated at fortnightly intervals by examining all plants at three sites, along a radius, each 30 cm. x 30 cm. The

FIG. 26



GENERAL PLAN OF EXPERIMENTAL PLOT

average number of lesions per leaf in each subsector was also recorded.

In the final detailed assessment of disease on November 1, when the plants were at the heading stage, 20 plants were taken at random from each subsector. Assessment was based on the following:-

(i) percentage of all leaves infected in each subsector,

(ii) percentage of flag leaves infected in each subsector,

(iii) percentage of second leaves infected in each subsector,

(iv) average number of lesions per leaf in each subsector.

Sheep grazed the plot at times during summer and the straw was broken up and partly consumed by the animals. The land was ploughed and cultivated in early May, 1970 and resown with barley on May 12. Disease development was assessed in mid-June.

ii. Air-borne conidia

A Burkard spore trap, with the orifice 60 cm. above ground level, was installed at the centre of the plot. The air spora was sampled continuously at 0.6 m^3 per hr (10 l per min.) from June 17 to November 24, 1969. The Melinex strip coated with petroleum jelly was changed weekly, the strips were cut into daily sections, mounted unstained in lactophenol-gelvatol, and examined at a magnification of 240x. The whole area of deposit for each day was examined, and the conidia deposited in each hour were counted.

Wind run at 1.25 m. above ground level, rainfall and temperature were recorded at the meteorological station, about 600 metres away. Daily wind run data were recorded as Km per day. Between October 27 and November 11, an estimate of the wind speed in each hour obtained from anemographs was assigned to one of four categories: calm, low, moderate or strong.

iii. Estimation of number of conidia produced in lesions

Barley plants naturally infected with R. secalis were transplanted from the field into pots and were kept in a glasshouse for 4 days before being used. Groups of 2 or 3 mature lesions on separate leaves were marked. Conidia from each group of lesions were washed into the glass funnell of a "Millipore" filter holder, containing a membrane of 8 μm pore size, with a dilute (0.1%) detergent solution and the lesions then rinsed in distilled water. After filtration under suction, the conidia were rinsed in distilled water, and stained by placing the wet membrane on a filter paper moistened with phloxine solution. The membrane was then dried at 40°C for 30 min. and mounted in immersion oil for microscopic examination. Conidia in the small squares at the four corners and centre of an eyepiece micrometer grid were counted at a magnification of 240x for each of 20 fields selected at random and the total number deposited on the membrane was estimated.

After the initial washing, plants with three separate groups of lesions were dried and placed under each of the following

conditions:-

(a) in a dew chamber, at 15°C, with mist injection for 3 seconds every 5 minutes,

(b) in a growth room at 15°C and a relative humidity of 85-95%.

After 48 hours, the lesions were again washed as already described. After drying, all the plants were immediately placed in the dew chamber with mist injection. The lesions were washed again after 48 hours.

iv. Wind tunnel studies

Forty eight barley seedlings with mature scald lesions, contained in 6 pots, were kept wet in a dew chamber at 15°C with mist injection as described above. After 48 hours, the plants were transferred to the upwind end of the working section of the wind tunnel (Carter, 1965) and spaced equidistant from the axis. An impactor was placed 3 m. downwind. The tests were conducted at six wind speeds (2, 3, 4, 5, 6 and 7 m/sec.) each for 3 min., during which time the impactor was operated at a suction rate of 10.0 l/min. The slide was changed at the end of each 3-min. test and examined under the microscope for R. secalis conidia. After the wind tunnel tests, three lesions were selected at random, washed and the conidia were stained and counted as described in (iii).

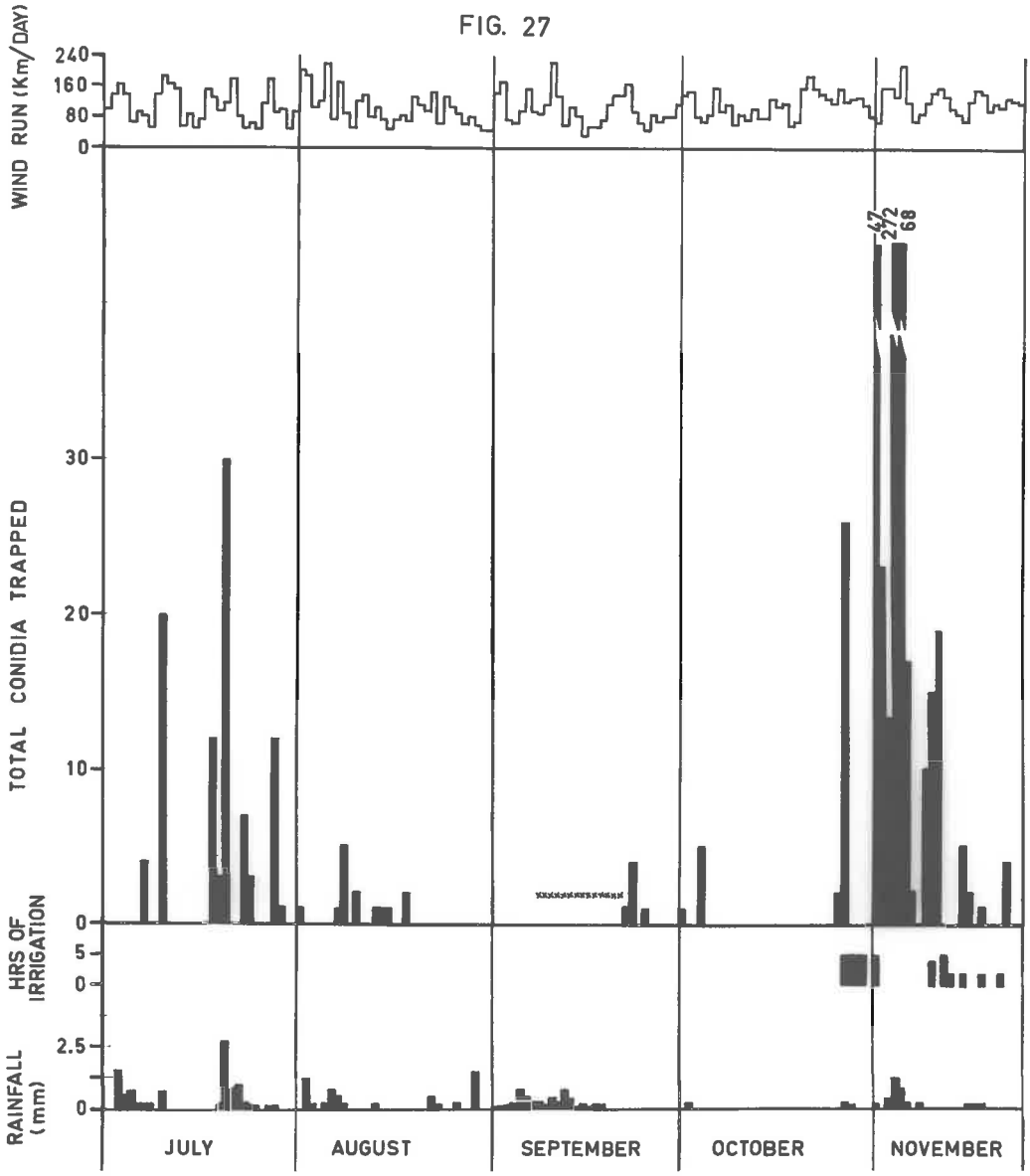
c) Results

i. Pattern of scald development

During the winter and early spring of 1969, frequent rain showers (Fig. 27) and mild temperatures [average maximum and minimum temperatures for the period were 64°F (18°C) and 50°F (10°C) respectively] provided ideal conditions for the development of scald. Lesions were first detected on a plant in the inner circle on June 29, 1969, 12 days after emergence of the seedlings. During the next two weeks the disease spread within the inner circle of the plot. Dates on which lesions were first detected outside the inner circle, and the maximum distance of an infected plant from the centre of the plot in each of the eight sectors, are recorded in Table 18. The percentages of plants infected, and the average numbers of lesions per leaf, in each subsector at the four dates of assessment are also tabulated.

On July 28, scald lesions were found for the first time in the plot outside the inner circle. The greatest distance of an infected plant from the edge of the inner circle was 2 m. By August 4, the disease had spread to a plant (in subsector G3) at least 8.8 m. from the nearest plant known to be infected in the previous week. On August 11, infected plants were observed within all of the outer subsectors, the greatest distance of an infected

FIG. 27



TOTAL R. SECALIS CONIDIA TRAPPED PER DAY, AND DAILY RAINFALL AND WIND RUN DATA FOR THE FIELD PLOT, JULY 1– NOVEMBER 24 1969

× SPORE TRAP NOT OPERATING

TABLE 18

Spread of Rhynchosporium infection within the experimental plot

Sector	Maximum distance from plot centre at which infection was detected			Percentage of plants infected				Average number of lesions per leaf			
	July 28	Aug. 4	Aug. 11	July 28	Aug. 11	Aug. 25	Sept. 8	July 28	Aug. 11	Aug. 25	Sept. 8
A 1				5	90	100	100	3	4	4	6
2	2.9			2	50	65	100	2	3	3	5
3		9.3	13.0	0	2	38	100	-	1	1	3
4				0	<1	1	100	-	1	1	3
B 1				4	90	100	100	3	4	4	5
2	2.7			<1	31	78	100	1	3	3	3
3		9.9		0	<1	2	100	-	1	1	2
4			19.8	0	<1	1	100	-	1	1	1
C 1				2	80	100	100	1	4	4	4
2	1.8			<1	30	67	100	1	2	2	2
3		10.6		0	<1	28	100	-	1	1	1
4			16.8	0	<1	1	100	-	1	1	1
D 1				2	85	100	100	1	4	4	4
2	1.7	4.6		<1	25	86	100	1	2	3	2
3				0	<1	24	100	-	1	1	1
4			14.9	0	<1	1	100	-	1	1	1
E 1				2	87	100	100	1	4	4	6
2	3.5			<1	21	55	100	1	2	3	5
3		9.9		0	1	10	100	-	1	1	3
4			16.2	0	<1	1	100	-	1	1	2

TABLE 18 (continued)

Spread of *Rhynchosporium* infection within the experimental plot

Sector	Maximum distance from plot centre at which infection was detected			Percentage of plants infected				Average number of lesions per leaf			
	July 28	Aug. 4	Aug. 11	July 28	Aug. 11	Aug. 25	Sept. 8	July 28	Aug. 11	Aug. 25	Sept. 8
F 1				4	90	100	100	2	4	4	5
2	2.6			1	13	73	100	1	2	2	4
3		11.7		0	<1	4	100	-	1	1	3
4			16.8	0	<1	1	100	-	1	1	3
G 1				4	80	100	100	2	4	4	5
2	2.6			1	19	80	100	1	1	2	3
3		12.3		0	<1	2	100	-	1	1	2
4			18.3	0	<1	1	100	-	1	1	2
H 1				5	80	100	100	2	4	4	5
2	2.5			2	50	70	100	1	4	4	4
3		8.3		0	3	3	100	1	2	2	3
4			17.8	0	4	1	100	1	1	1	2

plant from the centre of the plot being 19.8 m. (subsector B4). The nearest known source of inoculum to the latter plant was at least 9 m. away (in subsector C3).

The number of lesions increased rapidly within the inner circle whereas it spread much more slowly outside it. On September 8, every plant examined on the plot was found to be infected but the intensity of the disease was least in the outer subsectors (Table 18). Results of final disease assessment, when the plants were at the heading stage, are recorded in Table 19.

In the second year seedlings were examined for infection at the three-leaf stage in mid-June. Scald lesions were observed on some plants over the whole plot.

ii. Air-borne conidia

R. secalis conidia were not detected in the spore trap until July 7, eight days after the first lesion was recorded.

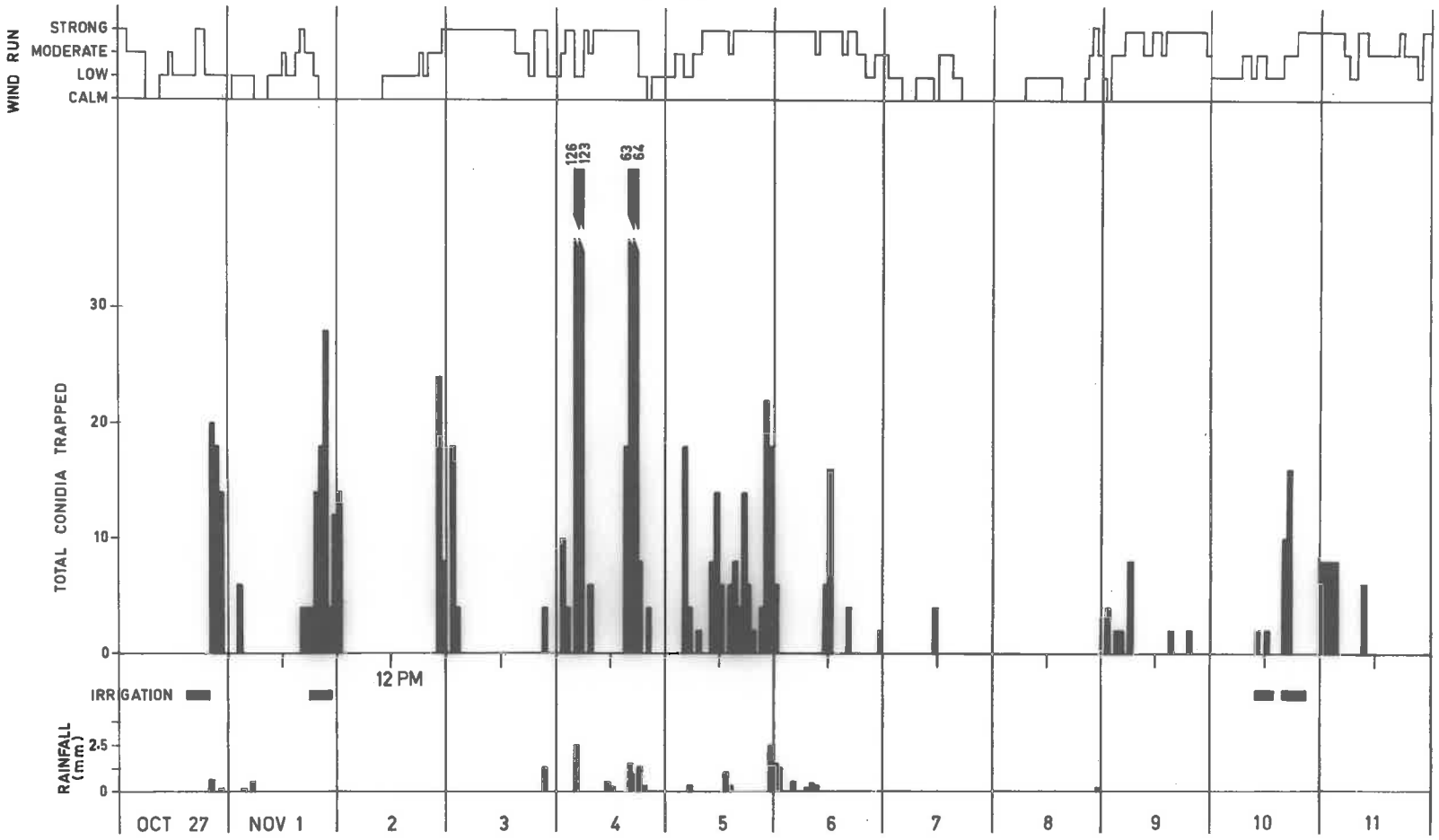
Generally, conidia were trapped during periods of rainfall or irrigation, or under windy but rainless conditions if the latter had been preceded by rain or irrigation (Figs. 27 and 28). The largest numbers were trapped during periods of rainfall (Figs. 27 and 28) and conidia were frequently in groups of two to ten. However, no conidia were trapped between August 22 and September 7

TABLE 19

Final disease assessment of scald infection made on November 1, 1969

Subsection	% of leaves (total) infected	% of flag leaves infected	% of second leaves infected	Average pattern of infection per leaf
A 1	82	27	95	More than 10 fused lesions
2	83	40	100	5 fused lesions
3 and 4	70	7	48	5 fused lesions
B 1	83	39	93	5 fused lesions
2	63	7	38	2 separate lesions
3 and 4	60	14	51	2 fused lesions
C 1	85	30	100	4 fused lesions
2	65	21	42	2 separate lesions
3 and 4	71	0	45	1 lesion
D 1	89	44	100	5 fused lesions
2	68	0	0	1 lesion
3 and 4	55	6	32	1 lesion
E 1	80	64	89	5-10 fused lesions
2	87	29	83	6 fused lesions
3 and 4	95	92	100	4 separate lesions
F 1	83	35	100	More than 10 fused lesions
2	66	18	67	3 separate lesions
3 and 4	90	39	68	More than 10 fused lesions
G 1	80	53	84	More than 10 fused lesions
2	68	12	57	5-10 fused lesions
3 and 4	60	10	37	5-10 fused lesions
H 1	80	52	100	More than 10 fused lesions
2	75	23	81	6 separate lesions
3 and 4	75	26	79	4 separate lesions

FIG. 28



HOURLY RECORDS OF CONIDIA TRAPPED, RAINFALL AND WIND SPEED ON SELECTED DAYS IN OCTOBER AND NOVEMBER 1969

(Fig. 27) although there were frequent rains. Conidia were caught at any time of the day or night but few were obtained at any one time.

iii. Estimation of number of conidia produced in lesions

The estimated numbers of conidia formed at 15°C in the dew chamber with mist injection are recorded in Table 20. No conidia were produced in lesions on plants kept dry in the growth room at 15°C and a relative humidity of 85-95%. On transferring these plants to a dew chamber at 15°C and with mist injection, each group of lesions produced an average of 1.3×10^6 conidia in 48 hours.

iv. Wind tunnel studies

No conidia were caught in the impactor at any of the wind speeds chosen for the tests. Each of the three lesions washed after the wind tunnel tests yielded conidia abundantly.

TABLE 20

Estimated numbers of conidia produced in a dew chamber at 15°C with mist injection

	Estimated number of conidia ($x10^6$)		
	Replicate 1	Replicate 2	Replicate 3
1st washing (conidia formed in field prior to removal of plants)	0.5	0.9	0.6
2nd washing (48 hr later)	1.1	1.7	1.0
3rd washing (48 hr later)	0.4	0.3	0.9

5. COMPARATIVE STUDIES OF PROCESSES IN THE INFECTION OF THREE CULTIVARS OF BARLEY BY R. SECALIS

a) Introduction

Studies on the relative resistance of barley cultivars to R. secalis isolates showed that Clipper was very susceptible to isolate SA-1, Atlas 46 was less susceptible, while Osiris was resistant.

The germination and growth of the fungus on the adaxial surfaces of leaves of the three cultivars at the 4-leaf stage was studied, using inoculum from 14-day old cultures of isolate SA-1 on 0.5% PSP agar and leaf-borne conidia from Clipper infected with this isolate.

b) Methods

i. Using the smear method to inoculate Clipper, Atlas 46 and Osiris

Marked areas at the middle portion of the second leaves of the seedlings were inoculated. The plants were sprayed with water and incubated in the usual manner at 15°C. After 48 hours, they were transferred to a glasshouse. At 4 and 8 days after inoculation, portions of three leaves of each cultivar were cut into small pieces

and cleared with a mixture of equal volumes of glacial acetic acid and absolute alcohol, stained with the periodic acid-Schiff reagent and mounted in dilute glycerine. Pieces cut from each inoculated spot were used together as individual replicates. Percentages of conidia that had germinated, percentages that formed appressoria (both appressoria sessile on conidia and appressoria at tips of germ tubes) and percentages that effected penetration were recorded. All counts were made from fields that contained thirty to sixty conidia.

ii. Using leaf-borne conidia to inoculate Clipper, Atlas 46 and Osiris

Leaves with scald lesions were thoroughly washed with tap water and floated on water at 10°C for 72 hours. Conidia produced on the lesions were used to inoculate marked leaves as described above in (i). The inoculated seedlings were sprayed with water and incubated for 48 hours at 15°C. Inoculated areas were cut into pieces, cleared and stained 2, 4, 6 and 8 days after inoculation. Examination of stained materials and assessments were also carried out as described above in (i). The percentage of conidia effecting penetration without superficial germ tubes or appressoria was also recorded. The germ tubes of 25 conidia were measured for each

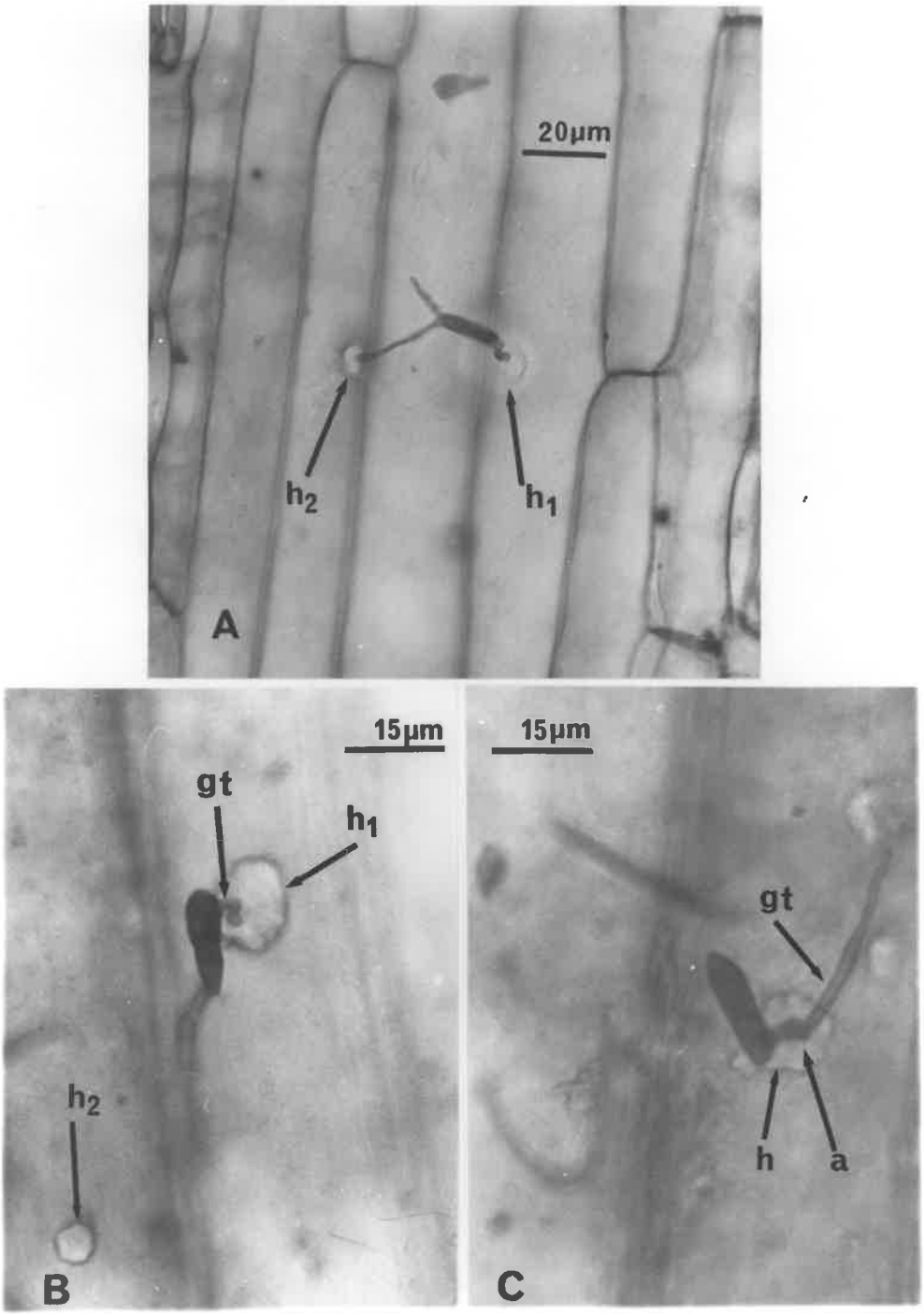
Figure 29.

A.- Initial stages in halo formation at positions (h_1 and h_2) in the cuticle of barley cultivar, Atlas 46, 4 days after inoculation.

B.- Halo (h_1) formed near the tip of a short germ tube (gt) in the cuticle of leaf of barley cultivar, Osiris, 4 days after inoculation. Note broadness of halo; h_2, halo after conidium had become detached.

C.- A germ tube (gt) growing from an appressorium (a) in the vicinity of halo (h) on leaf of barley cultivar, Osiris, 6 days after inoculation.

FIG-29



replicate.

iii. Using the spray method to inoculate Osiris

The second leaves of seedlings were marked and inoculated by the spray method. The plants were dried in a gentle current of air and sprayed with conidial suspension for a second time before being placed in a humid chamber for incubation at 15°C. They were removed to a glasshouse after 48 hours. Leaf pieces obtained from the middle portions of inoculated leaves were cleared, stained, mounted and examined as described in (i). In addition, the germ tubes of 25 conidia were measured for each replicate.

c) Results

Dark-staining haloes, observed on inoculated leaves of Clipper in previous studies, occurred on inoculated leaves of Atlas 46 and Osiris. In addition, other haloes which had not stained were present (Fig. 29). These occurred as depressions with definite edges and were found more frequently on Atlas 46 and Osiris than on Clipper. The haloes were formed as a result of gradual erosion of the cuticle (Fig. 29A, h_1 and h_2). They eventually became broad and often extended for about 15 μm from the germinated or ungerminated conidia which initiated their formation (Fig. 29B and C).

Where the cuticle had been degraded to the epidermal cell wall, the polysaccharide component of the cell wall reacted with the periodic acid-Schiff reagent to produce dark-staining complexes. Germinated or ungerminated conidia associated with unstained haloes did not form subcuticular hyphae. On the other hand superficial germ tubes often grew from appressoria in the vicinity of unstained haloes, crossing those haloes and growing entirely superficially on the cuticle (Fig. 29C).

The results for germination, appressorium formation, growth rate of germ tubes and frequency of penetration, based on the means of the various replicates, are summarised in Figs. 32-37. Detailed results are tabulated in Tables 21-28 (appendix).

Results obtained using all three methods of inoculation showed that the amount of germination and of appressorium formation, on all three barley cultivars, was extremely variable. Factors responsible for this variability in germination and appressorium formation were not apparent. Another complication in these experiments was that conidia were apparently washed from leaves of Atlas 46 and Osiris during the staining processes when the leaves had been inoculated more than four days previously (Fig. 32). Therefore it was not possible to determine whether host factors had any significant effects on germination and appressorium formation.

When leaf-borne conidia were used as inoculum, germination on all cultivars increased up to the 4th day after inoculation (Fig. 32), but on the 6th day the percentage germination decreased on Atlas 46 and Osiris. Germination on Osiris on the 8th day, reduced further but increased on Atlas 46. Germination on Clipper continued to increase up to the 6th day. No counts were made of germinated conidia on Clipper after the 6th day since subcuticular hyphae had grown profusely, making accurate counting of conidia impossible. The pattern of appressorium formation with time (Fig. 33), was similar to that of germination except that the values on Atlas 46 were the same on the 4th and 6th days.

When the smear and spray methods of inoculation were used, germination increased up to the 4th day on Clipper and to the 8th day on Atlas 46 and Osiris (Fig. 34). No counts were made on Clipper on the 8th day. Appressorium formation also showed a similar trend (Fig. 35) except that the percentage of appressoria formed on Osiris inoculated by the spray method was much less on the 8th day than on the 4th day.

Germ tubes grew to the same extent on the three cultivars inoculated with leaf-borne conidia (Fig. 36) but growth on Osiris inoculated by the spray method was significantly greater than that on the same cultivar inoculated with leaf-borne conidia.

In all the experiments, the percentage of conidia penetrating Clipper increased with time (Fig. 37); by the end of the 6th day after inoculation, the subcuticular hyphae had grown profusely, thus it was no longer possible to make further estimates of penetration. Subcuticular hyphae in Atlas 46 and Osiris grew to a maximum length of about 40 μm in 8 days and were generally unbranched. A significantly higher proportion of conidia effected penetration on Clipper than on Atlas 46 and Osiris (Fig. 37). There were no significant differences between the percentages of conidia that effected penetration on Atlas 46 and on Osiris. Penetration of the cuticle did not occur on leaves of Osiris inoculated by the spray method even though a high proportion of the conidia germinated and produced long germ tubes with appressoria (Figs. 30 and 36).

On all three cultivars, over 50% of the conidia that effected penetration did not produce superficial germ tubes (Fig. 31).

About 10 days after inoculation, conidia and germ tubes on all cultivars became moribund and many were detached from the leaf surfaces. At about the same time most of the subcuticular hyphae which formed in Atlas 46 and Osiris had become inactivated and shrunken.

Figure 30.

Germinating conidia on leaf of barley cultivar, Osiris, 8 days after inoculation by the spray method.

FIG·30

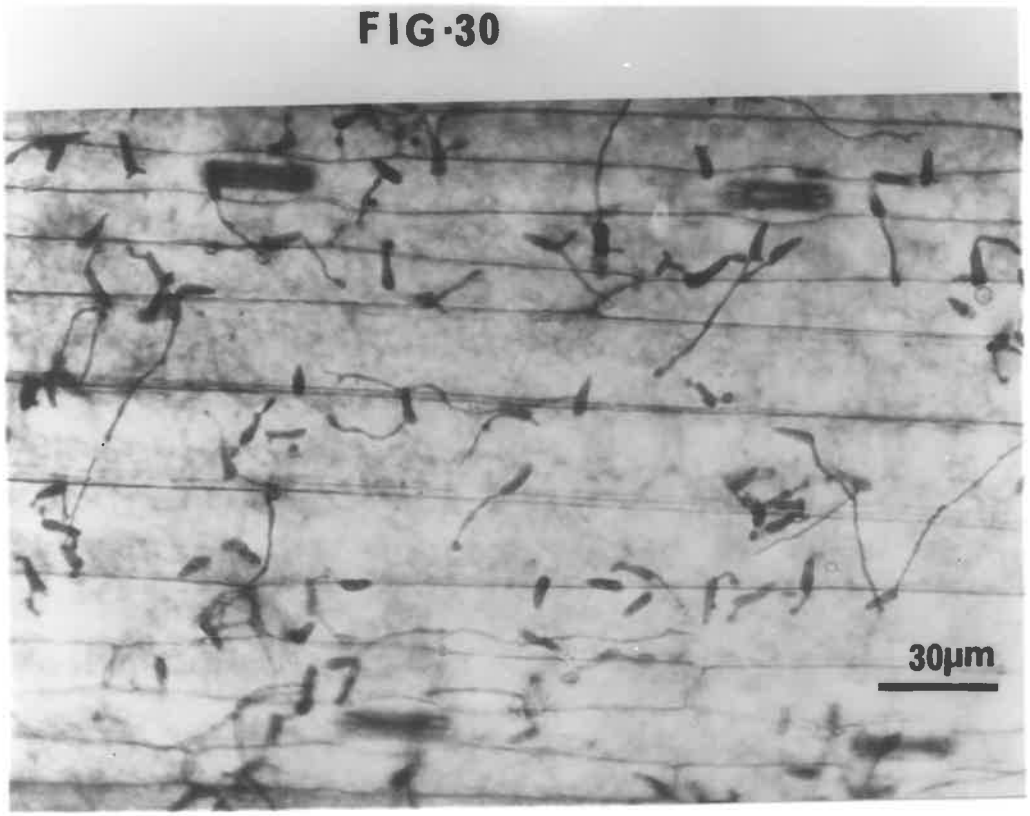


Figure 31.

A and B.- Conidia producing subcuticular hyphae without developing superficial germ tubes on barley cultivar, Clipper, 6 days after inoculation with leaf-borne conidia; s, subcuticular hypha; h, dark staining halo; c, conidium.

FIG·31

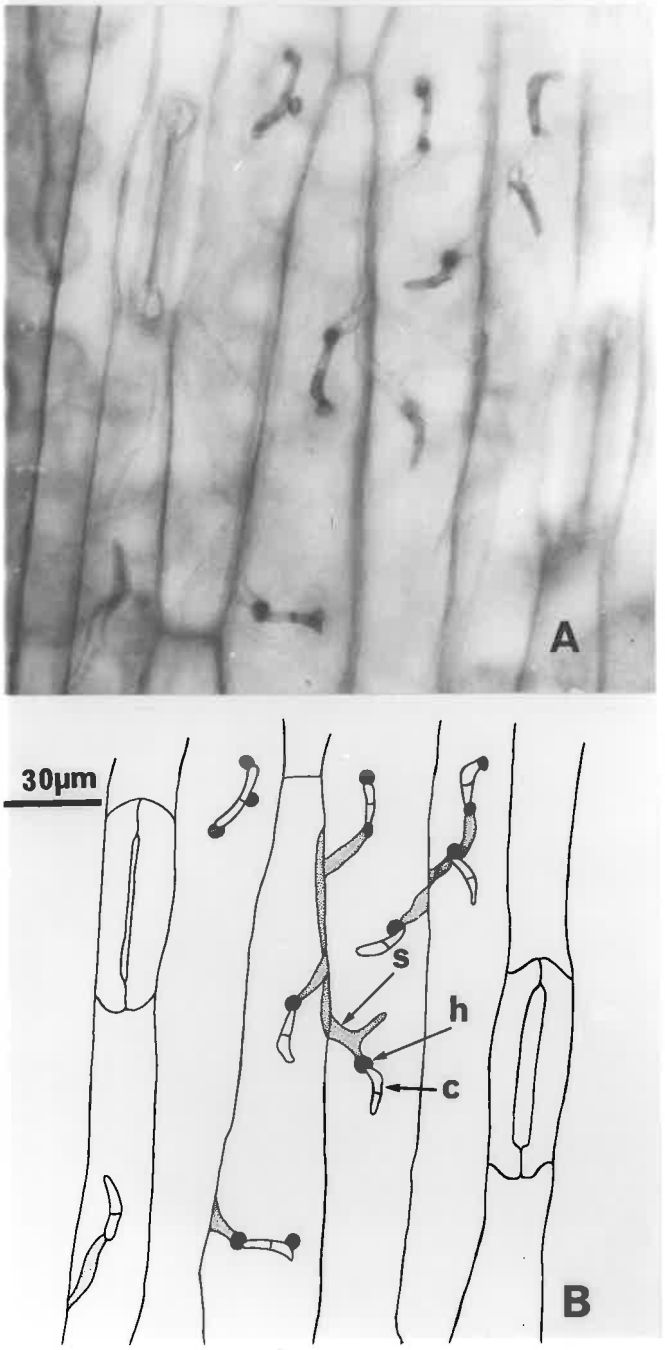
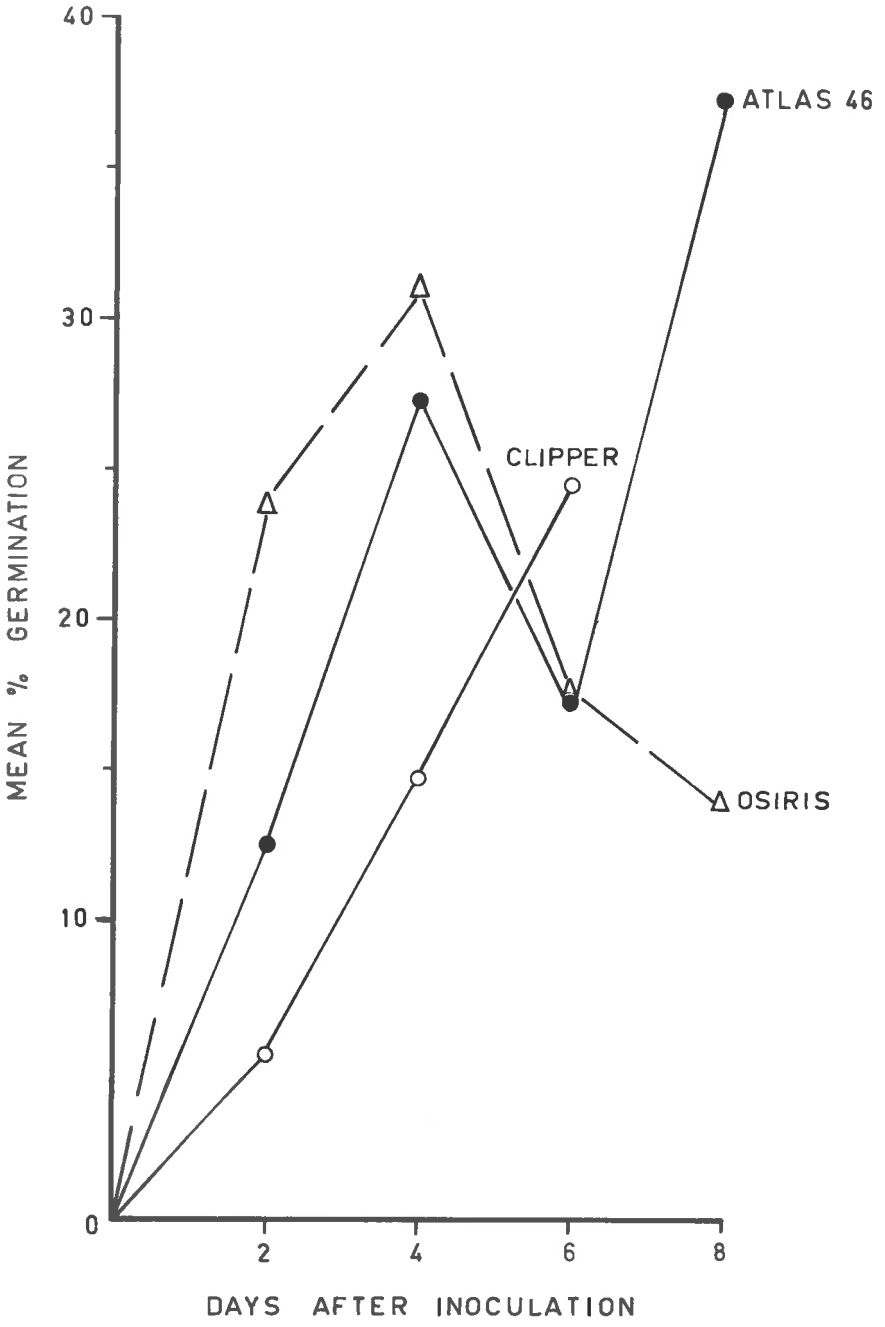
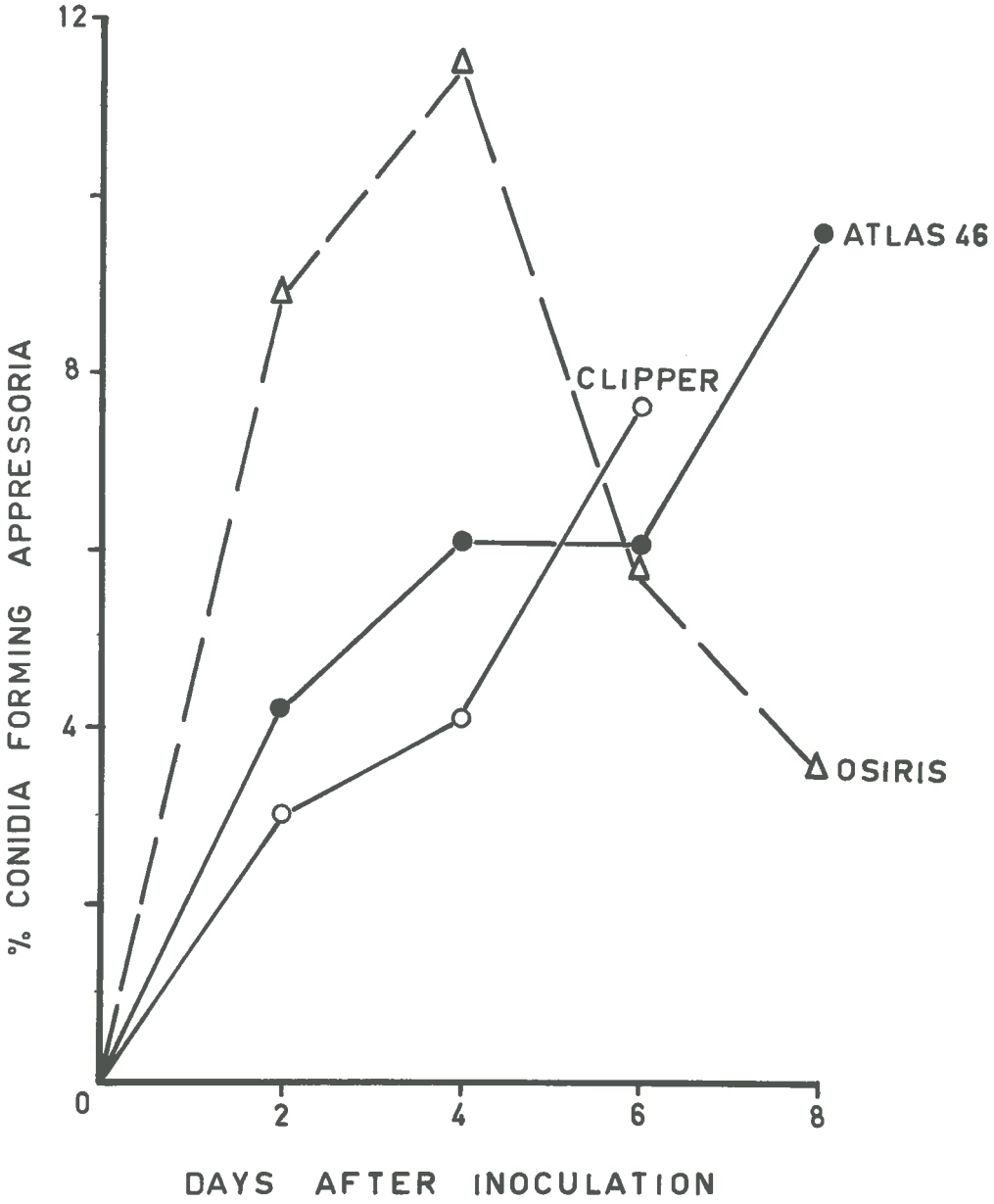


FIG. 32



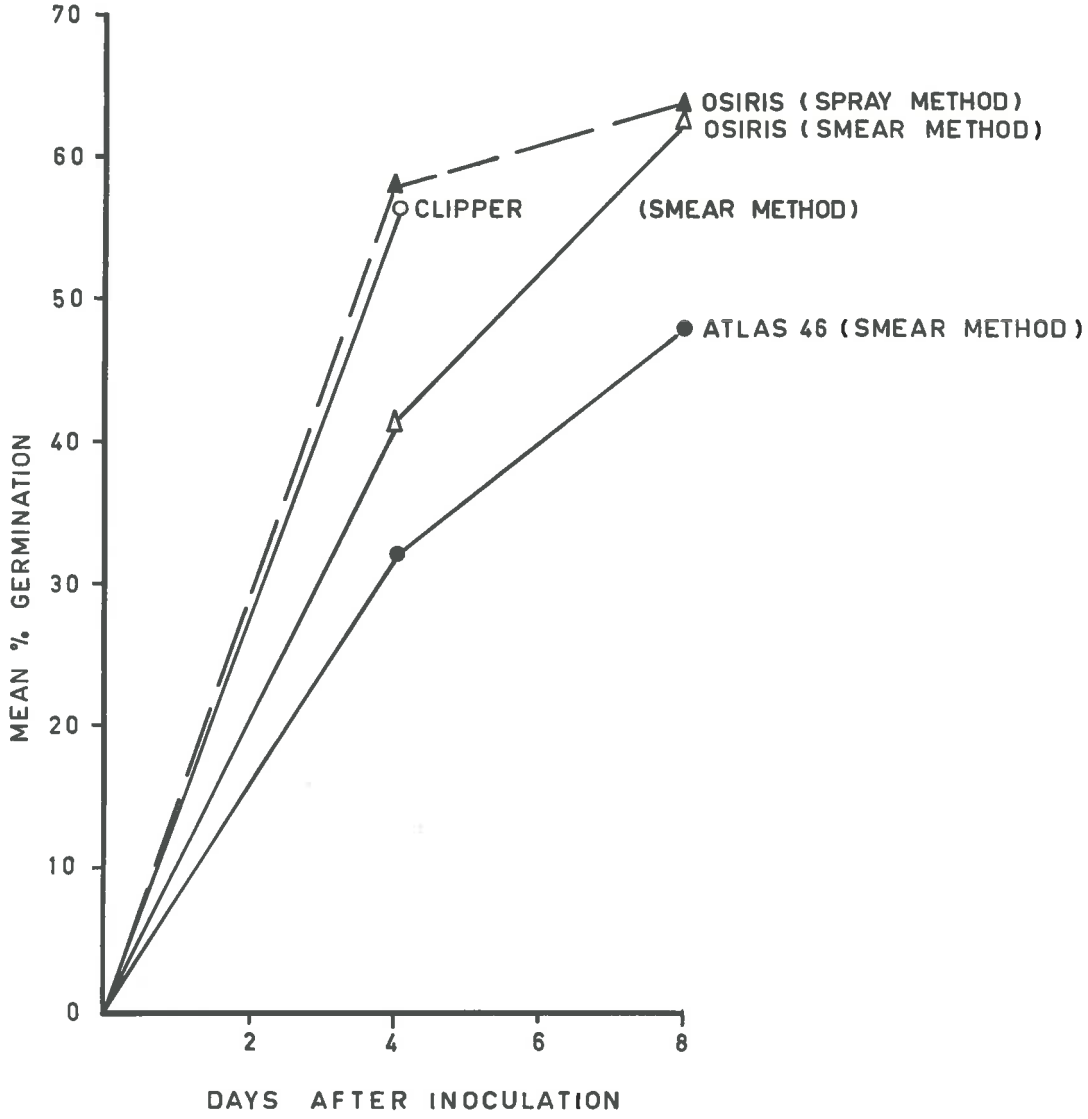
GERMINATION OF CONIDIA OF R. SECALIS ON LEAVES OF CLIPPER, ATLAS 46 AND OSIRIS, USING LEAF-BORNE CONIDIA AS INOCULUM.

FIG.33



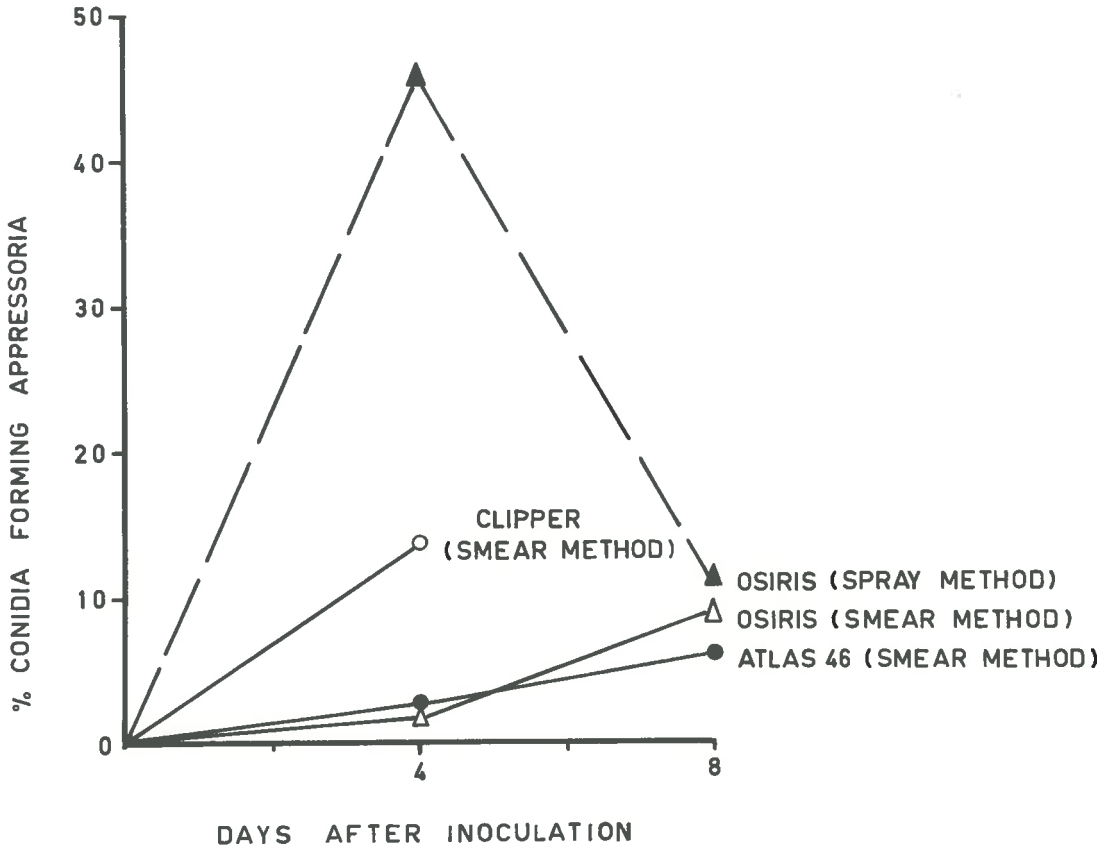
FORMATION OF APPRESSORIA ON LEAVES OF CLIPPER, ATLAS 46 AND OSIRIS INOCULATED WITH LEAF-BORNE CONIDIA.

FIG.34



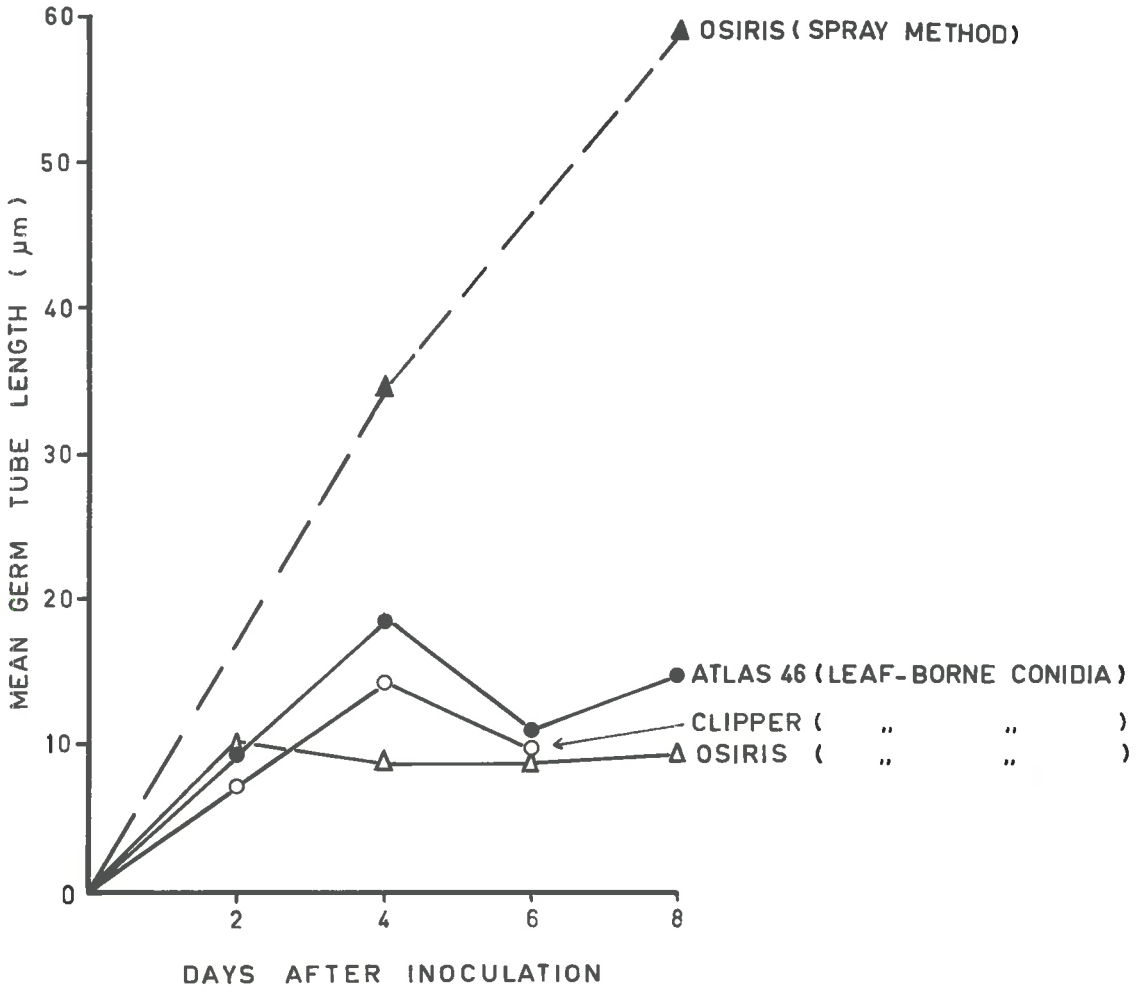
GERMINATION OF CONIDIA OF R. SECALIS ON LEAVES OF CLIPPER, ATLAS 46 AND OSIRIS USING CONIDIA DERIVED FROM PSP AGAR.

FIG.35



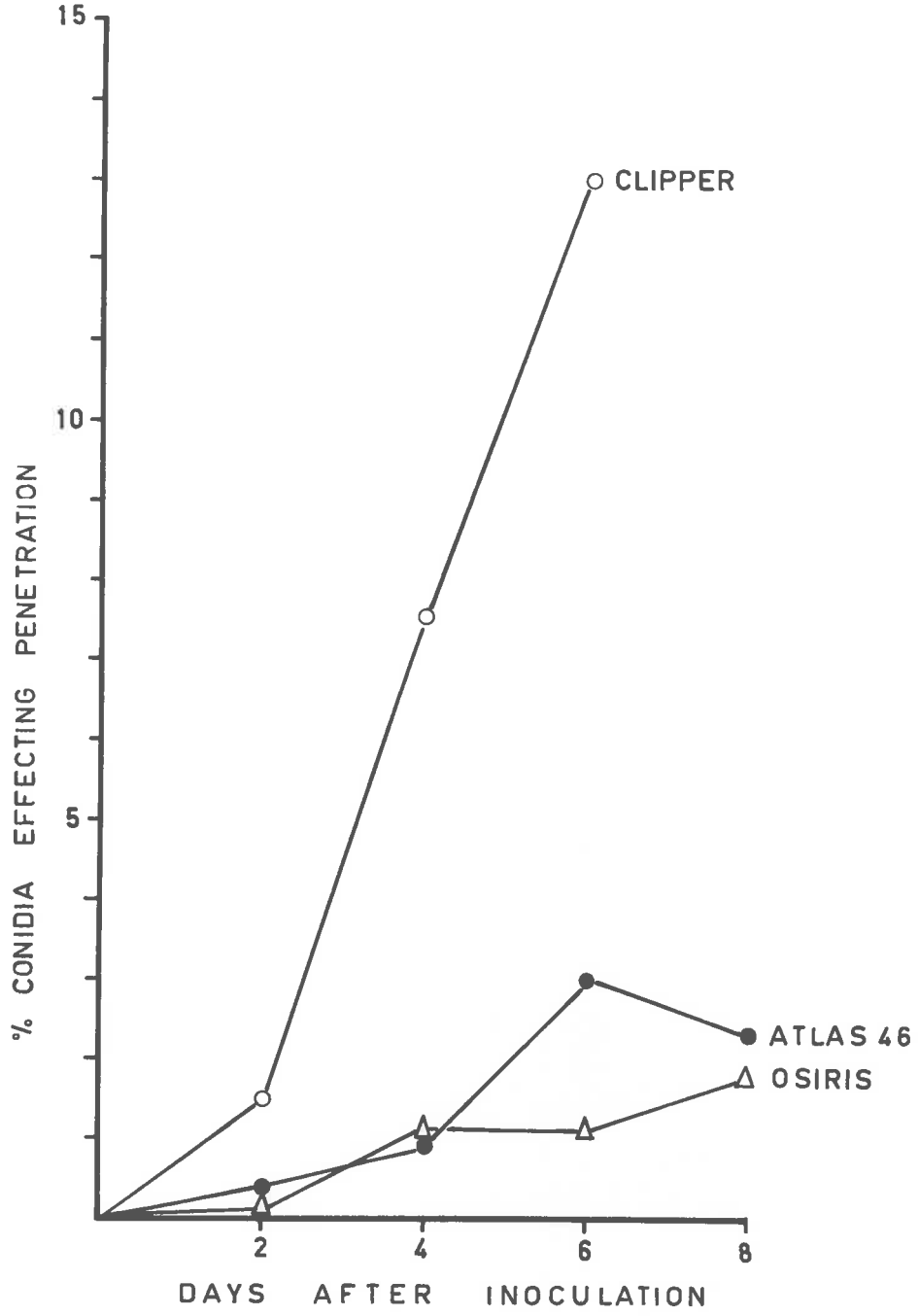
FORMATION OF APPRESSORIA ON LEAVES OF CLIPPER, ATLAS 46 AND OSIRIS INOCULATED WITH CONIDIA DERIVED FROM PSP AGAR

FIG.36



RATE OF GROWTH OF GERM TUBES OF R. SECALIS ON LEAVES OF CLIPPER, ATLAS 46 AND OSIRIS INOCULATED WITH CONIDIA FROM DIFFERENT SOURCES.

FIG.37



PENETRATION OF LEAF CUTICLES OF CLIPPER, ATLAS 46 AND OSIRIS BY R.SECALIS ISOLATE SA-1 (LEAF-BORNE CONIDIA WERE USED AS INOCULUM).

6. RHYNCHOSPORIUM ORTHOSPORUM CAUSING LEAF SCALD OF DACTYLIS GLOMERATA

a) Introduction

Rhynchosporium orthosporum Caldwell was described by Caldwell (1937) as causing leaf scald of Dactylis glomerata L. in the U.S.A. and has since been recorded on this host and on some other grasses in several countries (Sprague, 1950; Owen, 1952). This fungus, which until now has not been reported in Australia, was collected on Dactylis glomerata from an experimental field at the Waite Institute and it is briefly described below.

b) Description

Oval-shaped lesions, pale brown in the centre and dark brown at the margin, occur on leaf blades and leaf sheaths of the host. They measure 1-3 mm in width and 7-15 mm in length. Some lesions coalesce (Fig. 38).

Conidia are cigar-shaped, medianly septate, and measure 2.0-3.0 x 12.8-19.5 μm (Fig. 39, A).

The fungus grew slowly on PSP agar into a compact hyphal mat (Fig. 39, B). The colony was wrinkled and black in the centre with a pale yellow outer zone.

Figure 38.

Leaves of *Dactylis glomerata* showing scald lesions caused by natural infection with *Rhynchosporium orthosporum*.

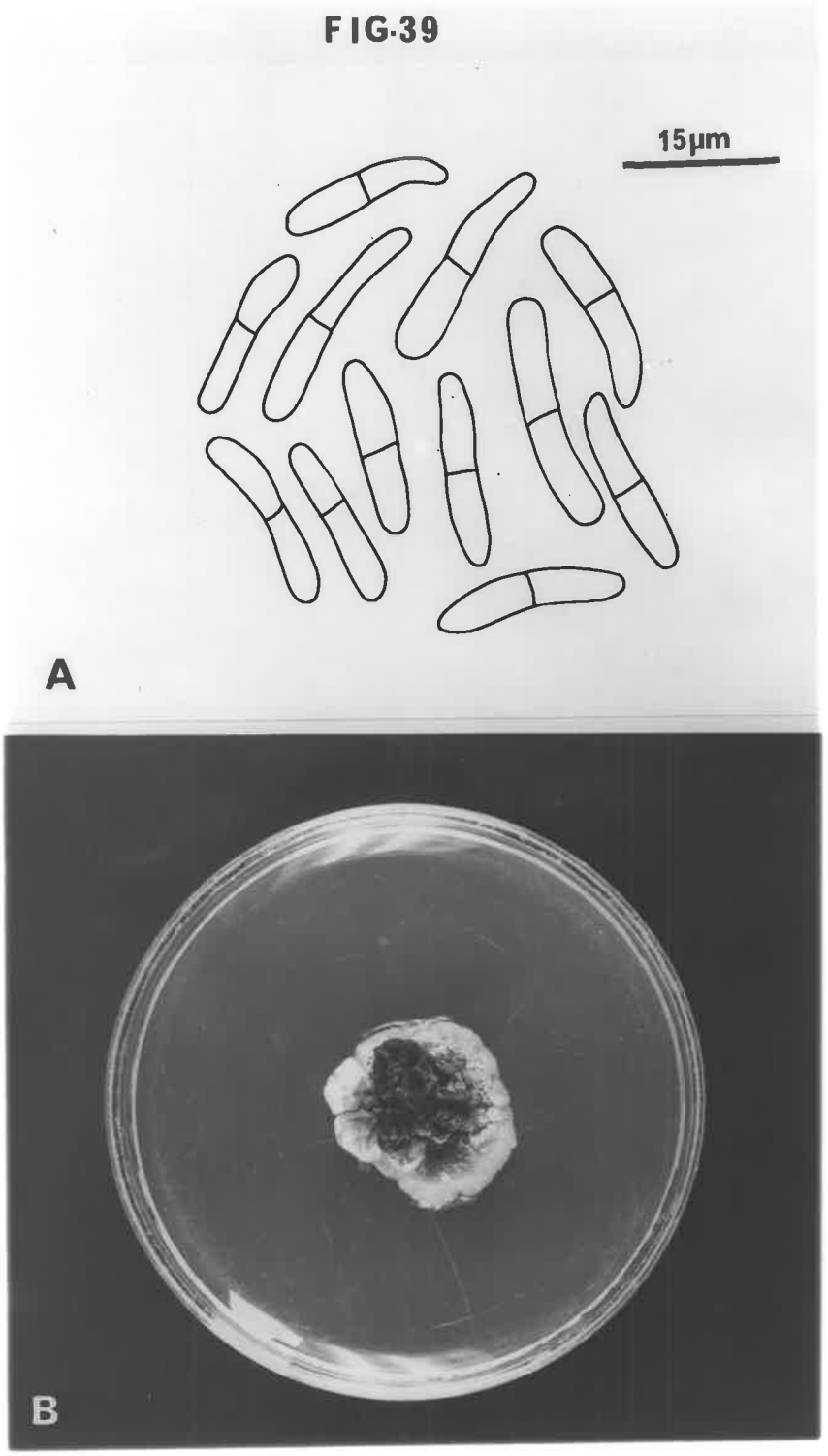
FIG·38

Figure 39.

A.- Camera lucida drawings of leaf-borne conidia of Rhynchosporium orthosporum.

B.- Six-week old colony of R. orthosporum on 1% PSP agar.

FIG-39



c) Pathogenicity

The pathogenicity of the mass isolate was tested on Dactylis glomerata, barley (all twelve differential varieties), and barley grass. Seedlings were inoculated by the spray method and incubated in the usual manner at 15°C. They were removed to a glasshouse (20-25°C) after 48 hours. Scald lesions appeared on D. glomerata 15 days after inoculation. Barley and barley grass seedlings were uninfected.

GENERAL DISCUSSION

On reaching host leaf surfaces, and in the presence of appropriate conditions for germination, spores of fungal pathogens form superficial germ tubes, appressoria and other infection structures. It is generally accepted that one or more of these processes occur before the outermost layers of the host leaves are penetrated.

Some conidia of R. secalis germinated and produced appressoria on the surfaces of barley leaves, and penetration of cuticle was initiated from a proportion of these appressoria. Other conidia effected penetration of the cuticle without producing superficial germ tubes or appressoria. As discussed later, germination and rapid elongation of superficial germ tubes may significantly reduce the frequency of penetration of the leaf cuticle of barley by R. secalis.

In the present study, the fungus consistently entered the host by direct penetration as described by Caldwell (1937) and branches from the subcuticular hyphae penetrated the epidermis and mesophyll. Direct penetration of stomatal pores was not observed but hyphae did aggregate above guard cells and their subsidiary cells and penetration was effected between the end walls of guard

cells and contiguous epidermal cells. This may have given the impression of direct stomatal penetration as reported by Bartels (1928) and Mackie (1929).

Evidence is accumulating that direct penetration of plant cuticles by fungal pathogens may in some cases be aided by chemical action. Heinen and Linskens (1960) reported the secretion of a cutin-dissolving enzyme by Penicillium spinulosum. Kunoh and Akai (1969) concluded that Erysiphe graminis produced cutinases which modified the cuticle of barley during infection, and Shishiyama et al. (1970) showed that cutin-esterase, secreted by Botrytis cinerea, hydrolysed minor side chains of fatty acids bound to cutin in tomato cuticles, thus reducing the mechanical strength of the cuticle. Evidence from the present investigations suggests that gradual enzymic erosion of the cuticle occurred on inoculated barley. Some of the cavities produced in the cuticles had diameters of a size which suggested that enzymes secreted by the germinated or ungerminated conidia were active over distances of about 15 μm (Fig. 29, p.107) from the conidia. The extensive modifications of epidermal cell walls suggest that R. secalis may secrete enzymes which degrade them, as occurs in other host pathogen interactions.

The positional relationship between the end walls of guard cells and adjacent epidermal cells may explain the frequency of

penetration around stomata. At these junctions the end of epidermal cells overhang the sunken guard cells forming concavities at the level of the upper surface of guard cells. It appears that hyphae entering these concavities force their way between the guard and epidermal cells.

Hyphae in the mesophyll did not spread laterally or longitudinally within leaves, thus lesions increased in diameter as a result of the growth of subcuticular mycelia. Similar observations were made by Caldwell (1937). However, hyphae did grow through the mesophyll from inoculated leaf surfaces to form substomatal stromata beneath the uninoculated surfaces. This contrasts with Caldwell's (1937) observations, as does the observation of conidia being extruded through stomatal pores from substomatal stromata. Davis (1922) also reported observing conidia protruding through stomata.

The appearance of grey patches on inoculated leaves probably marks the beginning of the reaction of leaf cells to toxic substances released by the fungus. At that stage the mycelium was confined to the subcuticular level and the mesophyll cells had collapsed before being reached by hyphae. The application of sterilized culture filtrates of R. secalis to barley leaves produced visible and physiological symptoms of scald disease, providing additional evidence that toxic metabolites caused the disease symptoms in infected plants.

Toxin production by plant pathogens is involved in a number of plant diseases (Wheeler and Luke, 1963; Owens, 1969). Litzenberger (1949) showed that victorin, a toxin produced by Helminthosporium victoriae produced the precise symptoms of the disease in susceptible varieties of oats. Other toxins such as colletotin, secreted by Colletotrichum fuscum (a pathogen of Digitalis lanata and D. purpurea) are not host-specific and are able to affect some plants which are not natural hosts of these pathogens. R. secalis toxins also are not host-specific since wheat, oats, Dactylis glomerata and some non-susceptible barley cultivars produced some of the visible symptoms of scald disease when treated with culture filtrates.

Barley cultivars show three categories of resistance to R. secalis, (a) resistance in incompatible combinations, (b) resistance against lesion enlargement, and (c) resistance against fungal penetration and establishment of subcuticular hypha.

Resistance in incompatible combinations was not investigated in the present work, but the lack of host-specificity of culture filtrates suggests that the fungus is unable to penetrate the cuticles or establish a subcuticular mycelium in resistant barley cultivars and other resistant species. This would explain the lack of symptoms

when these plants are inoculated.

The production of dark brown pigments, mainly in the epidermal cells, appears to be another type of resistance mechanism since lateral spread of R. secalis hyphae, which takes place between the cuticle and epidermis, does not proceed through the darkened margins of lesions nor through darkened epidermal areas resulting from hypersensitive reactions to attempted infection. Most barley cultivars respond to R. secalis infection by producing these pigments. The rate at which this inhibitory margin is produced determines the size of lesions and hence the extent of damage to the leaves. When Clipper barley was sprayed with cell-free culture filtrates, dark pigments appeared in epidermal cells. It appears, then, that the formation of darkened margins of lesions in infected plants is the host's response to the liberation of toxin(s) by the invading pathogen.

Dark brown pigmentation and collapse of epidermal cells resulted when culture filtrates were applied to barley leaf surfaces but not when cut stems of barley seedlings were placed in culture filtrates. This probably is a result of the rapid collapse and necrosis of epidermal cells when stems are immersed in culture filtrates. Culture filtrates applied to leaf surfaces would move relatively slowly through the cuticles and hence the epidermal cells could synthesize pigments before the cells were inactivated.

The processes involved in the infection of a highly susceptible cultivar (Clipper) and those of two cultivars with low susceptibility (Atlas 46 and Osiris) were compared, and the differences observed were related to possible mechanisms of resistance. When leaf-borne conidia were used to inoculate the cultivars, the apparent germination percentages on Osiris on the 6th and 8th days after inoculation and on Atlas 46 on the 6th day, were lower than the corresponding values on the 4th day (Fig. 32, p. 114). This was probably because germinated conidia, after using most or all of the nutrients available to them, were more easily washed off during staining. Reasons for a high percentage germination on Atlas 46 on the 8th day are obscure. When conidia derived from PSP agar were used to inoculate Atlas 46 and Osiris, percentage germination on both cultivars continued to increase with time (Fig.34 p. 116), possibly because nutrients derived from PSP sustained both germinated and ungerminated conidia for a few more days. On the other hand, percentage germination on Clipper continued to increase up to the time observation ceased, irrespective of the source of inoculum. This indicates that conidia were more firmly attached to leaves of this variety. For a foliage pathogen to infect its host, deposited spores must adhere to the plant surface in order to resist removal by wind and rain washing until penetration has occurred. It is possible that Clipper provided an additional

source of nutrients to the conidia which enabled them to maintain their hold on the leaves of this cultivar for longer periods than on the leaves of Atlas 46 and Osiris, or Atlas and Osiris produced superficial inhibitors whose effects were overcome by an exogenous nutrient supply. If this was the case, then the inability of Atlas 46 and Osiris to provide sufficient amounts of some nutrients required for continued attachment of conidia, operated as a mechanism of resistance. Unlike percentage germination, percentage of leaf-borne conidia that formed appressoria on each variety continued to increase with time. This was probably because appressoria acted as adhesive structures thus offering anchorage to conidia with appressoria. It is not clear why there was a marked decline in the percentage of conidia that formed appressoria on Osiris (inoculated by the spray method) after the 4th day.

It is generally believed that a high percentage germination of spores and stimulation of germ tube growth and formation of infection structures such as appressoria, prior to penetration, favour successful infection by many plant pathogens. The literature contains many examples of inhibition of spore germination and retardation of hyphal growth by materials exuded onto the surfaces of resistant varieties of plants (Walker et al., 1929; Hafiz, 1952; Topps and Wain, 1957). Ayres and Owen (1970) concluded that germination of R. secalis conidia on washed and unwashed leaves of three cultivars of barley (with differing susceptibility) was not

significantly affected by the susceptibility of the variety. From the observations made in the present study on the relationships between germination (formation of superficial germ tubes), rate of superficial germ tube growth, extent of appressorial formation and penetration, it appears that R. secalis conidia which produced long germ tubes on barley leaves, did not effect penetration, even if they developed appressoria. Evidence to support this is:
(a) Less than 50% of conidia that effected penetration on all three cultivars formed superficial germ tubes. (b) When the spray method was used to inoculate Osiris, penetration did not occur though there was some penetration when the smear method and leaf-borne conidia were used. But mean germ tube length at the end of 4 days was four times as long on leaves of Osiris inoculated by the spray method as on those of the same variety inoculated with leaf-borne conidia. (c) Low temperatures delay germ tube growth but the fungus effects greatest penetration under those conditions (Fig. 9 p. 59) Caldwell, 1937; Skoropad, 1966). Penetration of the cuticle without superficial germ tube formation is an important process because formation of superficial germ tubes and formation of subcuticular hyphae (direct from the conidium) are presumably the result of two distinct metabolic processes. Therefore, the factors that trigger off one but not the other of the two processes require investigation. Further study of the nutritional requirements for

the germination of the conidia is required. The results of such studies could lead to the development of treatments which could reduce or prevent infection by stimulating germination and rapid germ tube growth.

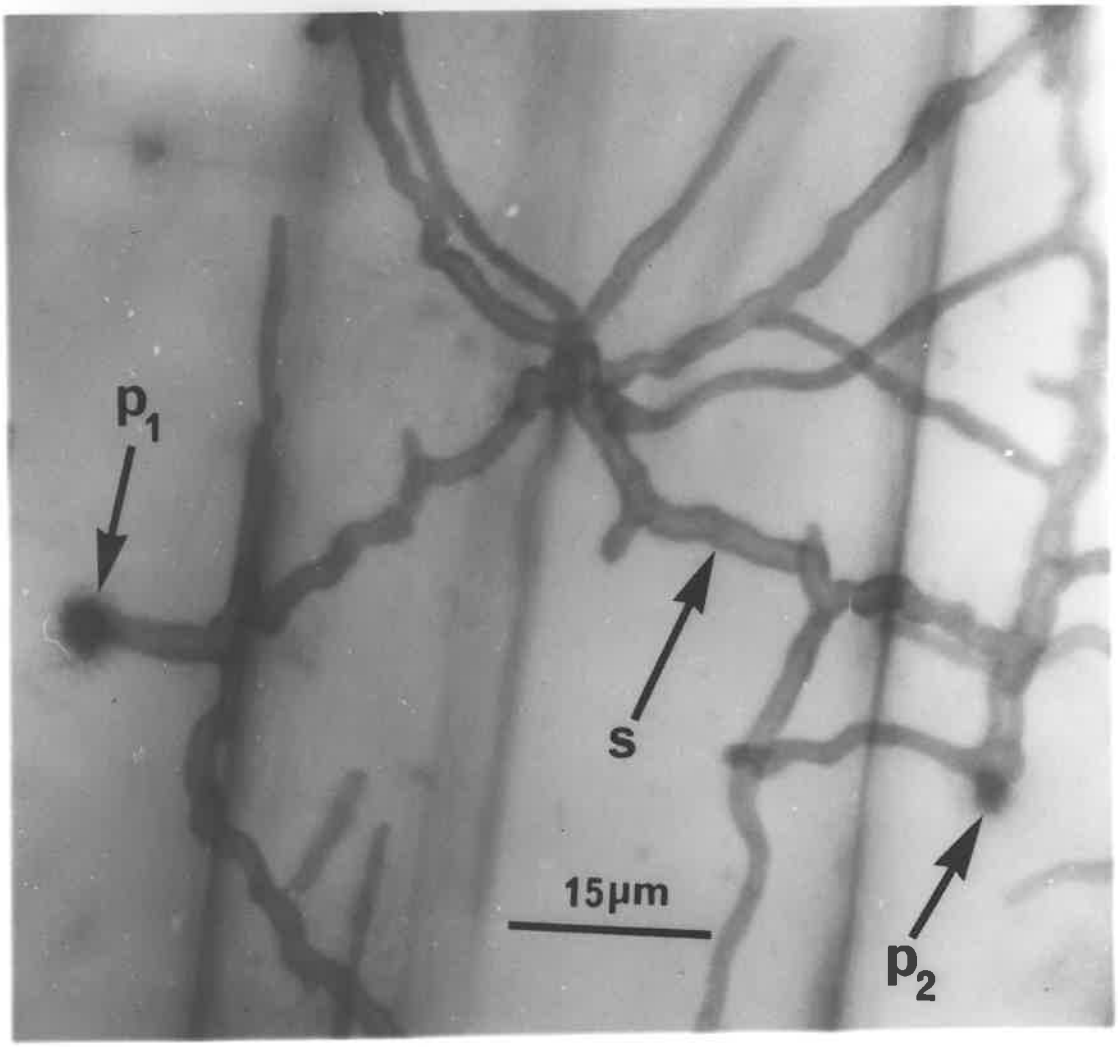
Conidia which effected penetration before they were washed off during staining left marks where penetration had occurred. The subcuticular hyphae also remained (Fig. 40 p. 133). The frequency of penetration of the three cultivars could therefore be compared with some accuracy. Recorded values of percentages of conidia that effected penetration [Tables 21-28 (appendix)], particularly on Atlas 46 and Osiris, would be higher than actual values since some conidia which did not effect penetration were dislodged.

The frequency of penetration was consistently higher on Clipper than on Atlas 46 and Osiris. Dark-staining haloes indicated that the cuticle had been degraded to the level of the epidermal cell wall, thus allowing the polysaccharide component of the cell wall to react with the periodic acid-Schiff reagent. Unstained haloes occurred more frequently on Atlas 46 and Osiris than on Clipper, suggesting that the lower layers of their leaf cuticles were more difficult to degrade. This would partly explain the lower frequency of penetration of Atlas and Osiria. Differences in the ability to resist penetration may be attributed to one or

Figure 40.

Clipper barley leaf inoculated with leaf-borne conidia, cleared and stained with periodic acid-Schiff reagent 6 days after inoculation; p_1 and p_2, points of penetration after conidia had been detached; s, subcuticular hypha.

FIG-40



both of the following: (a) Structural differences in the cuticles of the barley cultivars. (b) The presence of enzyme inhibitors in the cuticles of resistant cultivars.

Penetration of the outer layers of a host by a plant pathogen does not necessarily ensure successful infection, because substances may be present in the inner tissues which could reduce or prevent further growth of the fungus. After penetration of the cuticle, the subcuticular hyphae of R. secalis grew very fast and branched profusely in Clipper (Fig. 13 p. 67). Their growth in Atlas 46 and Osiris was limited, and the hyphae were shrunken 10 days after inoculation, suggesting the presence of fungitoxic materials. It is possible that both resistant and susceptible plants have the ability to inhibit the subcuticular hyphae, any differences being a matter of degree of inhibition. This would explain differences in susceptibility of Atlas 46 and Osiris since there were no significant differences between the frequencies of penetration of the cuticles of the two cultivars.

Further studies on the way in which lesion enlargement is limited and the presence and mode of action of fungitoxic substances could lead to the development of simple, rapid assay methods to determine the resistance of barley cultivars. At least two assay methods may be developed. One would involve the detection of

fungitoxic materials in resistant leaves. The other would involve determining the rate at which barley varieties are able to react physiologically to applications of exudates of the fungus in such a way as would limit lesion size and leaf damage following infection.

Results of the inoculation studies showed that R. secalis contains distinct physiological races. By using 12 differential barley cultivars, 35 isolates from barley and barley grass were separated into 20 groups (Tables 7 and 8 p. 34) several isolates being unique in their pathogenicity. Other workers (Sarasola and Campi, 1947; Schein, 1958) have demonstrated the existence of physiological races of R. secalis, but Skoropad (1960) failed to show any clear-cut differences in pathogenicity in tests involving 22 differential cultivars and 15 R. secalis isolates. It is difficult to evaluate Skoropad's work because he did not specify which differential cultivars he used. However, one explanation for his failure to detect distinct races might be insufficient genetic diversity in the isolates used, 14 of which were collected from Western Canada. The one isolate he obtained from Ontario was distinct pathogenically from the remainder of his isolates. The isolates used in the present work showed limited variations in virulence, possibly because most were collected from similar lesions. Other races with a wider range of virulence than the present work has

revealed, probably exist. The numerous pathogenic types, the extreme variability between isolates in their cultural characteristics, conidial morphology and growth rate of germ tubes are manifestations of the genetic variability within R. secalis.

The cultivars, Prior and Bussell, which have been grown commercially in Australia, and Clipper, derived from Prior and released recently for commercial use, were found to be the most susceptible. Bussell was susceptible to all isolates while both Clipper and Prior were immune only to SA-22, an isolate with very low virulence and a restricted host range. Cultivars with a wider range of resistance have not been used commercially in Australia. There was some positive correlation between pathogenicity and geographical origin of isolates (Fig. 2, Tables 7 and 8). These observations indicate that R. secalis shows adaptive selection.

Some of the cultivars tested had a high degree of resistance to infection by certain isolates but none was immune to all. Hence, where resistant cultivars are grown continuously, highly virulent races may be selected and perpetuated, and resistance could be short-lived, resulting in severe outbreaks of the disease. Some of the differential cultivars used in the present study are known to have specific genes for resistance to scald. In studies of inheritance of resistance to scald, Riddle and Briggs (1950) found that each of La Mesita and Turk had a single dominant gene

for resistance to six isolates of R. secalis, and that Turk had one or more additional genes for resistance to the disease. Dyck and Schaller (1961) found that Osiris also had a single dominant gene which was resistant to four races of R. secalis. Wells and Skoropad (1963) found that Nigrinudum had a single recessive gene for resistance. In the present inoculation studies, Turk was found to be immune to half of the South Australian isolates and very susceptible to the remainder. La Mesita and Osiris and Nigrinudum were found to be most resistant. Less than half of the 35 isolates attacked each cultivar and in almost all cases the mean lesion type was R. Osiris, La Mesita and possibly Nigrinudum are therefore promising as sources of resistance to scald in South Australia.

Although leaf scald has been recorded on rye, oats, wheat and other grasses in America, Europe and Asia (Sprague, 1950; Kajiwara and Iwata, 1963), it has been recorded in Australia only on barley and barley grass. The writer found a similar disease caused by Rhynchosporium orthosporum on Dactylis glomerata in South Australia but this pathogen did not infect barley or barley grass. There are conflicting reports on the ability of isolates of R. secalis obtained from Hordeum species other than barley (Hordeum vulgare) to infect barley (Caldwell, 1937; Vienot-Bourgin, 1949; Owen, 1958; Boyd, personal communication). Cross-inoculations in the present work showed that each of eight isolates from barley grass

could infect some barley cultivars (Tables 2, 3, 4 and 5) including Clipper, Prior and Bussell. Four isolates from barley grass infected this host, three of them giving severe infection, but only two of the five barley isolates used attacked barley grass (Table 11). Isolate SA-5, which was the most virulent and had the widest host range among the twelve differential cultivars did not infect barley grass. A thorough study of the taxonomy and genetics of barley grass in relation to its susceptibility to R. secalis isolates would be helpful. However, barley grass is a likely source of inoculum for infection of barley crops. This fact should not be overlooked in any programme aimed at controlling scald through modifications of farming practices.

Reports of several workers suggest that the conidia of most fungi imperfecti are released predominantly in the daytime (Hirst, 1953; Sreeramulu, 1959; Gregory, 1961; Kramer et al., 1963). Ozoe (1956) claimed that the abundance of R. secalis conidia in the air appeared to be greatest in the daytime, however, the results in Table 20 and Figs. 27 and 28 suggest that sporulation occurred most abundantly when free water was available and that conidia were released during rainfall or irrigation at any time of the day or night.

The conidia were frequently trapped in groups, as observed

by Skoropad (1959). The largest numbers were trapped during rainfall or irrigation, e.g. on July 20 (Fig. 27) and November 4 (Fig. 28). Fewer conidia were trapped under windy conditions without rain, e.g. on October 26, November 7, November 9 and November 22 (Figs. 27 and 28). Wind tunnel experiments showed that the conidia are not readily dislodged by wind alone. These observations support the view that release and dispersal of the conidia occur mainly as a result of water-splash. However, some conidia may be dislodged from lesions in strong winds, which cause plants to sway, vibrate, or rub against one another. Further experiments are necessary to gain more knowledge of the way in which conidia are dislodged.

Large numbers of conidia are produced whilst the lesions are wet and the total production of conidia in the plot must have been very large. However, the numbers trapped, when compared with the numbers of spores of other foliage pathogens which have been trapped within cereal crops, were very small, e.g. Hirst (1961) mentioned catches of up to 11,000 uredospores of Puccinia graminis per cu. m. air in infected spring wheat and Sreeramulu (1962) reported concentrations of as high as 14,300 chlamydospores of Ustilago nuda per cu. m. air over barley infected with the pathogen. In the present work, the maximum number of R. secalis conidia trapped in a day was 272 on November 4. The results in Tables 18 and 19 indicate that the disease spreads mainly between adjacent plants

but that pockets of infection may suddenly appear in a crop at isolated positions at greater distances from the nearest source of inoculum. The maximum such distance observed in the present work was about 9 metres. It is concluded, that unless barley crops are grown in adjacent fields, or linked by another host such as barley grass, R. secalis is unlikely to spread by natural agencies from field to field during a single growing season. Infected barley grass growing on the borders of barley fields may be a source of secondary inoculum.

Leaf scald may be controlled effectively through (a) a programme of breeding and cultivation of resistant cultivars, (b) cultural practices, (c) sanitary measures, (d) use of scald-free seeds.

Although further work is required to determine the form in which the fungus survives the summer season, the present work has confirmed earlier reports (Caldwell, 1937; Ozoe, 1956; Skoropad, 1959; Evans, 1969) that the disease originates from inoculum present on infected stubble. Continuation of the present practice of crop rotation in South Australia is therefore desirable, and barley crops should be grown in fields separated by at least 50 m. from those of the previous season.

Sanitary measures may include (a) destruction of barley straw and ploughing in of the remainder, (b) destruction of self-

sown barley, (c) clearing of the margins of barley fields before emergence of barley seedlings.

Seed infection was not studied in the present work, however, at 46°C and in a moist environment, the fungus is killed within 10 min. (Ozoe, 1956). Seeds may therefore be given a hot water treatment prior to sowing.

APPENDIX

TABLE 21

Comparison of processes in the infection of leaves of barley cultivars, Clipper, Atlas 46 and Osiris by R. secalis, 4 days after inoculation. Inoculation by the smear method with inoculum from 0.5% PSP agar.

Barley cultivar	Replicate number	Number of conidia observed	% conidia germinated *	% conidia with appressoria /	% conidia effecting penetration
Clipper	1	223	69.9	21.1	11.2
	2	385	52.9	12.8	2.9
	3	370	44.9	7.3	5.4
	Mean	-	55.9	13.8	6.5
Atlas 46	1	577	25.3	1.9	0.4
	2	313	40.3	3.5	0.3
	3	609	30.9	2.0	0.0
	Mean	-	32.2	2.5	0.2
Osiris	1	644	36.6	2.4	1.2
	2	570	48.5	3.3	0.0
	3	488	39.3	0.8	1.2
	Mean	-	41.4	2.2	0.8

* Include conidia penetrating without superficial germ tubes or appressoria and conidia with superficial germ tubes.

/ Include appressoria sessile on conidia and appressoria at tips of germ tubes.

TABLE 22

Comparison of processes in the infection of leaves of barley cultivars, Clipper+, Atlas 46 and Osiris by R. secalis, 8 days after inoculation. Inoculation by the smear method with inoculum from 0.5% PSP agar.

Barley cultivar	Replicate number	Number of conidia observed	% conidia germinated *	% conidia with appressoria /	% conidia effecting penetration
Atlas 46	1	406	29.1	4.9	1.2
	2	267	52.8	7.1	0.0
	3	332	61.4	6.6	1.2
	Mean	-	47.8	6.2	0.8
Osiris	1	354	69.2	9.9	1.4
	2	282	55.3	4.9	0.0
	3	347	68.3	11.8	0.9
	Mean	-	64.3	8.9	0.8

\+ Subcuticular hyphae had grown profusely on inoculated leaves of Clipper.

* Include conidia penetrating without superficial germ tubes or appressoria and conidia with superficial germ tubes.

/ Include appressoria sessile on conidia and appressoria at the tips of germ tubes.

TABLE 23

Comparison of processes in the infection of leaves of barley cultivars, Clipper, Atlas 46 and Osiris by R. secalis, 2 days after inoculation. Leaf-borne conidia were used as inoculum.

Barley cultivar	Replicate number	Number of conidia observed	% conidia germinated *	% conidia with appressoria ≠	% conidia effecting penetration	% conidia penetrating without germination +	Mean germ tube length (μm)
Clipper	1	909	6.1	3.0	1.2	0.7	4.8
	2	675	3.4	2.1	1.2	0.7	8.4
	3	463	6.9	3.9	2.2	1.1	10.5
	Mean	-	5.5	3.0	1.5	0.8	7.9
Atlas 46	1	631	10.8	4.6	1.0	0.6	10.5
	2	602	14.1	2.5	0.0	0.0	11.9
	3	548	12.6	5.5	0.2	0.0	5.6
	Mean	-	12.5	4.2	0.4	0.2	9.3
Osiris	1	824	23.7	10.8	0.2	0.1	9.6
	2	484	17.3	4.7	0.4	0.2	10.3
	3	485	30.4	11.4	0.0	0.0	8.7
	Mean	-	23.8	8.9	0.2	0.1	9.5

* Include conidia penetrating without superficial germ tubes or appressoria and conidia with superficial germ tubes.

≠ Include appressoria sessile on conidia and appressoria at tips of germ tubes.

\+ Penetrating without superficial germ tube or appressorium.

TABLE 24

Comparison of processes in the infection of leaves of barley cultivars, Clipper, Atlas 46 and Osiris by R. secalis, 4 days after inoculation. Leaf-borne conidia were used as inoculum.

Barley cultivar	Replicate number	Number of conidia observed	% conidia germinated *	% conidia with appressoria /	% conidia effecting penetration	% conidia penetrating without germination +	Mean germ tube length (µm)
Clipper	1	418	15.3	4.1	11.2	10.0	11.7
	2	460	16.1	3.9	5.7	4.3	16.1
	3	448	12.7	4.7	6.0	5.6	15.0
	Mean	-	14.7	4.2	7.6	6.6	14.3
Atlas 46	1	493	15.0	4.1	1.6	1.2	6.8
	2	475	38.1	6.9	0.2	0.2	29.6
	3	408	28.7	7.4	0.7	0.2	19.2
	Mean	-	27.3	6.1	0.8	0.5	18.5
Osiris	1	657	11.1	3.8	1.2	1.2	5.8
	2	524	28.1	9.7	0.4	0.2	9.5
	3	412	54.1	20.9	1.7	0.7	10.6
	Mean	-	31.1	11.5	1.1	0.7	8.6

* Include conidia penetrating without superficial germ tubes or appressoria and conidia with superficial germ tubes.

/ Include appressoria sessile on conidia and appressoria at tips of germ tubes.

\+ Penetrating without superficial germ tube or appressorium.

TABLE 25

Comparison of processes in the infection of leaves of barley cultivars, Clipper, Atlas 46 and Osiris by R. secalis, 6 days after inoculation. Leaf-borne conidia were used as inoculum.

Barley cultivar	Replicate number	Number of conidia observed	% conidia germinated *	% conidia with appressoria /	% conidia effecting penetration	% conidia penetrating without germination +	Mean germ tube length (μm)
Clipper	1	717	25.2	5.9	17.1	15.5	9.3
	2	617	26.0	8.8	12.2	6.2	9.5
	3	523	22.4	8.0	9.9	3.4	10.5
	Mean	-	24.5	7.6	13.1	8.4	9.8
Atlas 46	1	403	22.1	9.2	4.2	2.0	10.2
	2	579	11.4	2.6	2.8	1.6	13.6
	3	473	18.2	6.3	2.1	1.9	8.2
	Mean	-	17.2	6.0	3.0	1.8	10.6
Osiris	1	477	10.0	4.2	1.0	0.8	7.0
	2	418	28.0	8.1	1.4	1.2	10.6
	3	412	16.0	5.3	1.2	0.5	8.9
	Mean	-	18.0	5.9	1.2	0.8	8.9

* Include conidia penetrating without superficial germ tubes or appressoria and conidia with superficial germ tubes.

/ Include appressoria sessile on conidia and appressoria at tips of germ tubes.

\+ Penetrating without superficial germ tube or appressorium.

TABLE 26

Comparison of processes in the infection of leaves of barley cultivars, Clipper[x], Atlas 46 and Osiris by R. secalis, 8 days after inoculation. Leaf-borne conidia were used as inoculum.

Barley cultivar	Replicate number	Number of conidia observed	% conidia germinated *	% conidia with appressoria /	% conidia effecting penetration	% conidia penetrating without germination +	Mean germ tube length (μm)
Atlas 46	1	444	49.3	9.0	3.8	3.6	17.2
	2	457	36.8	6.6	1.7	0.4	15.2
	3	378	26.9	13.2	1.3	0.5	11.9
	Mean	-	37.7	9.6	2.3	1.5	14.8
Osiris	1	474	6.1	2.2	1.7	1.7	4.5
	2	486	12.3	4.3	1.2	1.2	9.0
	3	486	23.5	4.4	2.5	1.9	14.2
	Mean	-	14.0	3.6	1.8	1.6	9.2

x Subcuticular hyphae had grown profusely on inoculated leaves of Clipper.

* Include conidia penetrating without superficial germ tubes or appressoria and conidia with superficial germ tubes.

/ Include appressoria sessile on conidia and appressoria at tips of germ tubes.

\+ Penetrating without superficial germ tube or appressorium.

TABLE 27

Processes in the infection of leaves of barley cultivar, Osiris, by R. secalis; 4 days after inoculation. Inoculation by the spray method with inoculum from 0.5% PSP agar.

Replicate number	Number of conidia observed	% conidia germinated *	% conidia with appressoria /	% conidia effecting penetration	Mean germ tube length (μm)
1	172	61.0	40.1	0.0	31.7
2	181	57.5	13.3	0.0	38.4
3	189	56.6	84.7	0.0	33.7
Mean	-	58.3	46.0	0.0	34.6

* Include conidia penetrating without superficial germ tubes or appressoria and conidia with superficial germ tubes.

/ Include appressoria sessile on conidia and appressoria at tips of germ tubes.

TABLE 28

Processes in the infection of leaves of barley cultivar, Osiris, by R. secalis; 8 days after inoculation. Inoculation by the spray method with inoculum from 0.5% PSP agar.

Replicate number	Number of conidia observed	% conidia germinated *	% conidia with appressoria ∠	% conidia effecting penetration	Mean germ tube length (μm)
1	279	55.5	19.7	0.0	61.4
2	127	90.0	7.9	0.0	81.2
3	133	48.9	6.0	0.0	35.7
Mean	-	64.8	11.2	0.0	59.4

* Include conidia penetrating without superficial germ tubes or appressoria and conidia with superficial germ tubes.

∠ Include appressoria sessile on conidia and appressoria at tips of germ tubes.

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