THE INFLUENCE OF TEMPERATURE ON INFECTION OF *RHYNCHOSPORIUM SECALIS* ON RESISTANT AND SUSCEPTIBLE BARLEY VARIETIES

BY

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DECLARATION

I Arama Peter Futi declare that this thesis is my original work and has not been presented for a degree in any other University.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

DEDICATION

To Mary, Domtila, George and my mother Francisca.

ABSTRACT

The upsurge of barley scald has been of much concern to barley growers, breeders and pathologists in Kenya. Yield losses due to scald average 30% though losses upto 52% have been recorded. Varieties released in the past with resistance to scald have of late succumbed to Rhynchosporium secalis. In this study, infection by an isolate of Rhynchosporium secalis on two popularly grown barley varieties 'Bima' and 'Proctor' was investigated at different temperatures. 'Bima' was resistant whilst 'Proctor' was highly susceptible to scald in the field. The two varieties were grown in plastic pots in the greenhouse and inoculated 21 days later. Inoculated plants were placed in a Vindon growth chamber whose inside temperature was set at 10°C, 15°C, 20°C, 25°C and 30°C respectively. Barley varieties in the germplasm were also tested for scald resistance at two-leaf stage in the greenhouse using Njoro isolate of R. secalis.

Results showed that infection of R. secalis started with germination of germ tubes which grew along the leaf surfaces before forming appressoria. The differential stain periodic acid-Schiffs reagent stained deep red 'haloes' on leaf surfaces around penetration sites. These factors of conidia germination, appressoria formation and penetration were similar in the two varieties. Optimum temperatures for conidia germination, growth of germ tubes and scald lesion development were found to be 22.0°C, 23.5°C and 20.0°C respectively. Higher temperatures than optimum were not favourable and there was no infection.

occuring at 30°C. After successful penetration, sub-cuticular hyphae were formed thereafter branching profusely within 'Proctor' leaves and sparsely within 'Bima' leaves. Scald symptoms appeared earlier in 'Proctor' plants than 'Bima' between 10 and 20°C. Scalded leaf areas and sporulation within scald lesions were significantly less in 'Bima' than in 'Proctor' between 10 and 25°C. The factors of infection involving suppression of germ tube growth, sparce mycelial development, longer incubation period, chlorotic zones preceeding scald lesions, less percent scalded leaf areas and less sporulation within lesions of 'Bima' leaves than in 'Proctor' leaves were indicative of partial resistance in 'Bima' whereby the host allowed infection to occur but the rate of disease progress was lowered.

The study indicated that testing for resistance at two-leaf stage in the green house gave distinct reaction types. The techniques were recommended for future use in identifying resistance sources to barley scald as well as evaluating segregating populations. Complete resistance to scald was found in the varieties Bey, Forrajera and Abyssinian (CI. 3940). Resistance in one or two of these varieties should be incorporated into the agronomically well adapted and high yielding commercial barley variety 'Bima' to make it even more resistant.

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CHAPTER I

INTRODUCTION

Barley (Hordeum vulgare L. emend. Bowden) is one of the most important cereal crops grown in the world today. Many varieties of barley are adapted and grown under a wide ecological range. This is true from the fact that cultivation is widespread ranging from the Northern to the Southern hemisphere, from areas below sea level to high mountain ranges and during winter or summer. Barley grows beside frozen pools in highland Ethiopia and beneath date palms in the Sahara desert (Weaver, 1950) . Barley production ranks fourth worldwide in terms of area and is exceeded only by wheat. rice and maize. The successful cultivation of barley, however has required the solution of a number of specific problems. Frequently the most serious problem has been disease. Even under dry conditions, a number of diseases may be limiting factors in barley production.

Barley varieties were tested for adaptability for the first time in Kenya in 1934 (Ball, 1937). Growing of malt barley was started in 1947 (Owino, 1983). Kenya Breweries which is a grower and the sole buyer through contract farming is currently growing 17,200 ha of barley (Nyachae, 1987). The best malting barley

is grown on farms at altitudes of 2200 m and above. Barley requires an annual rainfall of between 700mm and 1200mm spread over at least five months as the crop grows. The best barley growing areas in Kenya are Mau Narok, Melili, Oloropil, Olorkuto and Timau. Other suitable areas include Bahati, Kinangop, Moiben, Molo, Mweiga, Nakuru, Njoro, Nyahururu and Subukia. Scald is the most important barley disease in Kenya high altitude areas where 70% of barley is grown (Owino, 1983).

Barley and its pathogens have co-existed for many centuries. Apart from scald, other diseases that attack the barley crop in Kenya are brown rust of barley (Puccinia hordei), stem rust (Puccinia graminis), yellow (stripe) rust (Puccinia striiformis), net blotch (Helminthosporium teres), spot blotch (Helminthosporium sativum) and Barley Yellow Dwarf Virus (BYDV). Brown rust caused by Puccinia hordei is the second most important barley disease in Kenya next to scald. Brown rust is favoured by warm dry weather. Temperatures of 15-20°C provide optimum conditions for infection.

The disease is serious at medium altitude and late in the growing season (Owino, 1983). Since 1980-81 growing season when serious epidemics were reported especially on the very susceptible variety, 'Tumaini', no new serious cases have been reported. Yellow rust is serious in high altitude areas. All the current commercial barley varieties are adequately resistant to yellow rust. Net blotch caused by Helminthosporium teres may come early or late. The variety 'Kenya Research' is more susceptible than the other varieties. No serious case has been reported since 1981 (Owino, 1983).

Rhynchosporium secalis (Oud.) Davis, the causal organism of barley scald is one of the pathogens most prevalent in semi-humid barley growing areas (Dickson, 1962) and in highlands with more severity at altitudes 2200-2700 metres above sea level (Gebre, 1981). Incidences of barley scald have increased tremendously due to changing cultural practices in agriculture today. Skoropad (1956; 1960) considered that shallow cultivation practices that left infested leaf and straw on soil surface,

wider use of combine harvesters which aid in spreading inoculum, failure of crop rotation, large acreage sown of a single cultivar and the introduction of susceptible varieties have contributed to the upsurge of scald. Yield losses due to scald have been recorded in various countries. Schaller (1951) reported losses in grain yields as high as 35% in California. Ali et al (1976) reported losses of 70% in Australia while in Kenya losses have been estimated at 30% (Nyachae, 1987), although higher losses have been observed. Yield losses estimated at 35-40% have been reported for spring barley (James et al, 1968; Jenkins and Jemmett, 1967). Yield losses of 1-10% are more common in many countries (Evans, 1969; Jenkins and Jemmett, 1967).

Barley scald is the major disease limiting barley production in Kenya. To reduce production costs (especially on fungicide applications) whilst increasing or maintaining high yields, Kenya Breweries started a research programme in conjunction with National Plant Breeding Station, Njoro. The breeding program involved introducing barley germplasm mainly from the International Maize and Wheat Improvement Centre (CIMMYT), Mexico, the International Centre for Agricultural Research in Dry Areas (ICARDA) Syria and Carlsberg Plant Breeding, Denmark (Owino, 1986). Varieties which performed well in different ecological zones were subsequently tested for malting qualities and considered for release as commercial varieties. Whereby a variety had some undersirable characteristic like disease susceptibility and poor malting qualities, it was used as a parent for crossing. The segregating populations (F_2 - F_8) were planted in Njoro, Mau Narok and Melili. The plots were not inoculated with disease at any stage of development (Owino, 1986). Inoculum was assumed to

be present in the environment. Scald scoring was done at boot and heading stages of development using the 0-9 Saari-Prescott Scale. There was no scald infections observed in experimental plots in all experimental sites at seedling and tillering stages of development (Nyachae, pers. comm.). Disease scoring was done on the basis of average scald infection on whole plots (Owino, 1985;1986). The selected advanced generations with low scald score of 0 to 4 on the Saari-Prescott scale were entered in Barley Preliminary Yield Trial (BPYT) for two years and finally into Barley National Performance Trial (BNPT) for adaptability, yield potential, resistance to scald and other diseases and resistance to lodging. The BNPTS were planted in Timau, Mau Narok, Melili, Molo, Njoro and Endebess. Disease scoring in BNPTS was done by Kenya Breweries researchers in collaboration with researchers from National Plant Breeding Station (NPBS), Njoro and National Seed Quality Control Services (NSQCS) Lanet. Lines were screened in BNPT for at least three years before being considered for commercial release. Through this joint programme, varieties like 'Hege magniff 11966', 'Tumaini', 'Kenya Research' and 'Proctor' were evaluated and sebsequently released for commercial growing. These varieties did well at first but in subsequent years of growing, they succumbed to scald to such an extent that farmers were forced to rely on intensive fungicide applications for control of scald in high altitude and humid areas of Timau and Mau escarpment.

Varieties 'Kenya Research' and 'Hege magniff 11966' had to be withdrawn due to their high susceptibility to the disease. New lines were subsequently introduced and this led to the release of the most highly scald resistant variety 'Bima' in 1984.

In 1985, there was ample rainfall in Timau, Mau escarpment and central Rift Valley areas. Even the variety 'Bima' grown in Timau and Mau escarpment during 1984-85 growing season showed susceptibility to R. secalis. Infection was mainly noted to occur around the sheath. This attack often led to premature leaf death. The breakdown in resistance to scald in these varieties may be attibuted to lack of adequate information on the pathogen variability of R. secalis in Kenya. Alternatively the screening method for resistance could have been inadequate as it relied heavily on field inoculum and the prevailing environmental conditions. These two factors may not have provided adequate disease pressure and optimum conditions for effective screening for resistance to scald. Thus it was with this in mind that this research project was formulated to:

1) Determine temperature influence on the germination and penetration of R. secalis on resistant and susceptible barley varieties.

2) Determine temperature effect on the lesion development and sporulation of R. secalis on resistant and susceptible barley varieties.

3) Apply the techniques used in this study to test barley germplasm for resistance to *R. secalis*.

CHAPTER 2

LITERATURE REVIEW

2.0 Nomenclature and Taxonomy of *Rhynchosporium secalis*.

The oldest preserved material of *Rhynchosporium secalis* (Oud.) Davis, is that which was collected in Romendal (Norway) on barley in 1889 by Hirsch (Shipton *et al*, 1974). It was identified as *Helminthosporium gramineum*. Jorstad re-examined the material and found *Pyrenophora teres* and *Rhynchosporium secalis* as well as *Helminthosporium gramineum*. Oudemans (1897) was the first to name and describe the organism. His collection was made in Netherlands on rye and he named the organism *Marsonia secalis*. Four months later, the fungus was isolated in Germany on barley and rye materials and was designated *Rhynchosporium graminicola* (Frank, 1897). Davis (1919) in the United States proposed the new combination *Rhynchosporium secalis* (Oud.) Davis, to overcome certain nomenclature difficulties. The description of the genus was thus ammended by both Davis (1922) and Caldwell (1937).

The genus *Rhynchosporium* (Heinsen) belongs to the Kingdom-Mycota, Phylum-Eumycota, Class-Deuteromycetes, Order-Moniliales and Family-Moniliaceae (Barnett and

Hunter, 1972). The fungus has creeping sterile hyphae, ascending fertile hyphae with curved, branched conidiophores bearing conidia on denticles (Grove, 1937). Conidia short, cylindrical to ovate, one septate, curved in profile, often beaked at the apex and terminated by a rather obtuse mucro at the lower end. The lower cell usually narrower than the upper. Some spores appear to have more than one septum (Grove, 1937). Spores measure 2.0-4.0µm by 12.0-20.0µm (Dickson, 1956).

2.1. Variation in R. secalis

2.1.1. Cultural Variation

Heinsen (1901) found that isolates of R. secalis sporulated abudantly on some media, but were highly mycelial on others. Caldwell (1937) presented evidence for specialization and recognised six forms on barley, rye and other grasses. After culturing nine monoconidial isolates from barley on various media, he found out that there were differences in colony growth, pigmentation and sometimes sporulation among isolates. Sarasola and Campi (1947) reported that several barley varieties resistant to barley scald in the U.S. were susceptible in Argentina. Schein and Kerelo (1956) found the media without sugar allowed greatest sporulation. They reported sporulation of the fungus to be practically nil on potato- dextrose-agar

and very high on lima bean agar. Isolates grown on lima bean agar had a pink colour in contrast to the brown and black colonies on other media used. Reed (1957) observed many mutants in cultures of most collections he made. He noted extreme variation in cultural characteristics among the mutant cultures, and between the mutants and the original isolates.

Habgood (1973) examined variation in aggressiveness and in conidial production in vitro in a number of single spores collected from within a single naturally infected plot of barley. Agressiveness varied significantly both between lesions in the plot, and between isolates. He concluded that conidial production is not under nuclear control. He related Rhynchosporium secalis to Phytophthora infestans in which an extra nuclear basis for some of the variability has also been suggested.

2.1.2. Pathogenic variation

Ali and Boyd (1974) showed that there is an existence of both inter-and intra-isolate variability in both the host reaction and isolate pathogenicity. This makes conventional identification of races of the pathogen difficult and shows that R. secalis has no strict host specialization. Hosts that consistently express resistance or susceptibility under different conditions and isolates which express their pathogenic characteristics consistently

have been identified. Jackson and Webster (1976) noted that there was a dynamic nature of disease population when five isolates of widely varying pathogenicity was carried out in two successive disease cycles. Hybrid type races were found which differed from the original population. There was a shift of the fungus population towards the more simple pathogenic races and away from the more complex one. Variability in pathogenic characteristics may likewise be differentially influenced by environmental factors.

Bartels (1928) carried out extensive cross-inoculation tests and concluded that the fungus was plurivorous. His suggestion was not unanimously accepted. Riddle and Suneson (1948) found no convincing evidence of physiologic races in field trials conducted in California. Skoropad (1960) did not find any clear evidence of pathogenic races among the 15 isolates tested on 22 differential barley varieties in Western Canada.

At present the literature reports two kinds of races of R. secalis

a) Those of Caldwell (1937) based on the ability of the fungus to attack members of different genera of plants, and

b) those of Sarasola and Campi (1947) based on the ability of the organism to attack different varieties of the same host species.

The existence of physiologic races of R. secalis has been claimed by a number of authors. Sarasola and Campi (1947) differentiated four races in Argentina. Schein (1958, 1960) placed the race identifications on a more conventional basis and identified seven races in the U.S. In Japan, Kajiwara and Iwata (1963) identified 10 races, whilst Dodoff (1963) identified four races in Bulgaria. Two races were identified in Britain (Williams and Owen, 1973) and Khan et al (1968) reported the existence of three field races in Western Australia.

2.2 Host range:

It has been shown that barley isolates of R. secalis can attack a number of non-cereal grasses. The organism has been isolated from Agropyron repens, A. smithii, A. ciliare, A. semicostratum, Avena sativa, Bromus unidoides, B. inermis, Elymus canadensis, E. triticoides, Festuca arundinaceae, Hondeum vulgare, H. jubatum, H. murimum, Holcus lanatus, Lolium multiflorum, L. perenne, Phalaris arundinacea, Secale cereale, Triticum aestivum, T. vulgare and other grasses (Ali, 1972; Bartels, 1928; Caldwell, 1937; Dodoff, 1963; Owen, 1958; Ozoe, 1956; Sarasola and Campi, 1947; Schein, 1958; 1960; Shipton et al, 1974;

Smith, 1937; Wilkins, 1973). Studies have shown that not all isolates from these grass hosts infect barley nor are all grass hosts infected by all barley isolates.

2.3 Life history of R. secalis

The major source of primary inoculum is infested plant debris (Ayesu-Offei and Clare, 1971; Caldwell, 1937; Jenkins and Jemmett, 1967; Ozoe, 1956; Skoropad, 1960). When cool and moist conditions prevail, conidia are produced on the superficial stromata present on infested plant debris. Free moisture or high relative humidity between 95-98% was necessary for conidial production (Ayesu-Offei and Clare, 1971; Caldwell, 1937; Jenkins and Jemmett, 1967; Ozoe, 1956; Skoropad, 1962). Sporulation took place over a wide range of temperatures but abundant conidia were produced between 10-20°C within 24 hours (Caldwell, 1937; Owen, 1958; Skoropad, 1962).

The fungus lost its sporulation capacity within 32 to 36 days at 10°C and 18°C respectively when infested debris was kept continously on a soil surface in the laboratory (Shipton et al, 1974). Following storage of infested debris in test bags in the field, Skoropad (1966) was able to demonstrate sporulation of the fungus

after 340 days. Skoropad (1962) showed that when infested debris was dried, conidial production ceased but a new batch of conidia was produced when free moisture was introduced in. Several (5-8) wetting and drying cycles depleted the reserve of the fungus and it then ceased to sporulate even under optimum conditions. Alternate wetting and drying and microbial saprophytes were apparently responsible for the early loss of sporulation in the field (Shipton et al, 1974).

Evans (1969) and Polley (1971) found a correlation between the amount of stubble debris from the previous crop and the amount and severity of R. secalis. Spring barley could become infected with R. secalis from the previous season's barley debris, even when attempts were made to bury all sources of inoculum by ploughing. Ozoe (1956) demonstrated that barley straw placed above the seed (in or on soil) gave rise to infected plants while straw placed beneath the seed failed to do so. Infection may also be initiated from inoculum produced in the seed or small pieces of trash carried with it (Reed, 1957). Volunteer barley crops have also been known to be very good source of inoculum (Jenkins and Jemmett, 1967).

The importance of seed-borne inoculum is not well understood. Bartels (1928) believed that the organism was seed-borne when he demonstrated that various seed dressings gave control of scald in the field. Smith

(1937) showed that the organism could infect barley kernels. He found conspicous lesions on floral bracts and believed that the presence of infective spores on the grain served as a source of inoculum for the new crop. Skoropad (1957) studied the infection of the pericarp in floral bracts, and seedlings in detail. He found out that coleoptiles are usually attacked when germination of infected seed took place under favourable conditions.

Sexually produced spores have not been associated with the conidial state of the scald organism. The possibility that a perfect state exists has been suggested by the discovery of microconidia (Skoropad and Grinchenko, 1957). More recently, Ali (1972) observed microconidia being exuded from flask-like mycelial branches. These were formed from mycelia on abraded leaf tissue on both resistant and susceptible hosts and occured in conjunction with normal conidial formation. Microconidia have not been observed to germinate. Newman and Owen (1985) showed evidence of high proportion (90%) of asexual recombination of R. secalis.

Ozoe (1956) and Skoropad (1960) suggested that after development of the first lesions, secondary inoculum was dispersed by wind-borne rain splash. Ozoe (1956) claimed that the number of conidia in the air appeared to be greater in the day than at night. However, Ayesu-Offei and Carter (1971) reported that conidia may be

released at any time of the day or night. Field observations showed that a low level of primary infection gave rise to a severe epidemic if subsequent weather conditions were favourable for secondary infection (Jenkins and Jemmett, 1967). R. secalis does not appear to be suited for long distance dispersal. As far as is known, the characteristic spores have not been identified as a component of the air spora except in the vicinity of infected fields. Observers noted that conidia were apparently transported over short distances and initiated infection in nearby crops (Ayesu-Offei and Clare, 1971; Ozoe, 1956; Skoropad, 1960). Ayesu-Offei and Carter (1971) found that conidia were most abundant in or near barley fields during the rainy and windy periods. Spores appeared in clusters of three to ten which indicated that water splash dispersal occured. During dry periods, conidia were few and occured singly. Long distance dispersal may also occur through the dissemination of small infected leaf fragments (Reed, 1957) or through distribution of infected grain (Skoropad, 1960).

Survival under field conditions has been investigated (Bartels, 1928) and Skoropad (1966) who found viability to be maintained for 6-9 months and 12 months respectively. Conidia themselves were apparently unable to overwinter (Caldwell, 1937) but Bartels (1928) contested that the stroma survived and resumed spore production on the return of favourable conditions. Polley (1971) believed that

in Britain, overwintering could be accounted for by the formation of sclerotia which gave rise to hyphae and conidia on return of favourable conditions. The fungus is capable of surviving high summer temperatures in Mediterranean areas. At present there is no substantial evidence that the fungus survives saprophytically in the soil.

2.4 Resistance to R. secalis

2.4.1 Sources of resistance

Studies on resistance to scald have been carried out. Ali (1974) observed that none of the 7 resistant barley cultivars studied was free from mycelium although no visible scald symptoms occured following infection by R. secalis. He also reported that resistance in the varieties 'Psaknon', 'Turk', 'Hudson' and 'West China' broke down at high temperature. This showed that the kind of resistance inherent in the cultivars was influenced by environmental factors. Auriol et al (1978) noted that isogenic barley lines with resistance to the pathogen were resistant to rhynchosporoside (1, 2-propanediol cellobioside) isolated from cultures of the fungus, while susceptible isogenic lines of these barleys to the enzyme were susceptible to the pathogen. Habgood (1977) showed that resistance was greater in older leaf tissues but that no differential effect between cultivars was detected.

Peresypkin and Drapatyi (1979) concluded that resistance was determined by peroxidase and polyphenoloxidase activity, thickness of leaf cuticle and fungicidal effect.

2.4.2. Genetics of resistance

Resistance to R. secalis in barley has been reported on numerous occasions. Mackie (1929) apparently was the first to study the inheritance of scald resistance. He found in an unnamed variety that resistance was controlled by a single recessive gene. Bryner (1957) found a dominant gene in 'Brier' (CI. 7157) responsible for resistance. He was the first to use designation Rha for the gene conferring resistance to R. secalis in 'Brier'. This was later amended to Rh by Robertson et al (1965) in accordance with accepted code of gene nomenclature.

Studies by Dyck and Schaller (1961) were probably the most extensive on the genetics of scald resistance. They used four pathogenic races of R. secalis namely US1, US7, US8 and US9 and 8 barley varieties 'Atlas', 'Atlas 46', 'Turk', 'Brier', 'La Mesita', 'Trebi', 'Osiris' and 'Modoc'. Apart from a single gene, designated Rh_2, conditioning resistance of 'Atlas 46', they also concluded that there was another dominant gene designated Rh_3 which also conditioned resistance. 'La Mesita', 'Osiris', and 'Trebi' were found to have a single dominant gene which was given the symbol Rh_4. Studies also showed

that the Rh_3 gene was closely linked with Rh_4 gene. An allele at the Rh_4 locus, Rh_4^2 was identified in 'Modoc'. Rh_5 gene which was independent of Rh_3 and Rh_4 genes was present in 'Turk'.

At Saskatoon, Baker and Larter (1963) used 4 varieties known to be resistant to scald to study their inheritance of resistance. They used a single Canadian isolate resembling US1. The varieties 'Jet' and 'Steudelli' both were found to have two complementary recessive genes for resistance which they designated rh6 and rh7. Temperatures above 25°C during the infection period caused a breakdown of resistance. Resistance in both 'Kitchin' and 'Abyssinian' was controlled by a single gene with incomplete dominance. In the homozygous state, this gene, designated Rh_9 conferred full resistance to either variety. Wells and Skoropad (1963) used an Alberta isolate on eight resistant varieties, 'Turk', 'Osiris', 'Bey', 36Ab1991, 'Rivale', CI. 3515, CI. 8256, and 'Nigrinudum'. A dominant gene Rh_3 was responsible for resistance in seven of the varieties. In the eighth variety, 'Nigrinudum', a recessive gene governed resistance. This gene was designated rh8. They also confirmed that the Rh_3 gene in 'Turk' was located on chromosone 3.

In a recent study on winter barleys, Starling et al (1971) recognised the predominance of the Rh- Rh_3- Rh_4 complex locus in 26 of 33 hosts examined. They speculated

that the locus complex was not infact a single locus and expressed doubt as to whether the number of loci identified was as great as the nine suggested in literature. Habgood and Hayes (1971) aided by their discovery that a single gene segregation apparent after a 14-day period could be interpreted as the action of two complementary genes if re-examined at the end of a 28-day incubation period, postulated that the action of certain genes was effective only in the presence of a complementary partner. These workers detected a gene in 'Osiris' which was complementary to the one effective 14 days after inoculation. This gene had not been previously reported since its effect was not apparent until well after the normal time of assessment. The symbol Rh10 was suggested for this gene, whilst rh11 was proposed for the recessive gene in CI.4364 and CI.4368. These workers also proposed revised symbols for the designation of some of the resistance genes identified. To summarise the work of Habgood and Hayes (1971) there are 5 alleles at Rh locus, two are dominant (Rh and Rh^2) two are incompletely dominant (Rh^3 and Rh^4) and one is recessive (rh^5). They are all found on chromosome 3.

Bockelman et al (1977) using trisomic analysis, determined chromosomal location of the resistance genes in 'Kitchin' and 'Jet'. They showed that 'Kitchin' possessed a single gene, Rh9, on chromosome 4, whereas, 'Jet' contained rh7 on chromosome 4 and 3 respectively. Habgood (1974)

studied inheritance of partial resistance. He reported that resistance was complex in inheritance. Results were incompatible with any hypothesis involving less than 4 genes. The F_2 studies showed that both dominant and recessive genes were active in conferring resistance and that there was significant effect.

2.5 Effect of environmental factors on infection and disease development

The expression of disease symptoms involving lesions and the rate of their development is determined by the combined influence of host, pathogen and environmental conditions prevailing. Variability in symptom expression resulting from the complex interraction complicates any genetic inferences that have to be made. It is therefore necessary to appreciate the non-genetic factors contributing to phenotypic variability of symptom expression.

2.5.1 Effect of light

Ryan and Clare (1975) found that light reduced the rate of germ-tube production and suppressed germ-tube elongation. This was usually reversed in darkness showing that light duration influenced infection and rate of disease development.

2.5.2 Effect of moisture and temperature

Both temperature and wetness (humidity) were found to be important factors for the infection of R. secalis on barley. Moisture was essential for the production of inoculum. While infected tissue did not need to be directly moistened, the atmosphere had to be humid enough for the tissue to absorb moisture (Caldwell, 1937; Ozoe, 1956; Skoropad, 1966). Optimum wetting periods for sporulation of R. secalis in barley lesions was investigated by Rotem et al (1976). They found that sporulation occured on wet lesions after leaf tissues had been necrotic. Wetting time required for maximum spore production decreased as temperature rose. Most spores were produced at 10°C after 72 hours wetting. Longer wetting periods than optimum led to lysis of spores at any given temperature. Partly responsible for the lysis of the spores were saprophytes of which the green Pseudomonas bacteria were the most common. Ryan et al (1974) found that maximum lesion development occured in plants kept wet for 14 hours or more at temperatures between 15-25°C.

2.5.3 Temperature

Temperature had a great influence on the behaviour of R. secalis. Sporulation took place in a wide range of temperature. Abundant conidia were produced at temperatures between 10°C and 20°C within 24 hours

(Ozoe, 1956; Skoropad, 1957; 1960; 1962). The optimum temperature for germination was in the range 18-21^{o}C (Bartels, 1928; Caldwell, 1937; Fowler and Owen, 1971; Ozoe, 1956). Conidia held at 27-31^{o}C often failed to germinate even when subsequently placed under optimal conditions (Caldwell, 1937; Fowler and Owen, 1971; Ozoe, 1956; Reed, 1957; Skoropad, 1960).

Infection of barley leaves occured readily over the temperature range 6-18^{o}C and infection was often well established in about 9-10 hours under cool moist conditions. Humid conditions above 92% relative humidity for about 48 hours was ideal for infection (Ayesu-Offei, 1971; Ozoe, 1956; Polley, 1971; Skoropad, 1957; 1960). Infection of seedlings from seed-borne inoculum depended to some extent on the rapidity of plumule growth. Temperature at 22-27^{o}C favoured rapid seedling growth but inhibited conidial production such that the plumule expanded beyond lesions on the coleoptile by the time sporulation took place. Such seedlings escaped infection.

Soil temperature at 16^{o}C was optimum for coleoptile infection. Infection decreased sharply with soil temperature at 20^{o}C and was almost absent at 22^{o}C. Dry soil conditions favoured infection (Ozoe, 1956) and so did poorly drained soils (Barradas, 1981).

Lesion development proceeded normally when post-inoculation temperature was 12-24^{o}C. Few lesions developed and development was slow when post-inoculation conditions were 6-12^{o}C or above 24^{o}C. Exposure to lower temperature initially followed by exposure to higher temperature favoured lesion development (Caldwell, 1937; Skoropad, 1957). Studies by Ali (1972) showed that the effect of temperature and symptom expression was dependent on the particular combination of isolate and host phenotypes examined. Using both susceptible and resistant cultivars, he noted that high diurnal temperature regimes (18^{o}C min./30^{o}C max.) greatly impaired the ability of certain isolates known to infect hosts normally susceptible to them, whilst other isolates could infect hosts normally resistant to them. It was also observed that at low temperature regimes (8^{o}C min./20^{o}C max.) the rate of symptom development proceeded most rapidly for certain isolates whilst higher temperatures (15^{o}C min./21^{o}C max.) favoured the rate of symptom development of others.

2.5.4 Symptom development

Scald symptoms produced on barley are usually distinctive for the pathogen on that host. Other fungi may induce similar symptoms on other hosts. Infected barley leaf tissue show extensive intercellular hyphal growth thus causing the cuticle above it to stretch but not rapture (Ryan and Grivell, 1974). Following this, the epidermal

cells collapse and chloroplasts in the underlying mesophyll cells show degenerative changes. The collapse of epidermal cells is preceeded by thickening of cell wall. This collapse is externally evident as water soaking and scalding of tissues. Lateral spread of the fungus is limited and according to Ayesu-Offei and Clare (1970) is intracellular at first then becoming intercellular among the collapsed cells. Permeability changes are associated with colonization as more nutrients leak into intercellular spaces in susceptible than resistant varieties (Ayres and Jones, 1972).

The development of conidia on stromata results in the separation and eventual cracking of cuticle, thus superficially exposing the stromata in the process (Ayesu-Offei and Clare, 1970). Infection is generally first evident on the auricles, leaves and sheaths as dark or pale grey, bluish-grey or simply cholorotic lesions. Lesions later assume a water soaked appearance. As invasion advances the centres of the lesions dry out and become light-grey to grey-green or grayish-white, the edges of the lesions are dark brown and may be surrounded by a chlorotic region.

The brown marginal zone bounding lesions were considered by Ayesu-Offei and Clare (1971) to limit lateral penetration of hyphae. The lesions may become zonated by successive enlargements; lesions coalesce when infection is severe

which may lead eventually to complete leaf death (Bartels, 1928; Caldwell, 1937; Sprague, 1950) and shredding of the leaves (Bartels, 1928; Skoropad, 1960). Infection in the region of the auricles may also lead to leaf death (Bartels, 1928; Rosen and Larsh, 1944). Most lesions occur on the laminae although lesions develop on sheaths, especially older ones (Brooks, 1928; James, 1967; Rosen and Larsh, 1944). According to the same authors, infections often appear to begin at the junction of lamina and sheath. Seedlings arising from infected grain may develop a typical scald lesion at the tip of the coleoptile 4-6 days after emergence or may remain symptomless (Habgood, 1971; Ozoe, 1956; Skoropad, 1959).

2.5.5. Summary

Studies on genetics of resistance to scald organims showed that both dominant and recessive genes were active in confering resistance. A total of 11 genes have been reported occuring on the Rh locus in different resistant cultivars. Resistance to scald could also be conferred by partial resistance genes. Environmental factors were also found to be important during infection and in disease development. Temperature and humidity conditions were found to be the most important factors for scald development. Temperatures between 12 and 24°C were reported to be favourable for infection. Past research has also shown that the effect of temperature and symptom expression was dependent on particular combination

of isolate and host phenotypes.

Until now there is no comprehensive research work on Rhynchosporium secalis of barley that has been undertaken in Kenya though it is the most important barley disease. Scald incidences have been found to be high in high altitude areas of Mau Narok, Melili, Molo, Njoro and Timau in years of high rainfall during the barley growing seasons. (Nyachae, pers. comm.; Owino, 1983). Scald incidences are generally low in these areas in years with rainfall of less than 600mm pa. Barley scald has not been observed to occur in hot and lower altitude areas of Ngorengore, Naivasha, Taita-Taveta and Mweiga (Nyachae, pers.comm.).

Field scores on barley nurseries grown in Timau, Njoro, Mau Narok, Melili, Endebess and Molo have shown that R. secalis populations in these areas have the same virulence (Nyachae and Owino, pers. comm.). Differences in scald severity in any particular year in these areas were mainly due to variation in weather conditions (Nyachae, pers. comm.). Some Kenyan isolates of R. secalis were sent to U.S.A. in 1980/81 for analysis using the U.S.A. differential set of cultivars (McDonald, unpublished data). Results indicated that the Kenyan isolates gave reaction types similar to race USI. This race was similar to that which was most prevalent in Canada (McDonald, pers. comm). It was not clear as to which areas the Kenya

isolates were collected and the methods used for sampling. Results from Barley National Performance Trials (BNPT) in 1983 and 1984 showed that 'Bima' and 'Proctor' had an average score (on the 0-9 Saari-Prescott Scale) of 2 and 5 respectively in five test sites namely Njoro, Mau Narok, Molo and Melili.

In the absence of artificial inoculation of barley nurseries in the field (Ch.1), scald was not observed infecting barley at seedling and early tillering stages (Nyachae, pers.comm.). Barley nurseries and especially segregating F_2 to F_8 populations could not be screened against scald during these early developmental stages. Seedling resistance, if evaluated and deployed could be an important factor in retarding scald infection in fields where barley is grown every year with a likelihood of inoculum build-up. Furthermore, through preliminary seedling screening for resistance in the greenhouse, breeders and pathologists can handle large numbers of barley lines and also be able to save on time and space. This research project was formulated due to the little available published and unpublished information on R. secalis of barley in Kenya. It was intended to highlight on infection and development of scald organism under controlled environmental conditions. The results so derived here would then be used in future to predict the behaviour of R. secalis in the field and also stimulate further intensive research on the organims in Kenya.

CHAPTER 3

MATERIALS AND METHODS

3.0 Source of R. secalis isolate

The isolate of Rhynchosporium secalis used throughout these experiments was obtained from National Plant Breeding Station, Njoro. A scald infected plant of the variety "Hege magniff 11966" growing in field 7 was taken. Infected leaves from the plant were cut and arranged between blotting papers in a herbarium press. These leaves were preserved until the time of isolation.

3.1 Isolation and storage of isolates

The artificial medium used for isolation of R. secalis was bacto lima bean agar (Difco). The medium consisted of 62.5g infusion from lima beans and 15g bacto agar per litre. To rehydrate the medium, 23g of lima bean agar was suspended in one litre cold distilled water in a 2 litre conical flask (pyrex) and heated to boiling so as to dissolve the medium completely. It was then sterilized in an autoclave for 15 minutes at 15p.s.i. at 121°C. The medium was cooled in a water bath set at 42°C. When it had cooled down to 42°C, 30ml streptomycin solution (prepared by dissolving 1g streptomycin sulphate powder in 750ml sterile water) was added using a sterilized.

pippete (capacity 10ml). The medium was then aseptically poured into petri-dishes (pyrex-9cm diam.) and let to solidify.

The method described by Schein and Kerelo (1956) was used for isolation of R. secalis from infected leaves. The preserved infected barley leaves of "Hege magniff 11966" were soaked for 5 min. in 100ml sterile water in a beaker. Using a sterilized scapel blade the infected leaves were cut into small pieces about 5 mm long and 3-4 mm wide. Each piece had at least half of its area infected. The leaf pieces were immersed using sterilized forceps into 70% ethyl alcohol in a watch-glass and wetted for 15-20 seconds. Tissues were transfered into another watch-glass containing 5% sodium hypochlorite solution where they were soaked for 90 seconds, making sure pieces were completely submerged. Pieces were finally transfered to agar medium in petri-plates without rinsing. Petri-plates were placed on the laboratory bench. Growth of isolates in the medium was observed after 5 days as light pink colonies at the edge of the leaf tissues. After 10 days, one colony was removed with a scapel blade (sterilized over a flame) and placed in a sterilized watch-glass. The colony was crushed using a rubber policeman in 1 ml sterile water. The spore suspension was added into 9 ml sterile water in a test-tube. After shaking to ensure complete mixing, 1 ml of the spore suspension was pipetted

into another test-tube containing 9ml sterile water. A series of four dilutions was thus prepared. From the last two dilutions in the series 1.0ml was pipetted from each and seeded into fresh lima bean agar in petri-plates. Each petri-plate seeded was swirled so that there was complete distribution of the suspension over the lima bean agar medium. Excess liquid was poured out. After 7 days, single, tiny colonies were observed growing. The colonies were sparse in plates seeded from the last dilution in the series. Only one colony was taken and crushed in a sterilized watch-glass using a rubber policeman. Small crushed pieces were picked using sterilized forceps and seeded into fresh medium for multiplication of inoculum. Rhynchosporium cultures to be used were sub-cultured into freshly prepared lima bean agar (Difco) medium and kept at room temperature. Petri-dishes containing Rhynchosporium colonies to be used later were stored in a refrigerator set at 10°C.

3.2 The plants

Breeder's seed of the two commercially grown barley varieties 'Bima' and 'Proctor' were obtained from National Plant Breeding Station, Njoro. 'Bima' was chosen for its high field resistance to scald whilst 'Proctor' was chosen for its high field susceptibility to barley leaf blotch. 'Bima' a two-row barley variety, was introduced from

Mexico by CIMMYT (Wheat Programme) in 1978 and had pedigree MN/RNR/7/78. 'Proctor' also a two-row barley cultivar was introduced from England after the 2nd world war. It was a cross between B225 x RSH and was given introduction number B102. Seed for the varieties 'Bima' and 'Proctor' used by Kenya Breweries Limited for research originated from NPBS,Njoro. Fourteen plastic pots (19cm diameter) were filled with sterilized soil composed of 1:1:1 of sand, loam and coffee husks respectively. The pots were divided into two halves and each was planted with 'Proctor' and 'Bima' seeds respectively. Each pot was planted with at least 14 seeds. Pots were placed in a greenhouse and were watered daily for 18 days. On the 19th day after planting the seedlings were removed from the greenhouse and placed in a Vindon growth chamber (Fig. 1.).

3.3. Inoculum preparation

Three week old cultures of R. secalis were used for inoculation. Plantings of barley and sub-culturing of the pathogen were done on the same day. Colonies were scraped off from the agar medium using a sterilized scapel blade. The colonies were put into an Osterizer blender after which 200ml of distilled water was added. Maceration of colonies was done for two minutes. Distilled water was added into the Osterizer blender to make upto 500ml. The resultant suspension of conidia, agar and mycelial pieces was filtered through two nematode filter papers (19cm. cotton wool - nylon) to remove mycelial

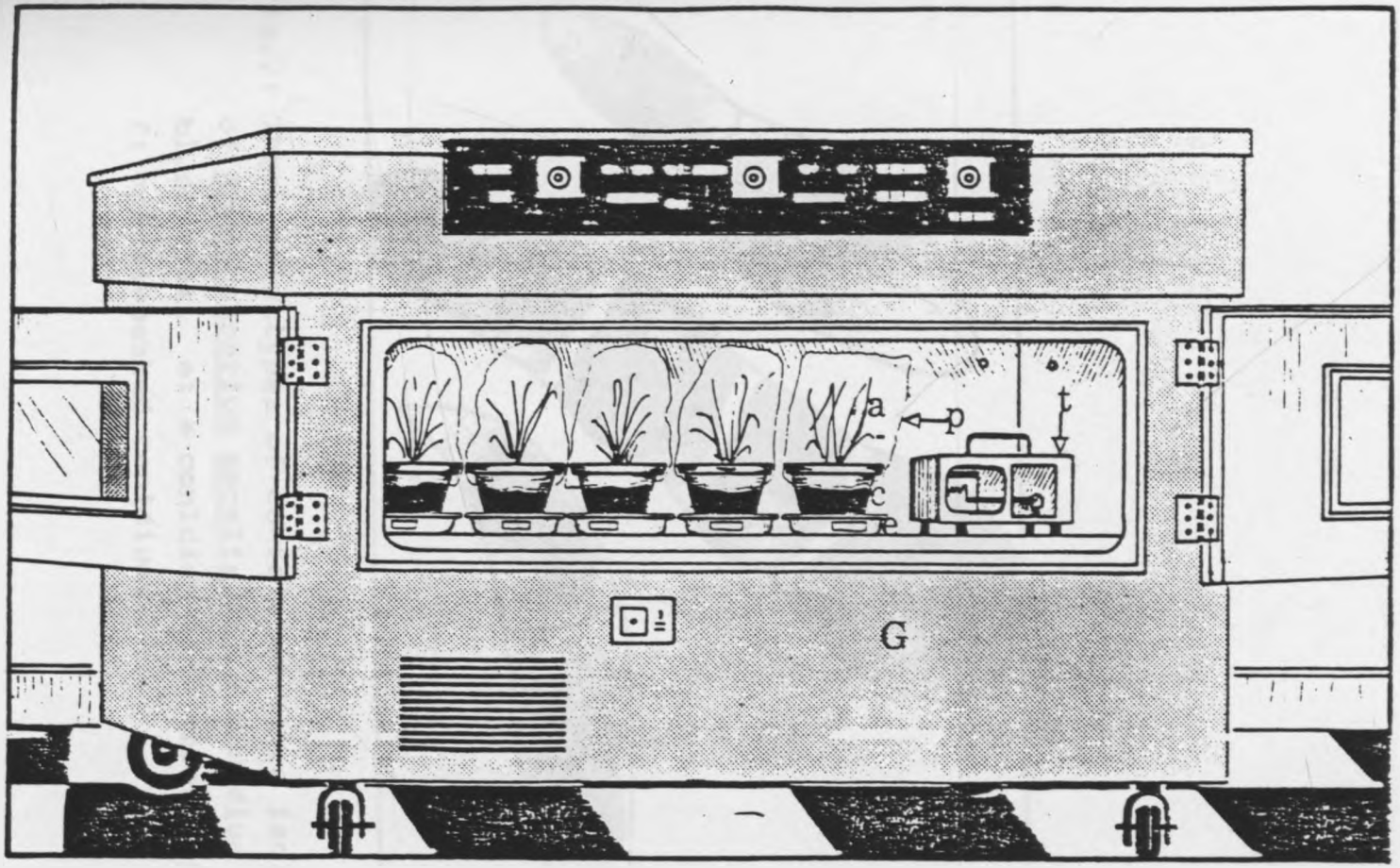

Fig.1: Vindon environmental growth chamber with lighting.
a: barley plants; p: polythene bag; c: plastic pot(19cm diameter);
e: aluminium tray containing water; t: hygrothermograph.

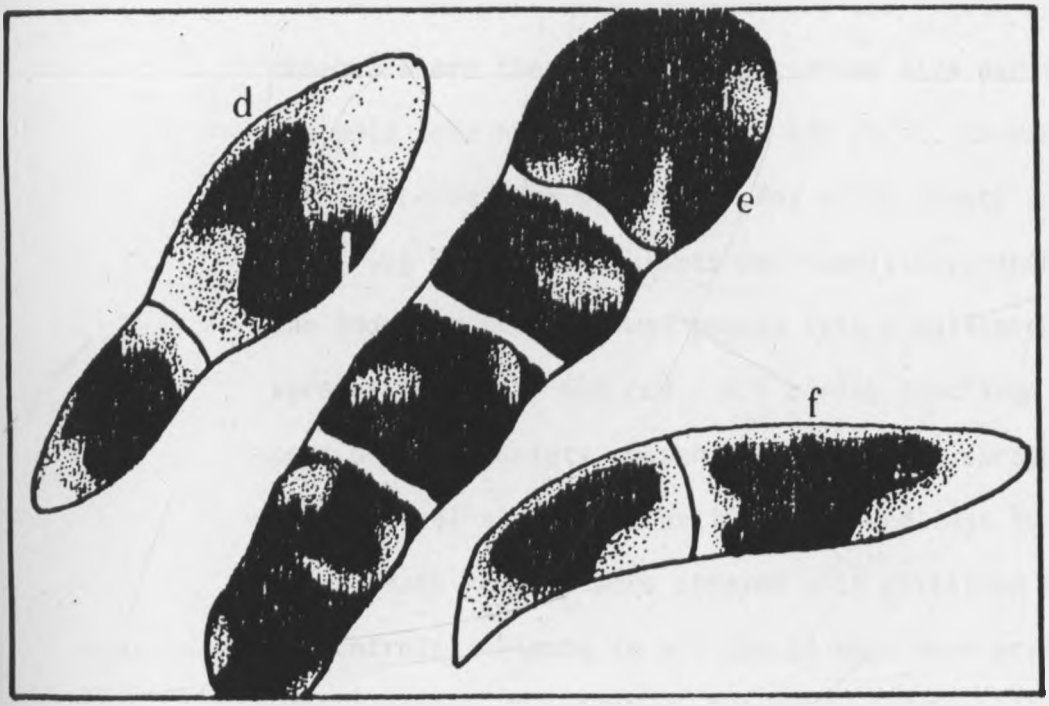

Fig.2: Three main types of conidia of Njoro isolate of Rhynchosporium secalis. d: a conidium with blunt apex; e: a conidium with three septations; f: apical-beaked conidium.

and agar fragments. The spore concentration was determined with a haemocytometer. It was then adjusted by diluting to a concentration of 500,000 spores per ml.

3.4 Inoculation procedure

All the pots were removed from the Vindon growth chamber where they had been placed two days earlier. The pots were numbered 01 to 14 and plants in each pot were numbered from 01 to 10. Any extra plants were removed. Numbering of pots and plants was completely randomized. The inoculum prepared was poured into a Gallenkamp liquid sprayer (capacity 600 cc). All barley seedlings in six pots of each variety chosen at random were sprayed with the conidial suspension until wet. Seedlings in one pot of each variety were sprayed with distilled water as controls. Plants in all the 14 pots were covered with transparent polythene bags (Fig.1) whose inside were sprayed with distilled water to help create high humidity conditions and also maintain leaf wetness for the first 24 hours. The pots were placed in aluminium trays containing water in numerical order. Trays were then placed in a Vindon environmental growth chamber (Fig. 1) with a constant temperature setting of $10^{o}C$ and 95-100% relative humidity. It was also set to give light for 12 hours and 12 hours of darkness. Subsequent experiments were carried out following the same procedure

with the temperature settings adjusted to 15°C, 20°C, 25°C and 30°C respectively.

3.5 Sampling and data collection

Sampling of barley leaves was carried out at specific time intervals at 0 hr, 12 hr, 24 hr and 48 hr respectively. The following procedure was carried out at all sampling time intervals. The lowest numbered seedling in each of the 12 pots (6 pots of each variety) was cut off just below the lowest leaf using a pair of scissors. Then the 2nd and 3rd leaves of the cut plant were cut off near the sheath. Using the pair of scissors the leaves were cut transversally into pieces of about 5-8 mm. long. These were dropped into a McCartney bottle half filled with 1:1 ratio of glacial acetic acid and 95% ethanol. Sampling time, pot number and plant number were marked on the bottle. Decolourized leaf pieces were removed after 48 hours and placed into a McCartney bottle containing methanol and this was preserved at 4°C. At the 48 hr sampling interval, the polythene bags covering the seedling were removed.

Decolourized leaf pieces were removed from methanol and let to dry for about 10 seconds. They were immersed in a watch-glass containing 1% cotton-blue in lactophenol and let to stain for 2 minutes. Excess stain was removed

by holding the leaf piece under running tap water. Differential staining of the leaf pieces was then carried out following the method described by Preece (1959). Leaf pieces were immersed in 1% periodic acid for 10 minutes, followed by washing the pieces in distilled water for 5 minutes. The leaf pieces were immersed in decolourized basic fuchsin for 3 minutes after which they were removed and immersed in sulphurous acid solution for 10 minutes. Finally the leaf pieces were immersed in a beaker containing distilled water for 10 minutes. Each of the leaf pieces was placed in a drop of distilled water on a microscope slide and examined under the light microscope for conidial germination and penetration. A microscope field was chosen randomly on the leaf surface. The number of conidia seen in that field were counted and recorded. Each conidium was recorded as either germinated or not germinated. For all germinating conidia, the presence of absence of an appressorium was noted, penetration or any attempt of penetration through the leaf surface was recorded and the germ-tube lengths were measured using a calibrated scale placed in the eyepiece of the microscope.

3.6 Incubation period and lesion development

At every sampling and every day there after all seedlings were observed for appearance of scald symptoms. After 7, 14 and 21 days scald symptoms were recorded. Leaves with scald had infected areas estimated using

the Rhynchosporium assessment key (Fig. 3) developed by James (1967) or by using tracing paper as described below. Infected areas were drawn underside the paper within the leaf diagram. On subsequent dates of recording, progress of the lesion margins were re-drawn after superimposing the respective leaf on the previously drawn diagram. Total percentage infected areas were determined by direct estimation from the Rhynchosporium assessment key (where it was used) and by using a planimeter on the leaf diagrams drawn.

3.7 Distribution of mycelia

Sub-cuticular mycelia distribution was studied on infected second leaves of barley seedlings numbered 10 in each pot. This was done 21 days after inoculation. Each leaf was cut using a pair of scissors into pieces, 5-8 mm long. These were dropped into a McCartney bottle containing 1:1 ratio of glacial acetic acid and 95% ethanol. The leaf pieces were removed after 48 hr and immersed in 1% cotton blue in lactophenol for 5 minutes. Excess stain was washed off under running tap water. Each leaf piece was mounted in 50% glycerine and examined under low power of the light microscope for mycelial spread below the cuticle.

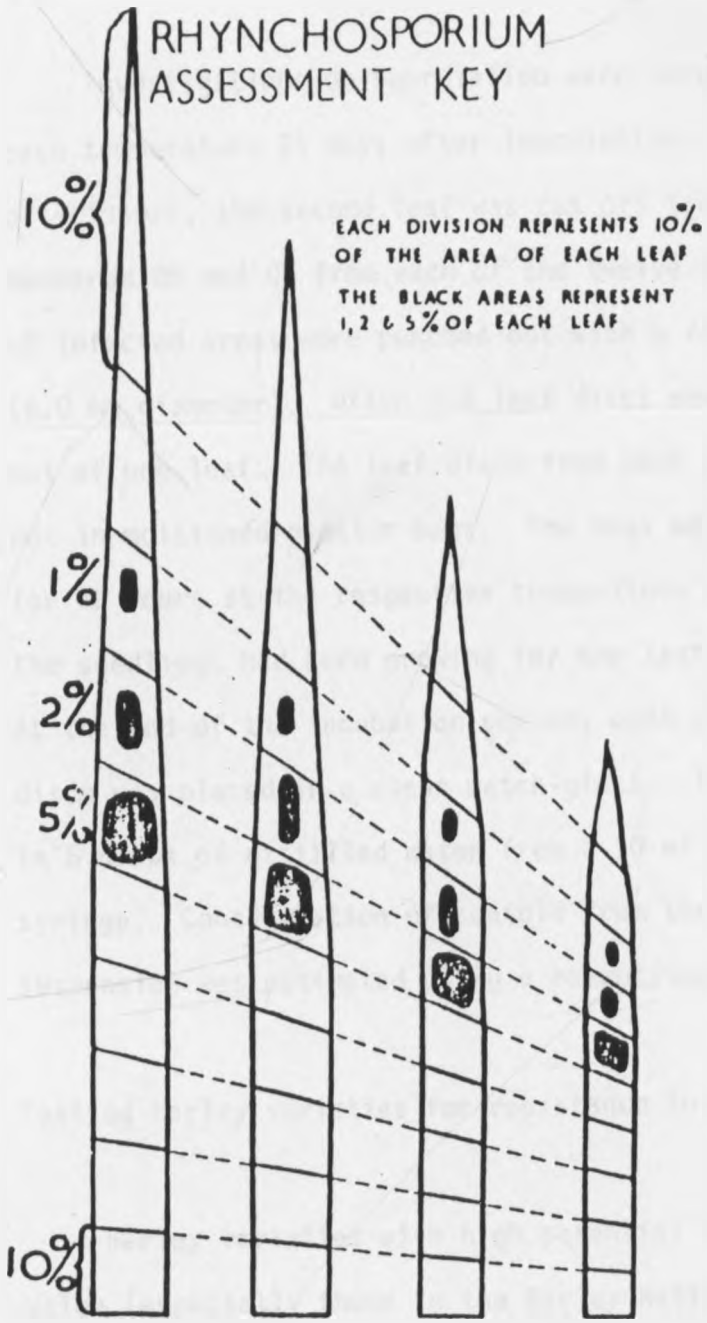

Fig. 3. Rhynchosporium assessment key. Each division represents 10% of the area of each leaf. The black areas represent 1%, 2% and 5% of each leaf (adopted from James, 1967).

3.8 Sporulation

Investigations on sporulation were carried out at each temperature 21 days after inoculation. With a pair of scissors, the second leaf was cut off from seedlings numbered 05 and 06 from each of the twelve pots. Centres of infected areas were punched out with a cork borer (6.0 mm diameter). Often 2-3 leaf discs were punched out of one leaf. The leaf discs from each plant were put in moistened plastic bags. The bags were incubated for 48 hours at the respective temperature for which the seedlings had been growing for the last 21 days. At the end of the incubation period, each of the leaf discs was placed in a clean watch-glass. It was macerated in 5 drops of distilled water from a 10 ml hypodermic syringe. Concentration of conidia from the resultant suspension was estimated using a haemocytometer.

3.9 Testing barley varieties for resistance to *R. secalis*

Barley varieties with high potential commercial value (especially those in the Barley National Performance Trial) from the germplasm were evaluated for resistance to *R. secalis*. Each variety was planted in eight plastic pots (19 cm diameter) filled with sterilized soil medium composed of 1:1:1 ratio of sand, loam and coffee husks.

Every pot had at least 6 seeds planted. The pots were placed in a greenhouse and watered daily. Inoculation was done when seedlings were 14 days old at the second leaf growth stage. Before inoculation all the pots and 6 seedlings growing in each pot were numbered. Numbering for both was completely randomized. The same Njoro isolate of R. secalis used in the other experiments was used for this experiment. Inoculum preparation was done as described in section 3.3. Of the eight pots planted of each variety, barley seedlings in 4 pots were sprayed with the spore suspension and the other 4 pots were sprayed with distilled water. Inoculation was carried out as outlined in section 3.4. The pots were placed in a greenhouse in which temperature fluctuated between 14^{o}C and 23^{o}C. Seedlings were watered daily. The type of reaction for the disease was scored 21 days after inoculation using the following method modified from Schein (1960).

0 - (highly resistant) no visible lesion or symptoms.
1 - (resistant) small lesions at the tip or on the margin and base of the leaf blades.
2 - (intermediate) narrow band of lesion extending
3 - (susceptible) broad well-developed lesions covering large areas.
4 - (highly susceptible) leaves wilted; no evidence of descrete lesions.

CHAPTER 4

RESULTS

4.1 Germination of conidia on leaf surfaces of two barley varieties

The infection of R. secalis started with the germination of conidia to form germ tubes. Germination of conidia was not influenced by the varieties or the interactions between varieties and time and interactions between varieties, time and temperature. Data on percentage germinated spores was transformed as $\sqrt{x + 0.5}$ before the analysis of variance was carried out. Germination was highly influenced by the interaction between temperature and varieties (Appendix 1). Conidial germination was low at 10°C on 'Proctor' leaves. The percent of conidia germinated on 'Proctor' leaves was 1% whilst on 'Bima' leaves it was 0.5%. The difference was however not significant (table 1). Increase in temperature to 15°C showed a slight increase in percent of conidia germinated on 'Proctor' and 'Bima' leaves. Further increase in temperature upto 25°C showed increase in percent of conidia germinated on both 'Proctor' and 'Bima' leaves (Fig. 4). Germination was not influenced by the two barley varieties as the percent germinated conidia on 'Proctor' and 'Bima' was not significantly different at 10°C, 15°C, 20°C and 30°C (table 1). Between 25°C and 30°C there was a drastic decrease of the percent of conidia

Table 1: Influence of temperature on percent germination of *Rhynchosporium secalis* on 'Bima' and 'Proctor' barley varieties.

Temperature (^{o}C)	Percent conidia germinated	
	Proctor	Bima
10	1.0 a	0.5 a
15	1.6 a	1.2 a
20	7.8 a	8.1 a
25	35.3 a	39.2 b
30	0.8 a	0.6 a

Means followed by the same letter in the same row are not significantly different ($P > 0.05$) using LSD.

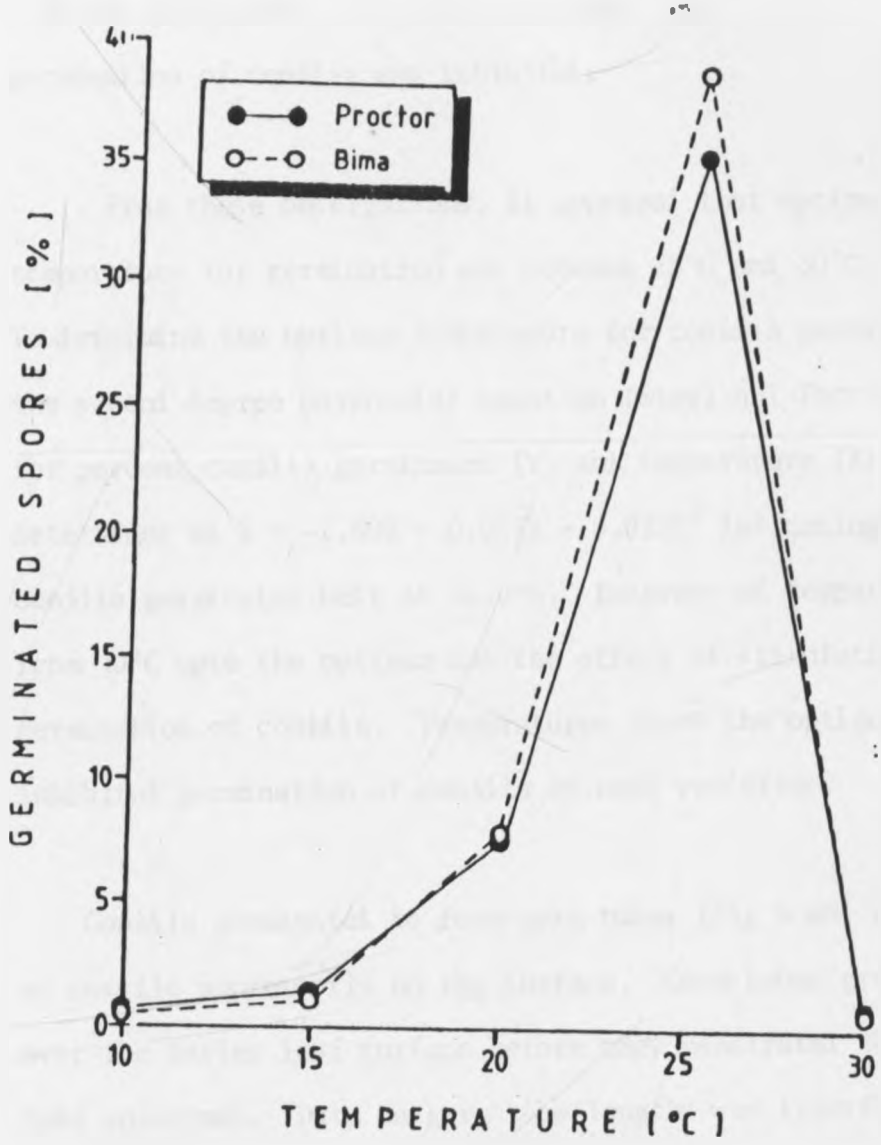

Fig.4. **The influence of temperature on percent germination of <u>Rhynchosporium</u> <u>secalis</u> on 'Bima' and Proctor leaf surfaces.**

that had germinated. It indicated that germination of conidia was inhibited.

From these observations, it appeared that optimum temperature for germination was between 20°C and 30°C. To determine the optimum temperature for conidia germination, the second degree polynomial equation (Steel and Torrie, 1980) for percent conidia germinated (Y) and temperature (X) was determined as $Y = -2.808 + 0.957X - 0.022X^2$ indicating that conidia germinated best at 22.0°C. Increase of temperature from 10°C upto the optimum had the effect of stimulating germination of conidia. Temperatures above the optimum inhibited germination of conidia on both varieties.

Conidia germinated to form germ tubes (Fig 6 and 7) or sessile appressoria on the surface. Germ tubes grew over the barley leaf surface before they penetrated the leaf epidermis. Data on germ tube lengths was transformed $\log (Y + 2)$ before analysis (Steel and Torrie, 1980). Results indicated that the varieties, temperature and the interaction between varieties and time were significant (Appendix 2) whilst the interaction between varieties, temperature and time and that between varieties and temperature were not significant. Germ tube growth increased with increase in time from 0 to 48 hours. Germ tube growth on 'Proctor' leaves for the first 12 hours average 6.8 µm whilst 12 hours

later increase in germ tube length was only 1.4 µm (table 2). Germ tube growth between 24 and 48 hours after inoculation was 3.2 µm on 'Proctor' leaves. The same trend was observed whilst examining germ tube growth on 'Bima' leaves (Fig. 5). This indicated that germ tube growth occured mainly within the first 12 hours after spores had landed on barley leaf surface and under optimum environmental conditions. There was no significant differences observed on germ tubes on the two varieties between 0 and 24 hours after inoculation (table 2). Germ tube lengths were however significantly more on 'Proctor' leaves than on 'Bima' leaves 48 hours after inoculation. The polynomial equation for germ tube length (Y) and temperature (X) was determined as: $Y = 1.89 + 0.20X - 0.004X^2$ indicating that growth was best at $23.5^{o}C$.

Germ tubes grew along the leaf surface before penetration occured. Germ tubes then enlarged at the tips to form appressoria (Figs. 6 and 7). Appressoria could also be formed sessile on conidia in which case no germ tubes were formed. Penetration was also effected by tips of germ tubes without formation of appressoria. Where an appressorium was formed, a penetration hyphae grew on the lower side of the appressorium and this penetrated the barley leaf epidermis. The aspects of conidial germination, germ tube growth, formation of an appressorium and penetration through the leaf epidermis was similar in 'Proctor' and 'Bima' plants. Penetration was direct through the leaf epidermis and no stomatal pore penetration

Table 2: The influence of 'Proctor' and 'Bima' barley varieties on Rhynchosporium secalis conidia germ tube growth (in µm) with time on inoculated leaves

Time (hr)	Germ tube lengths (µm)	
	Proctor	Bima
0	0.0 a	0.0 a
12	6.8 a	6.2 a
24	8.2 a	8.0 a
48	11.4 b	9.2 a

Means followed by the same letter in the same row are not significantly differently ($P > 0.05$) using LSD.

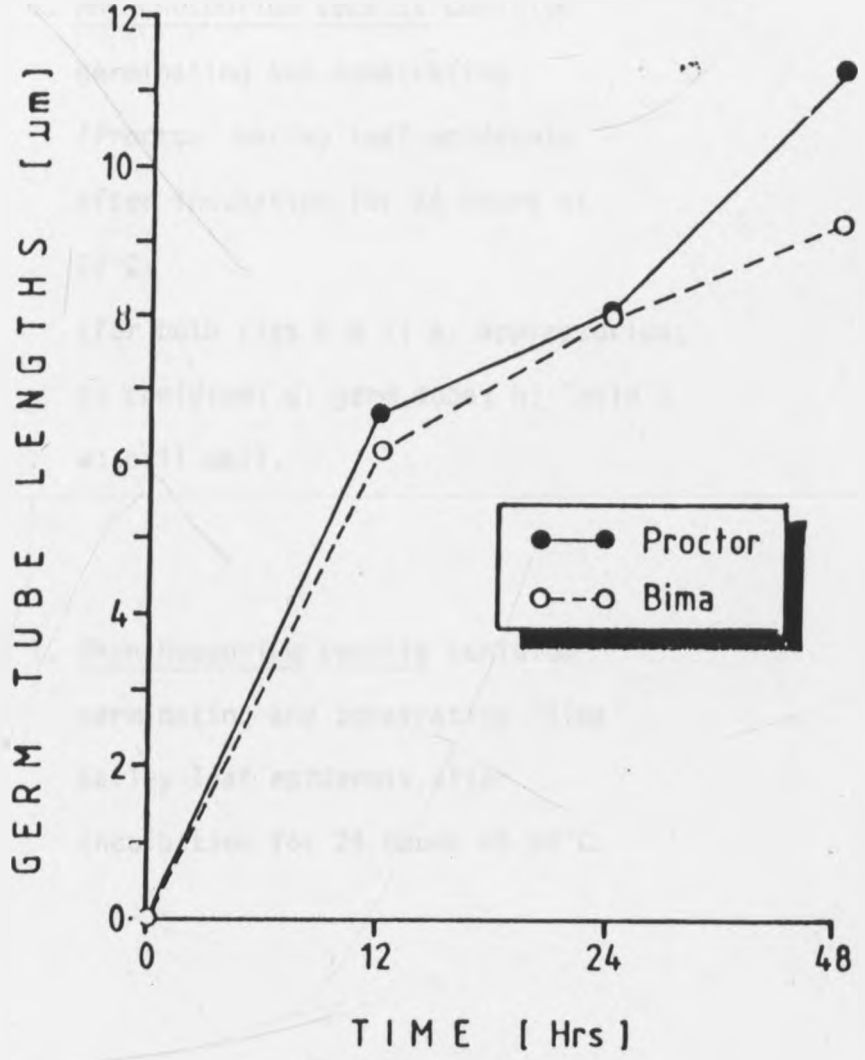

Fig. 5. **The influence of 'Proctor' and 'Bima' barley varieties on Rhynchosporium secalis conidia germ tube development with time on inoculated leaves.**

Fig: 6. Rhynchosporium secalis conidium germinating and penetrating 'Proctor' barley leaf epidermis after incubation for 24 hours at 20°C.
(for both figs 6 & 7: a: appressorium; c: conidium; g: germ tube; h: `halo´; w: cell wall.

Fig: 7. Rhynchosporium secalis conidium germinating and penetrating 'Bima' barley leaf epidermis after inculbation for 24 hours at 20°C.

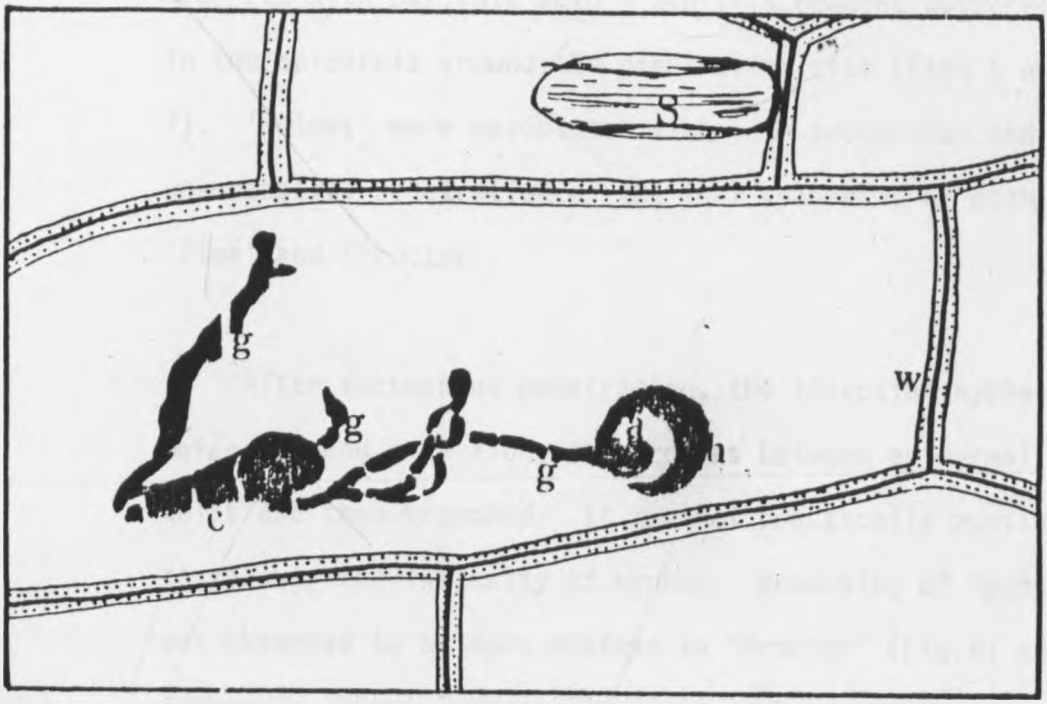

Fig.6.

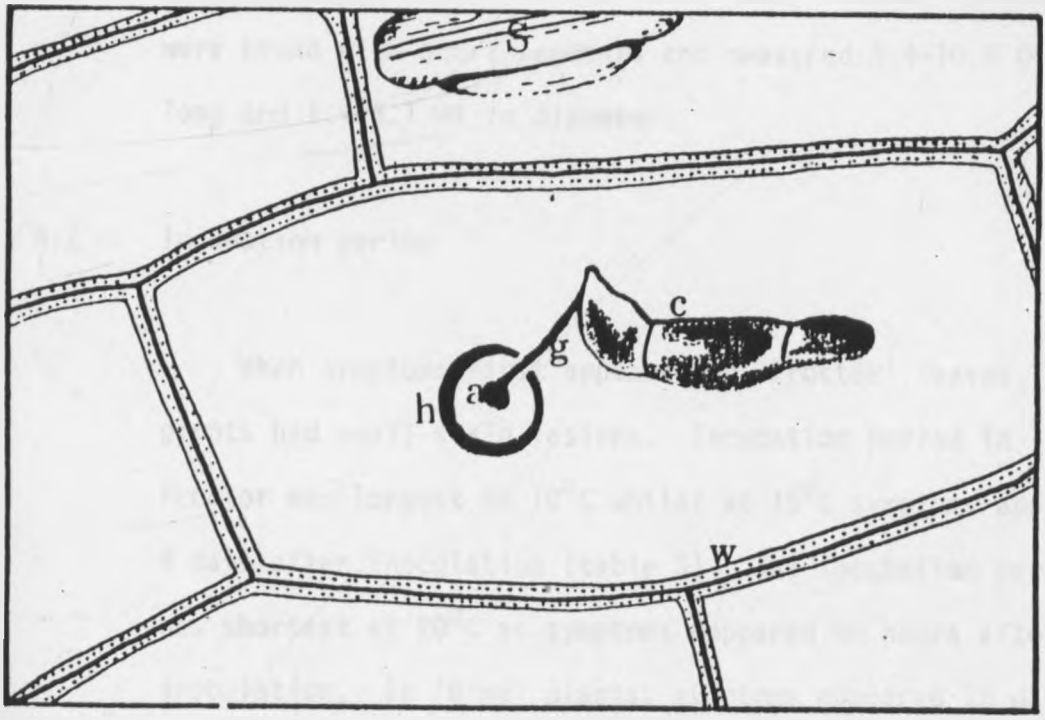

Fig.7.

occured on both varieties. Often, 'haloes' which stained deep red with periodic acid - Schiff's reagent appeared in the epidermis around the penetration site (Figs 6 and 7). 'Haloes' were associated with both successful and unsuccessful penetration of the leaf epidermis of both 'Bima' and 'Proctor'.

After successful penetration, the infection hyphae enlarged and grew along the grooves between epidermal cells and then branched. It was not practically possible to measure the intensity of hyphae. Branching of hyphae was observed to be more profuse in 'Proctor' (Fig 8) and sparse in 'Bima' leaves (Fig.9) at 10°C, 15°C and 20°C. Hyphal spread was sparse in both varieties at 25°C and no hyphae were observed in both varieties at 30°C. Rhynchosporium hyphae within 'Bima' and 'Proctor' tissues were broad with short segments and measured 5.4-10.8 µm long and 1.4-3.1 µm in diameter.

4.2 Incubation period

When symptoms first appeared on 'Proctor' leaves, all plants had small scald lesions. Incubation period in Proctor was longest at 10°C whilst at 15°C symptoms appeared 4 days after inoculation (table 3). The incubation period was shortest at 20°C as symptoms appeared 48 hours after inoculation. In 'Bima' plants, symptoms appeared 15 days, 6 days and 4 days after inoculation at 10, 15 and 20°C respectively. When symptoms first appeared, less than

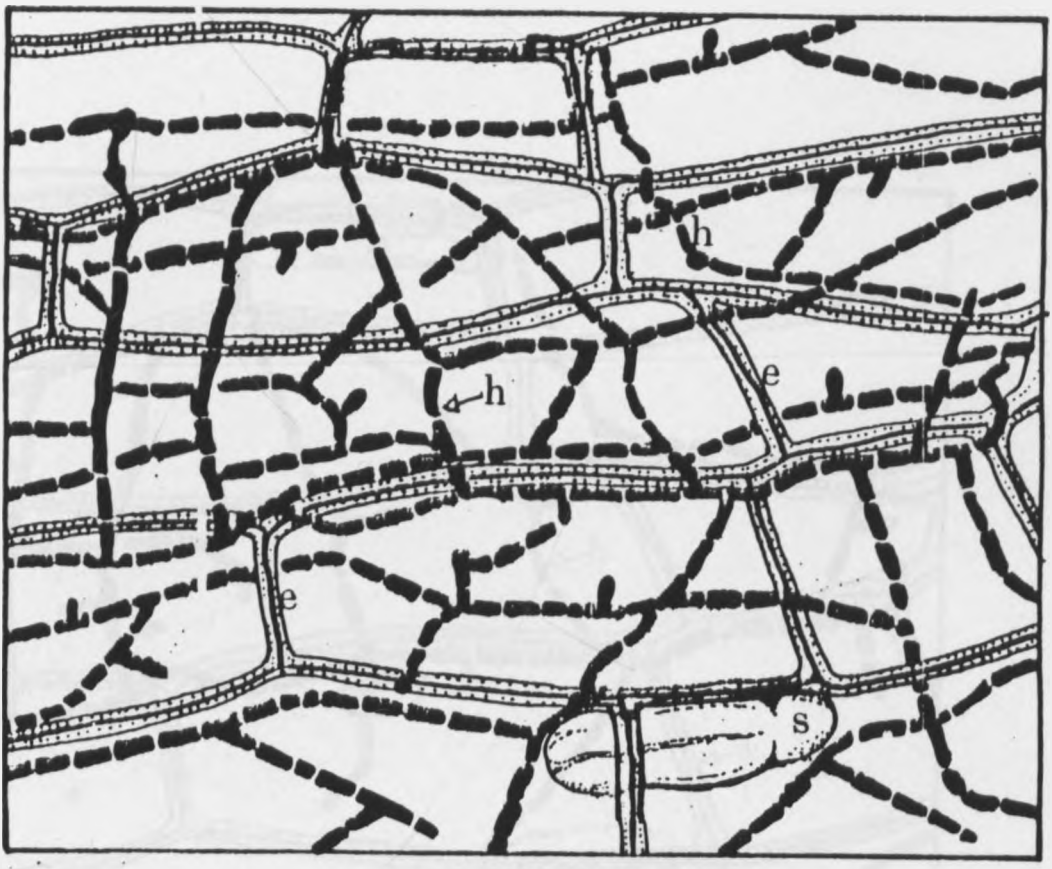

Fig.8. Subcuticular hyphae of Rhynchosporium secalis in 'Proctor' barley leaf at 20°C. h: hyphae; e: epidermal cell wall; s: stomata.

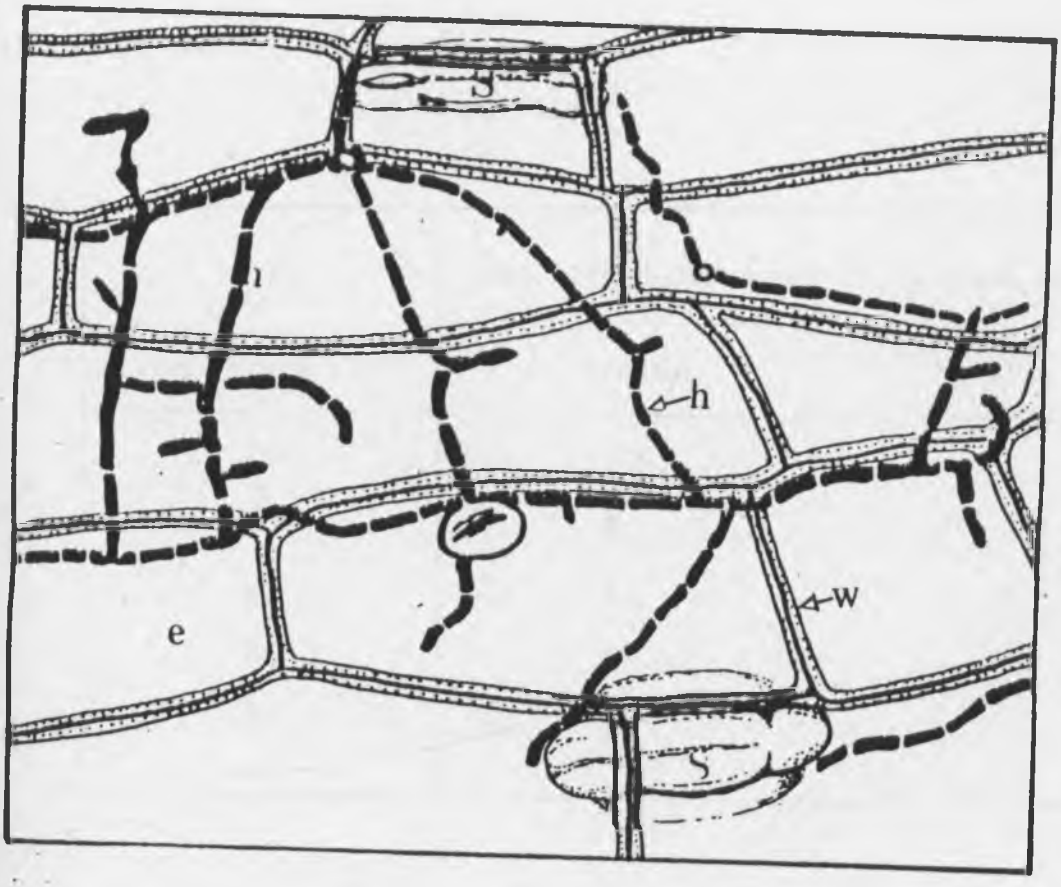

Fig.9. Subcuticular hyphae of Rhynchosporium secalis in 'Bima' barley leaf at 20°C. h:hyphae; w:epidermal cell wall; s:stomata.

Table 3: The influence of temperature and barley varieties on the incubation period (days) in the development of barley scald.

Temperature (^{o}C)	Days before appearance of symptoms on	
	Proctor	Bima
10	10	15
15	4	6
20	3	4
25	4	4
30	-	-

half the number of plants in each pot showed symptoms. There were no symptoms appearing in both varieties at 30°C. At that temperature, plants started yellowing 4 days after inoculation. Yellowing was more pronounced in 'Bima' plants which were more leafy than 'Proctor' plants. Incubation period was longer in 'Bima' than 'Proctor' at 10, 15 and 20°C (table 3). Incubation period decreased with increase in temperature from 10°C to 20°C.

4.3 Development of Scald

The data on scald development was transformed Arcsin$\sqrt{\%\ Y}$ (Steel and Torrie, 1980) before analysis of variance was carried out. Scald in 'Proctor' leaves at 10°C started as dull-white areas before turning into water-soaked dark grey lesions. Lesion development in 'Proctor' leaves was low at the low temperature of 10°C between 14 to 21 days. At 15°C percent leaf areas of 'Proctor' plants with lesions was higher than at 10°C between 7 and 21 days. It indicated that increase in temperature from 10°C to 15°C was favourable for lesion development.

Likewise, increase in temperature to 20°C showed further increase in percent leaf areas with scald lesions in 'Proctor'. The 2nd, 3rd and 4th leaves of 'Proctor' plants were wilted due to scald 21 days after inoculation at 20°C as no discrete lesions were observed. Scald lesions in 'Proctor' leaves at 15°C, 20°C and 25°C were

characteristically water soaked and pale to dark grey (Fig. 10). It was observed that scald lesions at 25°C were less than at 20°C during the same time interval between 7 and 21 days. No symptoms or lesions were manifested at 30°C.

In 'Bima' plants, there was no scald development at 30°C. Leaves had turned yellow 21 days after inoculation at that temperature. No symptoms had appeared 7 days after inoculation at 10°C. Less than 2% of the leaves of Bima had scald lesions 21 days after inoculation at 10°C indicating that lesion development was slow (table 4). Increase of temperature to 20°C showed marked increase on the total leaf surface with scald infection between 7 and 21 days. From 20 to 25°C there was a decrease in the development of scald. Scald lesions (Fig 10) in 'Bima' were often surrounded by chlorotic zones between 10°C and 20°C. It was realised that the optimum temperature for development of scald on barley leaves was between 15°C and 25°C. To determine the optimum temperature, the second degree polynomial equation for percent leaf areas with scald (Y) and temperature (X) was determined as:

$Y = -119.39 + 13.46 X - 0.34X^2$ indicating that scald developed best at 20.0°C. Increase in temperature from 10°C upto the optimum showed better scald development whilst temperatures above 20.0°C showed more inhibition

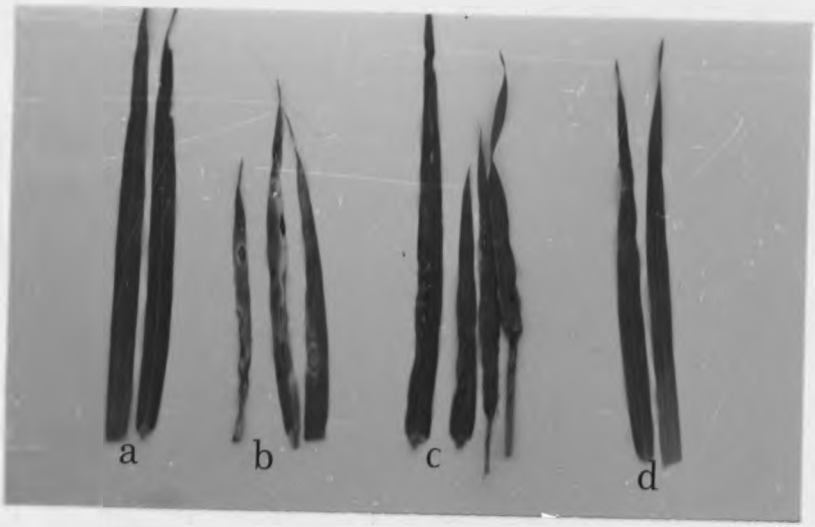

Figure 10: Scald symptoms on 'Bima' and 'Proctor' barley leaves at 20°C

a: healthy 'Bima' leaves

b: scalded 'Bima' leaves (note the chlorotic areas surrounding the lesions)

c: scalded 'Proctor' leaves

d: healthy 'Proctor' leaves

Table 4: The influence of temperature and time on percent Scald lesions on 'Bima' and 'Proctor' leaves

Temperature (^{o}C)	% Scald after 7 days					% Scald after 14 days				
	10	15	20	25	30	10	15	20	25	30
Proctor	0.00 a	6.33 b	4.01 a	1.33 a	0.00 a	0.36 a	21.26 b	76.02 b	17.39 b	0.00 a
Bima	0.00 a	0.25 a	3.32 a	1.10 a	0.00 a	0.54 a	1.56 a	48.64 a	10.72 a	0.00 a

Temperature (^{o}C)	% Scald after 21 days				
	10	15	20	25	30
Proctor	4.01 a	45.77 b	99.18 b	36.68 b	0.00 a
Bima	1.55 a	10.47 a	60.37 a	18.37 a	0.00 a

Means followed by the same letter in the same column are not significantly different ($P > 0.05$) using LSD.

of scald lesion development as the temperature increased. Between the two varieties, significant differences were not detected in percent scald leaf areas 7 days after inoculation (table 4). Significant differences between 'Proctor' and 'Bima' were observed 14 days after inoculation at 15°C, 20°C and 25°C. It was observed that lesions were 15 times and twice as much in 'Proctor' as in 'Bima' at 15°C and 20°C respectively (table 4). Whilst all leaves in 'Proctor' had wilted due to scald 21 days after inoculation at 20°C, leaves in 'Bima' plants were 60% scalded at the same time and temperature which had been determined as the optimum.

4.4 Sporulation

Sporulation of R. secalis on scald lesions were highly influenced by the varieties, temperature and the interraction between temperature and varieties (Appendix 4). There was no scald development in both 'Bima' and 'Proctor' plants at 30°C and there was also no sporulation at that temperature in both varieties. Increase in temperature between 10 and 20°C showed increase in number of spores within scald lesions followed by a decrease in sporulation with further increase in temperature in both varieties (Fig. 11). The largest number of spores was counted in 'Proctor' lesions at 20°C (table 5). At 10°C sporulation in 'Proctor' was

Table 5: Influence of barley cultivars on sporulation of Rhynchosporium secalis on scald lesions at different temperatures.

Temperature (^{o}C)	Number of spores per ml.	
	Bima	Proctor
10	65,000 a	130,000 b
15	130,000 a	370,000 b
20	360,000 a	705,000 b
25	10,000 a	65,000 b
30	0,000 a	0,000 a

Means followed by the same letter in the same row are not significantly different ($P > 0.05$) using LSD

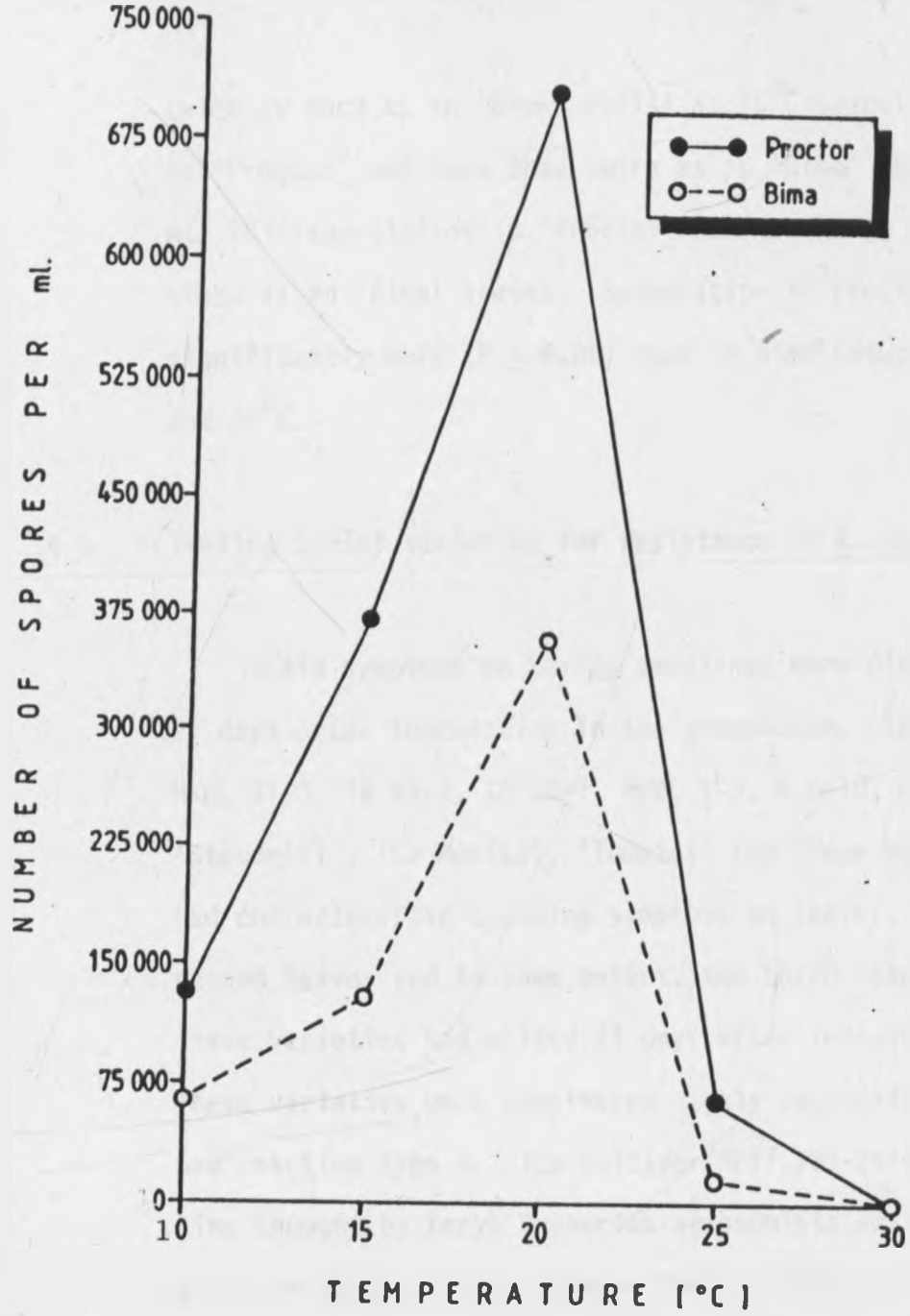

Fig. 11 **Influence of barley varieties on sporulation of Rhynchosporium secalis on scald lesions at different temperatures.**

twice as much as in 'Bima' whilst at 15°C sporulation in 'Proctor' was more than twice as in 'Bima' (table 5). At 25°C sporulation in 'Proctor' was more than six times as in 'Bima' leaves. Sporulation in 'Proctor' was significantly more ($P \leq 0.05$) than in 'Bima' between 10 and 25°C.

4.5 Testing barley varieties for resistance to R. secalis

Scald symptoms on barley seedlings were distinct 21 days after inoculation in the greenhouse. The varieties HKBL 31-3, IB 49-1, IB 66-1, HKBL 1-3, A.0-10, CI, 'Steudelli', 'La Mesita', 'Tumaini' and 'Hege Magniff 11966' had characteristic scalding symptoms on leaves. The second leaves and, to some extent, the third leaves of these varieties had wilted 21 days after inoculation. These varieties were considered highly susceptible and had reaction type 4. The cultivar 'MPYT 169-2Y' was a line thought by Kenya Breweries agronomists as being a sister line to 'Bima' due to their similarity in growth habits in the field. In consideration for release for commercial growing, 'Bima' was prefered because 'MPYT 169-2Y' was susceptible to scald in the field. In this study, 'MPYT 169-2Y' was highly susceptible whilst 'Bima' as a check variety was tolerant with reaction type 2. The variety IB9-2 (Entry no 29) is currently being considered by Kenya Breweries for release to farmers for growing

as malt barley. Recommendations were based on yield, desirable agronomic characters, local adaptation and field resistance to scald. This variety showed reaction type 4 which was considered to be highly susceptible. The varieties 'Bey', 'Forrajera' and 'Abyssinian' (Cl. 3940) were completely resistant as they had no scald lesions on leaves. Varieties that had reaction type 1 (resistant) like 'Jet', 'Trebi', 'Turk', 'Abyssinian 5 (Cl. 4354)' and 'Osiris', had small lesions on leaves covering less than 20%. Scald lesions on these cultivars and those with reaction type 2(intermediate) including 'Nigrinudum', IB 15-1, 'Atlas 46' and 'Abyssinian (Cl 668)' had chlorotic zones proceeding lesion. Chlorotic zones around lesions were not observed in varieties showing reaction types 3 and 4.

Table 6: Reaction of barley cultivars to the Njoro isolate of *Rhynchosporium secalis*

Entry No.	Cultivar	Reaction type
1.	HKBL 31-3	4
2.	Turk	1
3.	IB 41-1	3
4.	H.H 728	3
5.	Nigrinudum	2
6.	Forrajera	0
7.	HKBL 23-5	3
8.	IB49-1	4
9.	Modoc	3
10.	IB 73-1	3
11.	IB 66-1	4
12.	IB 15-1	2
13.	IB 26-1	3
14.	La Mesita	4
15.	B 476	3
16.	B 494	3
17.	Atlas 46	2
18.	Bey	0
19.	HKBL 397-1	3
20.	Abyssinian 5(CI.4354)	1
21.	Abyssinian (CI.3940)	0
22.	Steudelli	4
23.	IB 55-3	3

Table 6 contd.

Entry	Cultivar	Reaction type
24.	HKBL 397-3	3
25.	Osiris	1
26.	Kitchin	3
27.	IB 15-2	4
28.	IB 15-3	4
29.	IB 9-2	4
30.	Jet	1
31.	B 426	3
32.	Trebi	1
33.	Peruvian	3
34.	HKBL 1-8	4
35.	Abyssinian (CI.668)	2
36.	HKBL 23-11	4
37.	MPYT 169-2Y	4
38.	HKBL 31-2	4
39.	IB 61-8	4
40.	HKBL 1-13	4
41.	HKBL 1-14	3
42.	AO-10	4
43.	CI	4
44.	Tumaini	4
45.	Hege Magniff 11966	4
46.	Bima (check)	2
47.	Proctor (check)	4

Table 6 cont.

Key of the reaction types:

0 - highly resistant

1 - resistant

2 - intermediate

3 - susceptible

4 - highly susceptible.

CHAPTER 5

DISCUSSION

5.1 Infection by *R. secalis*

The experiments carried out in this study showed that infection by *R. secalis* on barley leaves started with conidia germination to form germ-tubes or by formation of sessile appressoria on the surface of the conidia under high relative humidity above 90%. Results indicated that conidia were capable of germinating between 10°C and 30°C. Conidia of the Njoro isolate of *R. secalis* were able to germinate at 30°C although the temperature was higher than the range reported by Mamluk (1981) between 6°C and 25°C and Fowler and Owen (1971) between 2.5°C and 27.5°C with an optimum of 20°C. They also observed that few spores germinated outside the range 5-25°C. In this study, increase in temperature from 10°C upto the optimum of 22.0°C stimulated conidia germination.

Several authors (Bartels, 1928; Caldwell, 1937; Fowler and Owen, 1971; Ozoe, 1956; Peresypkin and Drapatyi, 1977; Polley and Clarkson, 1978; Reed, 1957) working with different isolates of R. secalis reported optimum temperature for conidia germination to be between 18-21°C. The Njoro isolate R. secalis required a higher optimum temperature for germination of conidia than those reported by these authors. This could be explained by the fact that the isolate used had adapted to temperatures prevailing in Njoro. The average temperatures recorded at N.P.B.S., Njoro from 1971 to 1982 were 23.3°C (max.) and 9.1°C (min.). Higher temperatures above the optimum supressed germination of spores as was evident between 25°C and 30°C . At 25°C and 30°C conidia that had the ability to germinate did so within the first 12 hours after which no more significant number of conidia germinated. Results showed that factors of or associated with the varieties did not have influence on germination of conidia. Fowler and Owen (1971) also found no evidence that any aspect of spore germination upto and including the formation

of appressoria was influenced by host cultivar. Conidia of Erysiphe graminis D.C. germinated and appressoria were formed at the same rate on all cultivars of barley tested; cuticle was penetrated on highly resistant as well as on susceptible cultivars (White and Baker, 1954). White (1966) found that uredospores of Puccinia graminis Pers. germinated similarly on different wheat cultivars. Germination and stages upto penetration of spores of Venturia inequalis (Cooke) Wint. were comparable on leaves of susceptible, resistant and hypersensitive apple cultivars (Kuc, 1963). Fowler and Owen (1971) concluded that the earliest point at which resistance to R. secalis is manifested in barley leaves is at penetration of the cuticle. Observations made on germination of conidia in this study agreed with the findings of these authors.

5.2 Germ tube development

Germ tubes formed emerged from any free surfaces of the two cells of conidia. The first germ tube usually arose from the bigger cell; where two germ tubes were formed the second usually came from the other cell though both could develop from a single cell. Branching of germ tubes on leaf surfaces was infrequent and never elaborate. Production of more than one germ tube by a conidium observed here has been reported (Ayesu-Offei

and Clare, 1970; Caldwell, 1937). Germ tubes grew over barley leaf surface before penetration of epidermis was initiated. Appressoria formed at the tips of germ tubes initiated penetration. Germ tube tips could also penetrate leaf epidermis without prior formation of appressoria. The optimum temperature favourable for germ tube growth was 23.5 ^{o}C. Increased temperature from 10^{o}C to the optimum stimulated growth of germ tubes. Higher temperatures than the optimum supressed germ-tube growth. The optimum temperature for germ tube growth determined was higher than that required for germination of conidia 22.0 ^{o}C. From these results optimum temperature for germ tube growth was comparable to the average maximum 23.3^{o}C recorded at Njoro. Thus the average maximum temperature in Njoro was not favourable for germination of conidia but was optimum for germ tube growth.

Results from table 2 indicated that germ tube lengths were not significantly different in both varieties 24 hours after inoculation. After 48 hours, germ tube lengths were shown to be significantly less in 'Bima' than 'Proctor'. This suggested that factors within or associated with the variety 'Bima' supressed germ tube growth. After studying several resistant varieties, Ali (1974) concluded that resistance of a barley variety can be showed by rate of germ tube development. The findings from this study supported those of Ali (1974)

but differed from those of Fowler and Owen (1971) who found no evidence that any aspect of spore germination upto and including the formation of appressoria was influenced by host cultivar.

5.3 Penetration of barley leaf epidermis

After growing on the leaf surface germ tubes formed appressoria before penetrating the leaf epidermis. Tips of germ tubes also penetrated the leaf epidermis without formation of appressoria. Appressoria were also formed sessile on conidia as was observed here and reported for the first time by Ayesu-Offei and Clare (1971). Penetration by R. secalis on barley leaves was observed to be direct through the epidermis (Figures 6 and 7). No direct or preference of stomatal pore penetration was observed in this study. The aspect of penetration was similar in 'Proctor' and 'Bima' leaves. Direct penetration of fungal pathogens was first observed by Kuhn and De Bary (Stakman and Harrar, 1957). Bartels (1928) and Mackie (1929) reported that germ tubes of R. secalis entered through stomatal pores. Caldwell (1937) and more recently Jones and Ayres (1974) stated that the fungus entered the host by direct penetration of the cuticle and epidermis. Ayesu-Offei and Clare (1970) did not observe direct penetration of stomatal pores. They saw that the hyphae did aggregate above

the guard cells and the penetrations were effected between the end wall of guard cells and contiguous epidermal cells. They concluded that this aspect may have given the impression of direct stomatal penetration as reported by Bartels (1928) and Mackie (1929).

The deep red 'haloes' around penetration sites were observed by Ayesu-Offei and Clare (1970) who attributed them to toxic metabolites secreted by R. secalis. They concluded that the toxin secreted may be of polypeptide or glycopeptide type. Further research (Auriol et al., 1978; Beltran and Strobel, 1978; Mazars et al., 1983) revealed that the phytotoxin secreted by R. secalis was rhynchosporoside, a cellobioside of alpha-1,2-Propanediol. Penetration and presence of 'haloes' around penetration sites were not different in 'Bima' and 'Proctor' leaves between 10^{o}C and 25^{o}C. Penetration was not observed on both varieties at 30^{o}C neither were appressoria nor deep red 'haloes' observed at that temperature. This indicated that although conidia germinated, no infection occured at 30^{o}C.

5.4 Mycelial development

After successful penetration of the leaf epidermis, the penetrating hyphae enlarged and branched. Results in table 1 showed that at 25^{o}C, 35% and 39% of conidia

had germinated in 'Bima' and 'Proctor' plants respectively. Observations showed that there was sparse mycelial distribution within the leaf tissues of both varieties at that temperature. This indicated that most of the conidia that had attempted penetration did not succeed or those that managed to penetrate the leaf epidermis were not able to branch profusely. Leaves of infected 'Proctor' plants had profuse branching of sub-cuticular hyphae at 10°C, 15°C and 20°C within lesioned areas 21 days after inoculation whilst infected 'Bima' leaves had sparse branching of hyphae within scalded leaf areas. Though it was not practically possible to determine mycelial density, the observations made on both varieties indicated that 'Bima' plants possessed factors that limited lateral spread of R. secalis hyphae within the leaf tissues.

5.5 Incubation period

The trend observed was that incubation period was longer at 10°C and this decreased with increase of temperature to 25°C in both varieties. The incubation period of 3 and 4 days in 'Proctor' and 'Bima' respectively at 20°C differs from the observations made by El-Ahmed (1981) who reported incubation period of 10 days at 20°C. He was however screening lines of the 7th International Barley Observation Nursery (IBON) in the greenhouse where the temperature was always fluctuating.

That could account for the differences in both observations. No scald symptoms were observed in both varieties at 30°C which was shown to be too high for scald infection and development. Scald symptoms appeared earlier in 'Proctor' plants than in 'Bima' plants between 10°C and 20°C. This indicated that 'Bima' plants had resistance factors which delayed appearance of symptoms. To support this observation factors associated with 'Bima' suppressed growth of germ tube growth 48 hours after inoculation and also lateral mycelial development within 'Bima' leaf tissues was limited.

5.6 Scald lesion development

The optimum temperature required for lesions development of 20.0°C was below but near the average maximum temperature recorded at Njoro of 23.3°C. Results also indicated that increase in temperature from 10°C upto the optimum increased the rate of lesion development. The average temperatures prevailing in Njoro of 9.1°C min and 23.3°C max were favourable for scald lesion development. Of the two varieties investigated, 'Bima' plants were found to have less percent of scalded leaf areas than 'Proctor'. Limiting of scald lesion development in 'Bima' may be due to genetic factors within the variety. These factors were not exhibited in 'Proctor'. Probably contributing to the limitation of lesion development in 'Bima' were

the chlorotic zones observed surrounding scald lesions. Ali (1974) in his studies on infection, colonization and symptom expression in barley by R. secalis observed chlorotic regions ahead of the lesions in resistant cultivars studied. Mycelia were found to be sparse in chlorotic regions. The progression of lesions in these varieties were checked. Fowler and Owen (1971) and William and Owen (1975) observed occurences of chlorosis followed directly by necrosis in resistant cultivars studied. Disease progress was inhibited. Relying on the findings of these authors, chlorotic zones around lesions in 'Bima' and absent in 'Proctor' were indicative of resistance reaction in 'Bima'.

5.7 Sporulation

Results of sporulation in lesions of 'Bima' and 'Proctor' leaves indicated that sporulation occured between 10 and 25°C. There was no sporulation at 30°C. Wetness was necessary to enhance sporulation on lesion areas. Temperatures between 15°C and 20°C were favourable for sporulation. Mamluk (1981) reported that at temperatures above 24°C and dry conditions, no conidia were produced. Temperatures between 10 and 20°C have been reported to favour sporulation (Ayesu-Offei and Carter, 1971; Caldwell, 1937; Ozoe, 1956; Rotem et al., 1976; Skoropad, 1957; 1960). Results on sporulation in this study confirmed

the findings of these authors and that Njoro isolate was able to sporulate at 25°C on both varieties. This was above the limit of 24°C reported by Mamluk (1981). Sporulation in 'Bima' lesions were less than in 'Proctor' lesions between 10°C and 25°C. In this study on histopathological development of R. secalis in the two barley host cultivars, 'Bima' plants possessed factors that slowed rate of germ tube growth, limited mycelial development within scald lesions, lowered the percent of leaf areas with lesions and finally less sporulation in lesion areas than in 'Proctor'. These observations indicated that 'Bima' had factors which reduced the rate of disease progress. Parlevliet (1985) considered that such factors were indicative of partial resistance whereby infection occured but the rate of disease progress and spread was lowered. Partial resistance has been reported in stem rust of wheat (Wilcoxson et al, 1974; 1975), wheat leaf rust (Caldwell et al, 1970) and barley leaf rust (Parlevliet and Van Ommeren, 1975). According to these authors, factors that may affect the rate (r) of epidemic development may operate before or after the penetration and formation of infection hyphae. Most authors concluded that differences in resistance between cultivars were only observable after penetration (Parlevliet, 1985).

The rate of epidemic development is reduced when a given inoculum produces lesions that are less in

number, sporulates less and with long latent periods. The characteristics associated with 'Bima' as mentioned above generally show good agreement with partial resistance in the field (Parlevliet, 1975). From the observations, 'Bima' had partial resistance to R. secalis whilst 'Proctor' was highly susceptible. The observations as reported here and those made by Parlevliet (1975) confirm partial resistance in 'Bima' grown in commercial fields. Noteworthy to mention in this study was the observation made by Bockelman et al, (1977) who reported that California and Kenya had the most virulent isolates of R. secalis.

5.8 Testing barley varieties for resistance to R. secalis

The results showed that 'Bima' (check) gave reaction type 2 which was considered as intermediate or a tolerant reaction whilst 'Proctor' had reaction type 4 which was considered to be highly susceptible. This confirmed that 'Bima' had partial resistance. Of particular interest from table 6 were entries coded HKBL***, B*** and IB*** which were entries in the Barley National Performance Trial 1985-86. These cultivars had obviously been tested for the last 3 years at different sites (Molo, Njoro, Timau and Mau Narok) and have been previously selected for their good performance in yield, adaptability and very low scald infection in the fields. All these entries

except IB-1 showed susceptible and highly susceptible reactions contrary to their reactions when tested in the field and which was the criterion used for their selections. Subsequent release of any of these cultivars after field screening will be followed by high susceptibility in commercial fields and finally, withdrawal just like the previous varieties 'Kenya Research' 'Tumaini' and 'Hege Magniff 1966'. Riddle and Briggs (1950) reported that field screening of scald is difficult when other diseases are present especially Helminthosporium spp. and Xanthomonas campestris pv translucens which are also frequent on barley. These authors suggested that solution of this problem could be solved by screening varieties to seedling resistance in the green house.

Varieties showing reaction types 0 and 1 had good resistance to scald. However, these entries were introductions and have not been tested for adaptability, agronomic qualities and resistance to other barley diseases prevalent in Kenya. It is suggested that diallel crosses be made between one or two of these varieties and 'Bima' which is currently the most high yielding and best agronomically adapted barley variety grown. Segregating progenies (F_2 and F_3) of these crosses are to be evaluated for resistance to scald using the method applied here. Riddle and Briggs (1950) recommended that artificial inoculation of segregating materials should be part

of selection process rather than depending entirely on natural infection. To sum up, Saari and Prescott (1977) sees that the problems of breeding for resistance in barley will be a repetition of the history of wheat breeding. Once resistance is incorporated into a barley variety, it will not remain resistant forever. These changes reflect the dynamic ability of the micro-organisms to evolve. As a result the resistant varieties became susceptible and the frustrations of continually requiring to incorporate additional resistance will be necessary.

CHAPTER 6

CONCLUSION

The experiments carried out in this study showed that once landed on barley leaves and in presence of high relative humidity, R. secalis conidia germinated to form germ tubes or sessile appressoria. Barley varieties investigated were found not to influence germination of conidia. Increase in temperature from $10^{o}C$ stimulated more conidia to germinate. The Njoro isolate of R. secalis required $22.0^{o}C$ for optimum germination whilst higher temperatures supressed conidial germination. Germ tubes grew along the leaf surface before penetrating the leaf epidermis. Optimum temperature required for germ tube development was found to be $23.5^{o}C$ for the Njoro isolate. Factors associated with 'Bima' plants suppressed germ tube growth after 48 hours and germ tube development was less than in 'Proctor' plants. Germ tubes formed appressoria at the tips and these affected direct penetration through the epidermal cells. No penetration through stomatal pores was observed. Staining using periodic acid-Schiffs' reagent revealed deep red 'haloes' around penetration sites on leaf tissues.

After successful penetration, sub-cuticular hyphae were formed thereafter branching profusely in 'Proctor' and sparsely in 'Bima'. The incubation periods decreased with increase in temperature from $10^{o}C$ to $20^{o}C$. There were no symptoms development at $30^{o}C$. The incubation period was shorter in 'Proctor'

than in 'Bima' between 10 and 20°C. Scald was found to develop best at 20.0°C. Scald lesions in 'Bima' were preceeded by chlorotic 'haloes' that were not present in scalded lesions in 'Proctor'. Chlorotic zones in 'Bima' were attributed to disease resistance factors within the variety. Results on lesion development showed that lesions in 'Bima' were significantly less than in 'Proctor'. The slow disease progress in 'Bima' was attributed to partial resistance. Sporulation in 'Bima' was significantly less than in Proctor between 10 and 25°C. The factors of infection involving supression of germ tube growth, sparse mycelial development in 'Bima' plants, longer incubation period, chlorotic 'haloes' preceeding lesions, less percent scalded leaves and less sporulation within infected 'Bima' than 'Proctor' leaves were indicative of partial resistance in 'Bima'.

The studies indicated that testing for resistance to scald at two-leaf stage of barley growth gave distinct reaction 21 days after inoculation in the greenhouse. The techniques used here could be applied in future to test other barley varieties both at seedling and adult plant stage to screen barley germplasm for resistance against scald organism. Scald resistance in one or two of the varieties Forrajera, Bey and Abyssinian (C1. 3940) should be incorporated into the agronomically well adapated and high yielding commercial barley variety 'Bima' to make it even more resistant.

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APPENDICES

Appendix 1

Analysis of variance of percent germinated *Rhynchosporium secalis* conidia on barley leaves

Source	df	ss	Mean ss	Fc
Total	239	1169.75		
Treatments	39	1149.24	29.47	287.33**
Varieties (V)	1	0.01	0.01	0.07 NS
Temperature (P)	4	682.10	170.53	1162.76**
Time (T)	3	224.64	74.88	730.14**
V x P	4	1.60	0.4	3.89**
V x T	3	0.15	0.05	0.5 NS
P x T	12	239.75	19.98	194.81**
V x P x T	12	0.99	0.08	0.81 NS
Error	200	20.51	0.10	

C.V. = 13.99%

Key : * Significant ($P < 0.05$)

** Hightly significant ($P < 0.01$)

Appendix 2

Analysis of variance of Rhynchosporium secalis conidia germ tube lengths.

Source	df	ss	Mean ss	Fc
Total	239	145.24		
Treatments	39	141.26	3.62	182.42**
Varieties (V)	1	0.21	0.21	10.42**
Temperature (P)	4	18.64	4.66	234.69**
Time (T)	3	113.57	37.86	1906.64**
V x T	3	0.27	0.09	4.60**
P x T	12	8.04	0.67	33.75**
V x P	4	0.16	0.04	1.99 NS
V x P X T	12	0.37	0.03	1.55 NS
Error	200	3.97	0.02	

C.V. = 7.54%

Key: * Significant ($P < 0.05$)

** Highly significant ($P < 0.01$)

Appendix 3

Analysis of variance of percent scald lesion on barley leaves

Source	df	ss	Mean ss	Fc
Total	179	94573.78		
Treatments	29	91558.54	3157.19	157.06**
Varieties (V)	1	924.27	924.27	45.98**
Temperature (T)	4	4768.34	11992.08	596.57**
Days (D)	2	18974.77	9487.38	471.97**
V x T	4	3117.14	779.28	38.77**
V x D	2	131.61	65.81	3.27**
T x D	8	61.04	12.21	0.61 NS
D x V x T	8	955.84	119.48	5.94**
Error	150	3015.24	20.10	

C.V = 23.84%

Key: * Significant (P < 0.05)

** Highly significant (P < 0.01)

Appendix 4

Analysis of variance of sporulation of *Rhynchosporium secalis* on scald lesions.

Source	df	ss	Mean ss	Fc
Total	119	11.75		
Treatments	9	10.43	1.16	112.59**
Varieties (V)	1	0.87	0.87	84.26**
Temperature (P)	4	9.23	2.31	224.30**
V x P	4	0.33	0.08	7.97**
Error	110	1.13	0.01	

Key: ** Highly significant ($P < 0.01$)