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Rhynchosporium secalis (Oud.) Davis

and

Barley Leaf Scald

in South Australia

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Thesis submitted for Master of Agricultural Science,

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Abstract

Title: *Rhynchosporium secalis* (Oud.) Davis and Barley Leaf Scald in South Australia.

by J.A. Davidson

Mobile nurseries were used to identify races of *R.secalis* in the field. Results were influenced by environmental and genetic effects. Isolate race testing in the glasshouse produced inconsistent and extremely variable results. Cultivar infection rates in mobile nurseries and glasshouse tests were comparable when 'races' were disregarded.

Measurement of disease symptoms (incubation period, spore production, infection rate) in the glasshouse produced inconsistent results. The isolate used affected all factors measured, while barley genotype only influenced the cultivar infection rate. Other factors affecting results were the inoculum concentration, culture viability, culture age and variable glasshouse conditions. Correlations between the glasshouse cultivar infection rate and cultivar field disease levels (%Leaf Area Diseased (%LAD)) varied from nonsignificant to highly significant. The highest correlations occurred when glasshouse infection rates were compared to the %LAD recorded from midseason to late in the growing season. Best results occurred if 'races' were disregarded.

Measurement of disease in field trials resulted in a continuum of disease levels from 0 %LAD to 100 %LAD, rather than discrete groupings of resistant and susceptible types. Yield loss occurred as the result of disease at any stage of the growing season and all yield parameters may be affected. Field %LAD and field infection rates were well correlated, especially at midseason.

It was concluded that races should be disregarded in the study of *R.secalis*. While major genes are definitely involved in the inheritance

of scald resistance, the suspected presence of modifiers affects the expression of the disease, and this is further influenced by environmental factors.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and that, to the best of my knowledge and belief, the thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

I consent to the thesis being made available for photocopy and loan if accepted for the award of the thesis.

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AIMS

This study was designed to produce more information about the fungal pathogen *Rhynchosporium secalis* and the effect of the disease it causes, barley leaf scald, upon the barley crop in South Australia. Information from this study should aid in resistance breeding efforts against this pathogen.

Four aims were set out in this study.

1. The identification of races or pathotypes in the naturally occurring population of *R.secalis* in southern Australia.
2. To test the ability of known resistance genes to suppress disease development in the field when presented with a wide range of *R.secalis* pathotypes collected in southern Australia.
3. To develop glasshouse screening tests that would reflect the same order of resistance or susceptibility of barley lines as was seen in the field.
4. To identify how scald disease damages barley crops in South Australia, and the level of disease that is tolerable ie. does not result in a yield reduction. This would aid in the selection of partially resistant barley lines.

LITERATURE REVIEW

Introduction

Barley leaf scald is a leaf disease of barley which has been reported in many parts of the world. Several common names in use are leaf blight, leaf blotch, leaf spot and leaf scald. Designation of the disease as leaf scald was supported by North American workers who claimed that it was distinctive and would not be confused with other cereal diseases (Caldwell, 1937).

The disease began to increase in significance in different countries from the 1940's to '60's (Reed, 1957, Skoropad, 1960, Connold, 1963, Campbell, 1964, Doling, 1964, Hansen and Magnus, 1968, King, 1972), probably due to increased barley production. This in turn lead to earlier seeding and shortening of crop rotations, and the use of winter crops. Such measures provided a year round host for the fungus. Other factors probably contributing to the increased incidence of leaf scald were the use of combine harvesters which scattered straw and the release of mildew resistant but scald susceptible varieties (Skoropad, 1960, Doling, 1964, Jenkins and Jemmett, 1967, King, 1972).

The causal organism of leaf scald is the fungus *Rhynchosporium secalis* (Oud.) Davis. Its earliest recorded occurrence was in 1894 in Germany when Frank examined herbarium specimens of barley that revealed the presence of leaf scald (Caldwell, 1937). It was originally isolated from rye and described, in 1897, by Oudemans who named it *Marsonia secalis,* later changed to *Marssonina secalis.* Its genus was later transferred to *Rhynchosporium* because of its beaked shaped spores, and given the specific name *graminicola* Heinsen. Finally Davis in the United States named it *Rhynchosporium secalis* (Oud.) Davis, in accordance with the International Rules of Nomenclature (Brooks, 1928).

The fungus can attack any part of the barley leaf, from seedling to senescence (Skoropad, 1960), producing blotches of irregular shape. To a lesser degree the sheath is also attacked (Caldwell, 1937). The auricles are commonly attacked, possibly due to water being held in this region (Brooks, 1928, Smith, 1937, Gilmour, 1967). The blotches first appear as blue-green water-soaked areas and then produce dark-brown edges with lighter centres. These lesions can coalesce and destroy the entire leaf (Brooks, 1928, Caldwell, 1937, Smith, 1937, Gilmour, 1967, Ozoe, 1956). Successive enlargements of lesions may occur giving a zonate appearance (Caldwell, 1937). Occasionally elongated lesions appear which resemble those of barley leaf stripe (*Helminthosporium gramineum*) but leaf scald lesions can be distinguished from these by their dark margins (Smith, 1937). Awns and grain (lemma and palea) may also be infected (Ozoe, 1956, Skoropad, 1960, Gilmour, 1967). Studies on the development of leaf scald on field sown plants have shown that the distal parts of the seedling leaves are liable to infection, while in older plants the middle or basal parts are more commonly affected (Janakiram, 1980). Serious upper canopy leaf damage arises from the transfer of secondary inoculum from the first infected leaves, prior to stem elongation. About two weeks before anthesis there is an explosion of symptoms through the leaf canopy which may result in the defoliation of susceptible types (Janakiram, 1980).

Inoculum

(i) Host Debris

The disease can initially appear in crops due to infection from barley refuse (Caldwell, 1937, Ozoe, 1956, Reed, 1957, Doling, 1964, Ayesu-Offei and Carter, 1970, Polley, 1971). The fungus survives between seasons as dormant mycelium in plant debris and sporulates under the right conditions of moisture, temperature and light (Caldwell, 1937, Skoropad, 1960). The activity of the stroma within the debris is confined to sporulation. Saprophytic growth within or beyond lesions on dead leaves is not detectable. Seedlings may be infected as they emerge and touch the debris or by splashing of spores, via raindrops (Reed, 1957). Scald lesions appear in about 12 days following the first rain after the emergence of seedlings (Skoropad, 1960).

Skoropad (1965) in Canada, found that sporulation was induced by wet periods. Frequent wet periods during winter depleted food reserves of the stroma, by producing successive crops of conidia, without new growth of mycelium. A long freezing winter "preserves" the sporulating potential of the stroma by not inducing sporulation until spring when host crops are growing. Britain has milder winters than Canada, with frequent spells of wet and dry weather, and less complete freezing. Thus a different method of overwintering may occur, in the form of sclerotia like bodies (Polley, 1971). The fungus can also survive in the hot dry summers of Australia (Mayfield, 1982), although reports from Japan state that the fungus does not survive hot temperatures of 30-35^{o}C (Yamada and Shiomi, 1954). Studies seem to indicate that the fungus within the host debris is not viable for a second season (Reed, 1957). The length of time over which *R.secalis* can sporulate in lesions of naturally infected leaves of barley is also influenced by the location of leaves in relation to the soil (Ozoe, 1956, Skoropad, 1960, Skoropad, 1965,

Gilmour, 1967, Mayfield and Clare, 1984), being influenced by invasion of saprophytic micro-organisms (Skoropad, 1965).

(ii) Alternative hosts

Another source of inoculum and a base for further spread of the disease is the range of self-sown plants on which the fungus can survive between growing seasons (Gilmour, 1967). There are two views on the host range of *R.secalis*; first that there is strict host specialisation with different forms of the fungus attacking different host species and second, that there is no host specialisation and many grass species can act as alternative hosts for the barley attacking *R.secalis* (Gilmour, 1967). Plants which have been inoculated with *R.secalis* and so postulated as alternative hosts are species of *Agropyron, Agrostis, Bromus, Cynosurus, Dactylis, Danthonia, Elymus, Festuca, Holcus, Hordeum* other than barley, *Lolium, Milium, Phleum* and *Poa* (Bartels, 1928, Brooks, 1928, Caldwell, 1937, Sarasola and Campi, 1947, Schein, 1958, 1959, 1960, Kajiwara and Iwata, 1963, Kay and Owen, 1973).

In contrast, Caldwell (1937) reported that strict physiological races occurred on six hosts; rye, barley, *Agropyron repens, Bromus inermis, Elymus canadensis,* and *Hordeum jubatum.* Isolates from *H.murinum* could also attack barley. Owen (1958) found strict host specialisation of isolates collected from barley, rye, *Hordeum murinum* and *Agropyron repens*, with *H.murinum* isolates able to attack barley. Kajiwara (1968) collected isolates from barley and rye in Germany. No barley isolates attacked rye and rye isolates showed only very small lesions on barley. This disagreed with work done by Heinsen (1901) and Bartels (1928) and Kajiwara suggested their isolates were not pure. Ali and Boyd (1974) noted that no host outside the genus *Hordeum* developed symptoms under natural field conditions in the presence of plant debris infected with *R.secalis.* They concluded that differences in environmental

conditions, between the field and glasshouse, contributed much to the variability and that under favourable conditions *R.secalis* can develop in the tissue of many host genera.

(iii) Seed transmission

Skoropad (1959) found that seed infection under natural conditions commonly occurred when the seed had filled more than half the space enclosed by the floral bracts. Typical scald lesions appeared on the outer surface of the chaff in 6-10 days after infection. Infection during late-dough stage did not always express scald symptoms on the outer surface of the lemma or palea, but mycelium present on the under surfaces of the floral bracts served as a source of primary inoculum. Scalded seed has been recorded at levels of 2% (Skoropad, 1959) and as high as 36.5% (Kay and Owen, 1973a, Jackson and Webster, 1976). The lesions tend to be on the distal end of the seed, away from the embryo, having got there by way of water droplets running down the awns.

Infection of the new seedling occurs when the plumule breaks through the pericarp at the end of the seed where there is a lesion. The coleoptile develops a prominent scald lesion on its tip 4-6 days after emergence. This location allows entrance of spores into the leaf whorl and so infects the first leaf. From here secondary spores can disseminate. Transmission of the fungus to the seedling has been recorded at 0% (Caldwell, 1937) to 26.2% (Jackson and Webster, 1976c). Environmental effects appear to be important as seen by contrasting reports of little or no seedling infection from heavily infected seed (Caldwell, 1937, Habgood, 1971) to the rapid development of leaf scald in a similar experiment reported by Reed (1957). Optimum soil temperature for infection from seed has been reported as 16°C, with decreasing infection occuring at 20°C, and very slight infection at 22°C (Ozoe, 1956).

Yield Loss in Barley associated with Leaf Scald

James *et al* (1968) presented evidence to suggest that there is a linear relationship between yield loss and the percentage of leaf area affected by leaf scald on the flag and second leaf at growth stage 11.1, Feekes scale (milky ripe). The percentage loss is equivalent to 2/3 the percentage infection on the flag leaf, or 1/2 the percentage infection on the second leaf. Only the two top leaves were assessed since physiological evidence shows that, in Europe, most of the carbohydrate for the grain was produced by these leaves. Yield losses were 30-40% and a decrease in grain weight explained 74% of this loss. Fertile tillers per linear yard were reduced and in one variety the number of grains per head was reduced. James *et al* also noted that sheath infection was related to leaf infection, possibly due to spores being washed down from the leaf to the sheath. Evans (1969) used James' relationship to estimate yield losses in a survey of barley crops in West Sussex, where stubble debris was plentiful. The average percent of flag and second leaves affected, for all crops was 22% at growth stage 11.1, and so the estimated yield loss was 11%. Where stubble debris was hard to find the average amount of disease at the same growth stage was 4%, giving an estimated yield loss of 2%. When barley crops did not follow barley crops *R.secalis* levels were less than 1%, so that yield losses were negligible. Surveys in South West England (Melville and Lanham, 1972) estimated yield losses from 1-4% over 3 years. Fungicide trials in the UK estimated yield losses to be in the range of 30-35% (Jenkins and Jemmett, 1967), and that disease levels at late growth stage had a major effect upon yield (Jenkins and Melville, 1972, Mercer *et al*, 1982).

King (1972) commented on the wide fluctuations in incidence of leaf scald, such as the complete absence in England and Wales, during 1970, thereby indicating the importance of weather during the growing

season. His estimate of yield losses, using the formula devised by James, was between 0% and 1.3%. Brown *et al* (1981) using James' scale concluded that scald reduces barley grain yields by 2-20% each year in Victoria. Because of the epidemic pattern observed there, with scald becoming visible in the latter part of the growing season, it was concluded that most of the yield reduction would be through grain weight loss. Fungicide application was found to significantly increase yield by 24% and the highest correlation was between the amount of disease on the flag leaf at growth stage 11.1 (Feekes scale) and yield loss. This relationship was significant at only one site and so Brown suggests that using such a critical point model might be misleading. Barr and Mayfield (1981) found a relationship between yield and disease levels on the second and third leaves at milky ripe stage. In Japan yield losses have been estimated at 30% due to scald disease (Yamada and Shiomi, 1954). Khan and Portmann (1980) evaluated yield losses in the susceptible cultivar, Clipper, by fungicide application. Significant negative correlations were found between mean leaf area infected with scald at mid-dough growth stage and grain yield and also seed weight. Reduced infection at earlier growth stages (prior to booting) was of further significance in avoiding yield losses. It appeared that genotypes with less than 60% mean Leaf Area Diseased (LAD) and showing a markedly slow rate of disease development are of potential value in Western Australia, with respect to control of leaf scald. Jackson and Webster (1981) in California found that yield increases, brought about by fungicide application, were the result of increased grain number and grain weight. Thus disease pressure occurred early and severely enough to affect floret set as well as grain filling. Schaller (1951), also in California, noted that yield losses were the result of reduced seed number as well as seed weight. Ozoe (1956) noted that scald brought

about reductions in plant height, tillering, number of heads, number of seeds per head, and delayed maturation. Yield was reduced by as much as 45%.

In a controlled environment experiment Mayfield (1982) found that greatest losses occurred when plants were inoculated after stem elongation. This was associated with reduction in root weight, leaf area, water use and a delay in anthesis. Yield losses, at a maximum of 30%, were due to a reduction in the number of heads produced per plant. Earlier inoculations lead to fewer primordia being initiated. In field trials disease in early crop growth stages was more effective in reducing yield than disease later in the season. This may have been associated with a low level of disease in the trial, which was rarely above 10% LAD.

Environmental Factors Affecting Growth and Development of the Fungus

Temperatures of 15-20°C are optimal for infection (Ozoe, 1956, Ryan and Clare, 1975), with a maximum at 25-30°C and minimum at 0°C. Greater infection occurs if the plants are incubated at 10-18°C for at least 6 hours after inoculation (Ozoe, 1956). Ryan and Clare (1975) state that the optimum period of leaf wetness after inoculation is 14 hours, with a minimum of 2 hours. With a relative humidity (RH) below 87% no infection occurs, the optimum is above 92% (Ozoe, 1956). Light, immediately following inoculation, reduces the total lesion area (Ryan and Clare, 1975), thus shading plants in the field enhances susceptibility (Ozoe, 1956). Water congestion of leaves and low temperature (eg. 10°C) for one day predispose plants to infection (Skoropad, 1962a).

(i) Germination

R.secalis spores are hyaline and sickle shaped, with a beak at the apex, and 12.4-20 *u*m x 4.9*u*m in size. Usually they consist of only two cells (Ozoe, 1956) and the upper cell germinates first, with the germtube swelling from the side of the cell or the tip of the beak. The lower cell may then send out a tube (Caldwell, 1937).

Germination of spores is reduced by light (Ozoe, 1956) but resumes when spores are transferred to the dark (Ryan and Clare, 1975).

The optimum temperature for germination is 15-20°C (Caldwell, 1937, Yamada and Shiomi, 1954, Ozoe, 1956, Schein, 1960, Fowler and Owen, 1971, Ryan and Clare, 1975). The minimum ranges from 0-2.5°C and the maximum from 25-32°C (Caldwell, 1937, Ozoe, 1956, Fowler and Owen, 1971). The thermal death point of conidia is 44°C in moist conditions for 10 minutes. Conidia can survive at -20°C for 24 hours and -30°C for 3 hours. In dry heat conidia die at 95°C in 10 minutes (Ozoe, 1956). Conidia of all isolates fail to germinate at the extremes of 2°C and 31°C, but if they are then transferred to 19°C, those at the lower temperature will germinate (Reed, 1957).

Spores germinate most readily between pH 4 to pH 6 (Ozoe, 1956, Reed, 1957, Schein, 1960), although germination still occurs at a reduced rate at pH 1.6.

The optimal RH for germination is between 98.2% and 100%; no germination occurs below 91.2% (Ozoe, 1956).

On agar films, increasing drop size and spore concentration decrease the percent spore germination. *R.secalis* spores produce a self-inhibitor of germination, the effectiveness of which is increased by increasing the concentration of spores, and decreased by increasing the availability of exogenous nutrients. Barley leaves supply a source of exogenous nutrients irrespective of their susceptibility to *R.secalis* (Ayres and

Owen, 1970), so no decline is seen on intact plants or on detached leaves (Fowler and Owen, 1971).

(ii)Germ tube growth and penetration

As with spore germination, growth of the germ tube is retarded by light (Ryan and Clare, 1975).

The optimum temperature for germ tube growth is 15-21°C (Caldwell, 1937, Yamadi and Shiomi, 1954, Reed, 1957, Fowler and Owen, 1971, Ryan and Clare, 1975), within the range of 2°C to 31°C (Reed, 1957).

The germ tube forms a round structure at the end, an appressorium, from which penetration occurs. Beneath the appressorium the outer epidermal wall thickens to form a rounded papilla, several times the thickness of the wall, which projects into the lumen of the cell. The penetrating hypha grows through the outer epidermal wall, possibly aided by enzymic degradation (Ayesu-Offei and Clare, 1970), and then into the subcuticular position (Caldwell, 1937). Penetration occurs between the end walls of guard cells and contiguous epidermal cells (Ayesu-Offei and Clare, 1969). This may explain why Bartels (1928) and Mackie (1929) reported entry via the stomata directly to the mesophyll. Jones and Ayres (1974) found that any hyphae directly entering the stomatal pores were superficial hyphae which developed from mycelial fragments inoculated with the atomised spore suspension. Penetration may occur on either side of the leaf and usually within 48 hours of the spore germinating (Caldwell, 1937).

The host shows an increase in stomatal opening where the leaf has been infected. This may be due to a drop in turgor pressure of the epidermal cells, or dead epidermal cells, which do not oppose the opening of the guard cells (Ayres, 1972).

(iii) Hyphal growth and lesion development

Following penetration, the infecting hyphae branch profusely (Caldwell, 1937), growing between the pectic layer and outer layer of the epidermal wall. (The adaxial surface consists of wax, cuticle, pectic and outer and inner layers of the epidermal cell wall). The pectic and cuticular layers remain intact until conidia are produced (Ayesu-Offei and Clare, 1970, Ryan and Grivell, 1974), whereas the cell wall is degraded and replaced by hyphae (Ryan and Grivell, 1974). The cuticle over the hyphae is stretched but not ruptured, and any cell wall degrading enzymes the fungus secretes at this stage act in a very localised area (Jones and Ayres, 1974).

Hyphae are small for several days, 0.6 *u*m diameter (Caldwell, 1937), while the normal diameter is 2-3.0 *u*m (Caldwell, 1937, Ozoe, 1956). They are septate and can form wide globular cells or dumb-bell shaped cells (Ozoe, 1956). The subcuticular mycelia are responsible for the grey appearance of the area of infection on the leaf (Caldwell, 1937). The hyphae grow in regions rich in pectic substances, often along lines where adjacent epidermal cells meet. Infections cause localised thickenings of cell walls in regions close to the hyphae. Close to subcuticular hyphae, short lengths of the plasma lemma of epidermal cells may be separated from cell walls (Jones and Ayres, 1974). This action may be responsible for changes in permeability of the membrane reported by Jones and Ayres (1972). This in turn causes concentration of nutrients to increase in the free space between cells of susceptible cultivars, and also to some extent in resistant cultivars.

The outer epidermal wall collapses first and then the inner. The hyphae then pass through epidermal cells to the mesophyll (Caldwell, 1937). Thus the mesophyll cells are not affected until epidermal cells have begun to collapse. This accords with carbon-dioxide fixation rates not

being affected until this stage (Jones and Ayres, 1972). Hyphal growth in the mesophyll is inter-cellular. Cells collapse and cause typical lesions (Caldwell, 1937). Hyphae in the mesophyll do not develop laterally, so increase in lesion diameter is due to growth of subcuticular mycelia (Ayesu-Offei and Clare, 1970). The mass of fungus here exceeds that in the mesophyll (Caldwell, 1937).

Cell collapse may be brought about by toxic substances (Ayesu-Offei and Clare, 1971). When cut stems of susceptible barley seedlings are immersed in sterile culture filtrates, leaves develop grey water-soaked patches similar to those produced on infected seedlings, within one hour of treatment. Microscopic examination shows the collapse of cell walls, and respiration rates increase with this treatment (Jones and Ayres, 1972). A toxin has been isolated, named rhynchosporoside, from diseased plants, which is able to cause marginal necrosis of the leaf tip and chlorosis of the entire leaf (Auriol et al, 1978). Binding between rhynchosporoside and barley membrane proteins increases with the susceptibility of the host (Mazars et al, 1983).

As with conidial germination and germ tube growth, mycelial growth of *R.secalis* is more rapid in the dark, than the light (Ozoe, 1956).

The optimum temperature for growth is 16-18^{o}C, with a maximum at 25-30^{o}C (Ozoe, 1956, Fowler and Owen, 1971), and a minimum of 0^{o}C (Ozoe, 1956). The thermal death point for mycelium is 46^{o}C for 10 minutes. Hyphae are resistant to low temperatures: -20^{o}C for 24 hours and -30^{o}C for 3 hours. (Ozoe, 1956).

Optimum pH ranges from 5.17 to 6.93 (Yamada and Shiomi, 1954, Ozoe, 1956, Schein, 1960), and growth does not occur outside pH 2.35 and pH 11.5 (Ozoe, 1956, Reed, 1957, Schein, 1960).

Different isolates are capable of utilising sucrose, glucose or maltose and starch, different nitrogen sources and vitamins as food sources. A

starch hydrolysing enzyme is produced in advance of the fungal mycelium, when grown on agar, the active agent being either an alpha-amylase or a dextrinising starch enzyme (Schein, 1960). *R.secalis* is reported to produce cellulolytic enzymes that enable it to use cellulose as the sole carbon source (Olutiola and Ayres, 1973a). The fungus can grow and sporulate in liquid nutrient media that contain glucose, galactose or galacturonic acid, or any pair of these, as the sole carbon source. Glucose is the most common carbohydrate in the free space of the leaf, galactose the most common aldose, after glucose, in barley leaf cell walls and galacturonic acid is the primary constituent of pectic substances (Olutiola and Ayres, 1973b). Growth is greatest on glucose and least on galactose, and increases with a mixture of glucose and galacturonic acid (Ayres and Olutiola, 1973, Olutiola and Ayres, 1973b). The same workers found nitrogen to have no effect upon mycelial growth. Jenkyn and Griffiths (1976) found that the greatest proportions of glucose and peptone (ie. Carbon and Nitrogen) gave the most mycelial growth. On media with high C and low N, mycelia was dark, sporulated less, was closely septate and cells were often thicker walled and rounded, such as were found on corn-meal agar, barley grain and straw. They postulated that these conditions may lead to the production of sclerotia-like bodies which overwinter in Great Britain.

Large quantities of manure or nitrogenous fertilisers are reported to increase the occurrence of the disease (Ozoe, 1956). Other workers say there is no clear relationship between nitrogen levels and leaf scald (Jenkins and Jemmett, 1967), while Jenkyn and Griffiths (1976) found higher nitrogen levels decreased lesion number, though lesions did develop sooner. Susceptibility of cultivars was found to be negatively correlated with water soluble carbohydrate (WSC) and positively correlated with nitrogen, at growth stage 10.4 (Feekes scale) (Jenkyn

and Griffiths, 1978). Application of nitrogen, which reduced WSC, increased the severity of scald.

(iv) Sporulation

Sporulation occurs after the complete breakdown of the leaf tissue in the infected spot. It is most abundant in the central and most collapsed area of the lesion (Caldwell, 1937). Conidia form directly from hyphae, without conidiophores (Brooks, 1928, Caldwell, 1937, Ozoe, 1956), though some early reports (Davis, 1922) suggested conidiophores did occur in the substomatal stroma. The cuticle above the subcuticular stroma was reported as remaining intact until masses of conidia bud and protrude through the cuticle, cracking it (Brooks, 1928, Ayesu-Offei and Clare, 1970). Thus the stroma is protected until conditions are favourable for conidial production (Ayesu-Offei and Clare, 1970). Sporulation occurs only on the side of the leaf where infection has occurred, owing to the manner in which the stroma develops (Caldwell, 1937). However, hyphae may also grow through the mesophyll from inoculated leaf surfaces to form substomatal stroma beneath uninoculated surfaces. Conidia then extrude through stomatal pores (Ayesu-Offei and Clare, 1970).

Relative humidity above 95% and temperatures between 15°C and 20°C are optimal for spore production of *R.secalis* (Caldwell, 1937). The fungus sporulates readily and abundantly when infected material is floated in water for 48 hours at 10-18°C. Higher temperatures, 27-30°C, under moist conditions, prevent production of new spores and cause old ones to swell and rupture (Skoropad, 1960). Light has not been reported as having any effect upon sporulation (Boyd, Khan and Shearer, 1969).

Olutiola and Ayres (1973b) found sporulation was inhibited by high concentrations of glucose and galacturonic acid. Nitrogen concentration affected sporulation in a complex manner. Jenkyn and

Griffiths (1976) found spore production tended to be greatest with high peptone (Nitrogen) and low glucose (Carbon).

More spores are produced on leaves when discrete lesions develop, than when considerable leaf area becomes chlorotic via atomising spore suspensions, or if there are two or more lesions per leaf (Fowler and Owen, 1971).

(v) Spore Dispersal

The earlier the crop is sown, the greater is the disease intensity (Ozoe, 1956, Jenkins and Jemmett, 1967, Khan *et al*, 1968, Marshall *et al*, 1971, Melville and Lanham, 1972). This may be associated with spore production being at a maximum earlier in the season (Mayfield, 1982), as higher inoculum concentrations increase the disease levels on susceptible cultivars (Evans and Griffiths, 1971, Habgood, 1972).

Ozoe (1956) found numerous conidia in the air adjacent to the soil surface between the rows of barley plants. The conidia decrease in number with height; there being very few in the air at one and half times the height of the crop. The number in the air is influenced by atmospheric conditions, being closely correlated with rain. Numerous conidia were contained in the drops of rain dripping from the surface of diseased leaves. Ozoe found numbers of conidia in the air to be higher during the day than night but Ayesu-Offei and Carter (1971) found no difference in spore numbers released during the day or night. Spread from an infection point is not large; the maximum distance reported is 15 metres (Ozoe, 1956, Ayesu-Offei and Carter, 1971). Ayesu-Offei (1971) found sporulation occurred most abundantly when free water was available and conidia were released with rainfall or irrigation. They were frequently trapped as groups of two to ten. Fewer were trapped in windy conditions without rain. Wind tunnel experiments showed that the conidia are not readily dislodged by wind alone, but strong winds

cause swaying and vibrations so the plants rub together dislodging conidia. These observations support the view that release and dispersal of conidia is mainly due to water splash. The maximum number of spores produced in a field experiment in one day was 272 (Ayesu-Offei 1971), a number far below that of rusts or mildews, and so it is unlikely that *R.secalis* will spread from field to field in a single growing season. Polley (1971) found epidemic development to be closely associated with the frequency of periods of 12 hours or more when relative humidity did not fall below 90% and precipitation occurred at least 9 hours before the end of the period. The associated temperature mean was not less than 10°C during the period. Stedman (1980) used a rotorod to sample spores in the air, during rain. The number of spores was not associated with the duration or quantity of rainfall, nor to the maximum or mean rate of fall. Most lesions were found when rain ceased during the late evening or continued through the night so that plant surfaces remained wet for several hours.

Races of *Rhynchosporium secalis*

The first study indicating variability in virulence of a *R.secalis* population was by Sarasola and Campi (1947) when they found four races of the pathogen in Argentina. Reed (1957) confirmed these results and Schein (1957, 1958, 1960) established a total of seven races in USA. Race studies have been carried out in Canada (Skoropad, 1960), Bulgaria (Dodov, 1963), Great Britain (Owen, 1963), Japan (Kajiwara and Iwata, 1963), Australia (Ayesu-Offei, 1971, Ali et al, 1976) and California (Jackson and Webster, 1976a).

Elsewhere such a high degree of variability in virulence was found, that identifying the isolates as specific races was abandoned and in Great

Britain the original eight races (Owen, 1963) were simplified to only two. Jackson and Webster (1976b) found variation could be produced in the glasshouse. Using a mixture of five isolates representing five races of widely varying virulences they carried them through two successive disease cycles and an intervening saprophytic stage. Besides the five original races being re-isolated, 14 hybrids were formed. The original races differed in their sporulating ability in culture and host tissue but this was not related to virulence.

Further variability may be apparent due to aggressiveness of the isolates decreasing with successive transfers on agar. No changes are seen if isolates are passed through hosts of differing resistances (Kajiwara and Iwata, 1963).

The high degree of variability in the virulence of the Australian *R.secalis* population has been attributed to the influence of the genetically diverse barley grass (*H.leporinum*) population, since few resistant genes occur in locally grown cultivars of barley (Ali, 1981).

Variation in virulence between spores from the same lesion was found by Hansen and Magnus (1973). Habgood (1973) found variation in aggressiveness and conidial production of single spore isolates collected from a single lesion. Since this variation within isolates would affect epidemiological studies on virulence and aggression, Habgood suggested that a population approach was needed rather than using single spore isolates as representatives of particular races.

Resistance

Genetic studies of the host reaction to scald have been reported over the past 50 years, indicating that both specific and nonspecific resistance occurs within the barley genome. Studies done at the microscopic level have shown that any one *R.secalis* isolate will germinate similarly on all hosts (Fowler and Owen, 1971, Ayres and Owen, 1971, Ali, 1974). One study concluded that leaf washings and intercellular fluid from a susceptible barley cultivar stimulated germination of conidia, while those from a resistant one partly retarded conidial germination (Doken, 1981). Resistance can be expressed at penetration, such that specific resistance allows only very slight penetration and non-specific resistance may show reduced penetration (Fowler and Owen, 1971). However, spores of *R.secalis* may establish subcuticular hyphae in resistant cultivars, though visible symptoms may not occur (Ayres and Owen, 1971, Ali, 1974). The frequency and rate of extension of hyphae is greater in susceptible than resistant cultivars (Ayres and Owen, 1971) and in general the extent to which mycelium proliferates below the cuticle is positively correlated with severity of symptoms (Fowler and Owen, 1971).

Cuticle thickness does not contribute to resistance (Ayres and Owen, 1971), though abrasion of the leaves gives earlier development of symptoms in susceptible lines, and greater mycelial development in resistant lines (Ali, 1974). After the fourth day from inoculation the susceptible cultivars support more mycelia and a faster rate of mycelial growth than in resistant cultivars (Ayres and Owen, 1971). Extracts from the darkly pigmented lesion margins inhibit germ tube elongation and oxygen uptake of hyphal suspensions. *R.secalis* mycelium does not penetrate through pigmented areas (Ayesu-Offei, 1971) which appears to be a resistant mechanism. Possibly, its usefulness is reduced in

susceptible cultivars where many cells are destroyed before pigment is produced (Ryan, 1975).

In resistant cultivars, a few abnormal conidia may be produced when there are no symptoms. Host age, duration of incubation period and temperature alter the expression of resistance in some cultivars. Temperature, as well as affecting resistance, can impair the virulence of an isolate. Spore concentration ($2.5x10^5$ to $1x10^7$) and culture age (21-70 days) does not alter resistant reactions (Schein, 1960, Ali, 1974). However, cultivars that are not immune may show increased lesion development with increased spore concentration (Evans and Griffiths, 1971, Habgood, 1972). Janakiram and Boyd (1980a) suggest that all genotypes can exhibit symptoms of scald infection but differ in the extent and rate to which symptoms occur. Variability of symptom expression is not qualitative, but is subject to a number of influences such as environment and genetic background effects.

(i)Specific Resistance

Resistance was first discovered by Mackie (1929) in an unspecified cultivar, and was found to be controlled by a single recessive gene. Riddle and Suneson (1948) established that resistance existed in the cultivars Trebi (CI836), Algerian (CI1179), Wisconsin Winter (CI2159), Telli (CI194), Osiris (CI1622), Sheba (CI4359) and Abyssinian (CI1233). A composite cross involving the first three resulted in 17 resistant selections, including Modoc (CI7566). Turk (CI5611-2) and Atlas (CI4118) were backcrossed to produce the resistant Atlas type, CI7189. This was later crossed with the mildew resistant Atlas (Hanna x Atlas) to produce Atlas 46 (CI7323). This was released in 1947, but by 1956 was susceptible to 39% of *R.secalis* races in California (Webster et al (1980).

Turk has the strongest form of resistance (Riddle and Briggs, 1950), controlled by a single dominant gene (Riddle and Briggs, 1950, Baker and Larter, 1963, Wells and Skoropad, 1963, Evans, 1969a, Starling et al, 1971). This was identified as Rh3 (Dyck and Schaller, 1961a, Evans, 1969a). Crosses with Atlas revealed the presence of one or more additional genes (Riddle and Briggs, 1950) and this was identified as Rh5 by Dyck and Schaller (1961a)

Riddle and Briggs (1950) found that La Mesita differs from Atlas, in scald resistance, by a single dominant gene. Trebi and California No.1311 have a dominant and recessive gene for resistance, Trebi being one of the parents in the composite cross that produced 1311. The dominant gene is the same as that in La Mesita. One of the genes in Turk appeared to be identical to the one in La Mesita, California No.1311, and Trebi. These three show a moderate amount of disease, which may be due to modifying genes, multiple alleles or different but closely linked genes.

Bryner (see Dyck and Schaller, 1961a) found a single dominant gene in Brier (CI7157), which he labelled Rha, later changed to Rh. Dyck and Schaller (1961a) found five dominant genes conferring resistance against the seven US races: Rh2 in Atlas and Atlas 46, Rh3 in Atlas 46, Turk, and possibly in Brier, Rh4 in La Mesita, Trebi and Osiris, an allele of Rh4, $Rh4^2$, in Modoc, and Rh5 in Turk. They found Rh3 and Rh4 to be closely linked and on chromosome 3 (Dyck and Schaller, 1961b). Evans (1969a) confirmed the presence of Rh3 in Atlas46 and Turk, and Rh4 in Osiris. Wells and Skoropad (1962) found a single dominant gene in Turk, Osiris, Bey (CI5581), 36Ab1991, Rivale, CI3515, and CI8256, and a single recessive gene, rh8, in Nigrinudum (CI2222). All seven of these may have had Rh3 or more than one gene with close linkage. Rh3 was confirmed as being on chromosome 3. Baker and

Larter (1963) found Jet (CI967) and Steudelli (CI2266) to have two complementary recessive genes, which they designated as rh6 and rh7. These are temperature sensitive and breakdown above 25°C. Abyssinian (CI668) and Kitchin (CI1296) have an incompletely dominant gene Rh9, which is complete when in the homozygous form, but not in the heterozygous form.

Frecha (1967) found Psaknon (CI6305) and Osiris (CI1622) carried a common dominant factor for resistance. Atrada x Atlas (CI7189) carries two factors, one of which is the same as in Psaknon and Osiris. Starling *et al* (1971) found Trebi, Atlas, Turk, Brier , LaMesita, Modoc and CI8256 to segregate on the basis of a single gene, and Atlas 46 and CI3515 on two genes. The gene in Hudson was found to be at the Rh-Rh3-Rh4 locus. The Atlas gene (Rh2) segregated independently from this locus. The gene in CI8618 is not allelic to Rh2 or the Rh-Rh3-Rh4 locus (Evans, 1969a). There is a single dominant gene in Atlas 46, Turk and Osiris. Since these are allelic or closely linked it is probable that Rh3 is in the first two and Rh4 in the last.

Habgood and Hayes (1971) investigated the genetic resistance in 18 cultivars, ten of which had been studied before. The recessive gene, rh11, was allocated to CI4364 and CI4368. They suggested that the Rh3 gene, of Turk and Atlas 46 genome, was in fact the Rh gene of Brier, and so dropped the 3 suffix. They found five alleles occurred at this Rh locus, two were dominant (Rh and Rh^2), two were incompletely dominant (Rh^3) and Rh^4) and one was recessive (rh^5). Two pairs of complementary genes were found to exist in this list of 17 cultivars; Rh^4 and Rh10 , and rh^5 with rh6. On the basis of this multiple allelism and complementary gene explanation , Habgood and Hayes (1971) suggested new symbols be used to designate the resistance in the cultivars.

Ali (1975a) identified three genes in Psaknon, two genes in Atlas 46, Atlas 57, and Hudson, and one in Turk. It was suggested that these have identical alleles (Ali, 1974). La Mesita, West China and Sakigake have resistance genes at different loci from each other. These genes are greatly affected by environmental conditions. One of the genes in La Mesita, Rh4, appears to be linked to Rh3 (Ali, 1975b). Habgood and Hayes (1971) claim that these two genes are alleles.

(ii) General Resistance

Some cultivars have a general resistance which enables them to show less disease than other cultivars, against isolates to which they are not specifically resistant (Fowler and Owen, 1971). These have been found to be penetrated less by the fungus than other cultivars, and more mycelia is associated with any particular level of severity of external symptoms than with the more susceptible lines. Some non-specific resistance has been shown to result in less sporulation (Fowler and Owen, 1971, Habgood, 1977).

In general, nonspecific resistance can be characterised by less leaf area damage from the same spore dosage (Habgood, 1972, 1977, Williams and Owen, 1975) This reduction of leaf damage has been found to be due to a reduction in lesion number, rather than lesion size or time to lesion development (Williams and Owen, 1975, Habgood, 1977).

Evans and Griffiths (1971) showed percentage infection to be associated with resistance levels. Habgood (1972) used ED50 (conidial concentration required to give a 50% leaf area diseased) to study Vulcan, which is highly resistant in the field, but not in the glasshouse. Precise tests with graded inoculum levels showed Vulcan had an ED50 that was 4.8 times that of the susceptible Maris Otter and 4.1 times that of Maris Puma. He hypothesised that this resistance was under monogenic control. The

inheritance of partial resistance of other cultivars was more complex involving more than four genes (Habgood, 1974).

Jackson and Webster (1981) found that the life span of leaves was a key factor in non-race-specific resistance. The shorter the life span, the less time there was for spores to establish and spread up the plant.

Habgood (1975b) used infection rates, calculated from proportion of leaf tissue infected and the time between assessments, to compare cultivars. The lowest infection rate occurred on most resistant types. However cultivars with a prostrate early habit had an infection rate higher than would be expected from the resistances that were known. Such a high infection rate is difficult to detect in the glasshouse or in microplot tests.