Rhynchosporium secalis (Oud.) Davis

and

Barley Leaf Scald

in South Australia

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Abstract

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

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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.

CHAPTER 1

A SURVEY OF THE VARIATION IN VIRULENCE OF *RHYNCHOSPORIUM SECALIS*, THE CAUSAL PATHOGEN OF BARLEY LEAF SCALD, IN SOUTH AUSTRALIA.

INTRODUCTION

Before a resistance breeding programme can be undertaken it is necessary to know the range of virulence in the natural population of the pathogen concerned. Studies of this nature by Ayesu-Offei (1971) and Ali *et al* (1976) indicated the extensive range in virulence of the *Rhynchosporium secalis* population in southern Australia, with the latter author identifying 35 pathotypes. As so many pathotypes would make a screening programme cumbersome, it was decided to study the pathogen population in South Australia further to identify the most frequent and stable pathotypes that occurred. The feasibility of combating these by a resistance breeding programme can then be considered. Two methods were used to study the virulent range of *R.secalis* i) mobile nurseries and ii) glasshouse testing.

GENERAL MATERIALS

Ali *et al* (1976) used 21 barley genotypes as the set of differentials for testing isolates for their virulence range. It was decided to use this same set (Table 1, page 3) as any other differential set described in the literature appeared to be a subset of that used by Ali. Galleon, a commercial cultivar in South Australia, was also included as it appeared to have some resistance when grown in the field, though no resistance gene was known in its genotype.

TABLE 1

CULTIVARS USED AS DIFFERENTIALS TO PATHOTYPE ISOLATES OF *R.SECALIS*

Cultivar	**CI number**	**Resistance gene**
Abyssinian	CI 1233	Rh9
Atlas	CI 4118	Rh2
Atlas 46	CI 7323	Rh2Rh3
Brier	CI 7157	Rh
Clipper	CI 14844	nil
Galleon	CI 2331b	nil
Gospeck	CI 9094	minor genes
Hudson	CI 8067	Rh2Rh3
La Mesita	CI 7565	Rh4Rh14
Modoc-California	CI 7655	Rh4 [2]
Nigrinudum	CI 2222	rh8
Osiris	CI 1622	Rh4
Psaknon	CI 6305	Rh2Rh3Rh13
Sakigake	CI 7388	Rh12
Sultan	CI 5577	Rh3 [a]
Turk	CI 5611-2	Rh3Rh5
WestChina	CI 7556	Rh15Rh16
WisconsinWinterxGlabron	CI 8162	Rh [3]
	CI 3515	Rh3
	CI 4364	rh11
	CI 8618	unknown

A. MOBILE NURSERIES

Materials and Methods

Seeds of the 21 differential cultivars were surface sterilised with sodium hypochlorite and germinated on wet filter paper over two days. They were then treated with Milstem (a.i. ethirimol) to protect them against *Erysiphe graminis hordei* (powdery mildew) infection. A plastic tray of the dimensions 45cms x 50cms x 10cms was used for the mobile nurseries. Six half centimetre diameter holes were drilled in the bottom of the trays to allow water drainage. The trays were filled with potting mix to one centimetre below the lip of the tray and the soil was dampened. Holes, two centimetres deep, were made in the soil to receive the germinated seeds. There were 23 rows, slightly less than two centimetres apart, and ten holes per row, three centimetres apart. Ten seeds per cultivar were planted in a single row, one seed per hole. Three rows of the susceptible cultivar, Clipper, were sown: one at each end of the tray and one in the middle, to assess the efficacy of the test. When the plants were at the 1-2 leaf stage the trays of plants were placed in barley crops, or barley stubble from the previous year's crop, at sites within the state's barley growing area. (See Map 1., next page) This was done during autumn-early winter, when crops were being established, and again in spring, when crops were at growth stage 30 (Zadoks scale) ie. stem elongation. The lip of the tray was embedded to soil level so that the splash of rain droplets, containing spores, was not obstructed by the sides of the tray. These trays were left in the field for two weeks to enable infection to occur. They were then returned to the glasshouse to await the development of leaf scald over the next three weeks. Disease was scored as a frequency of the number of plants per cultivar with scald lesions.

MAP 1

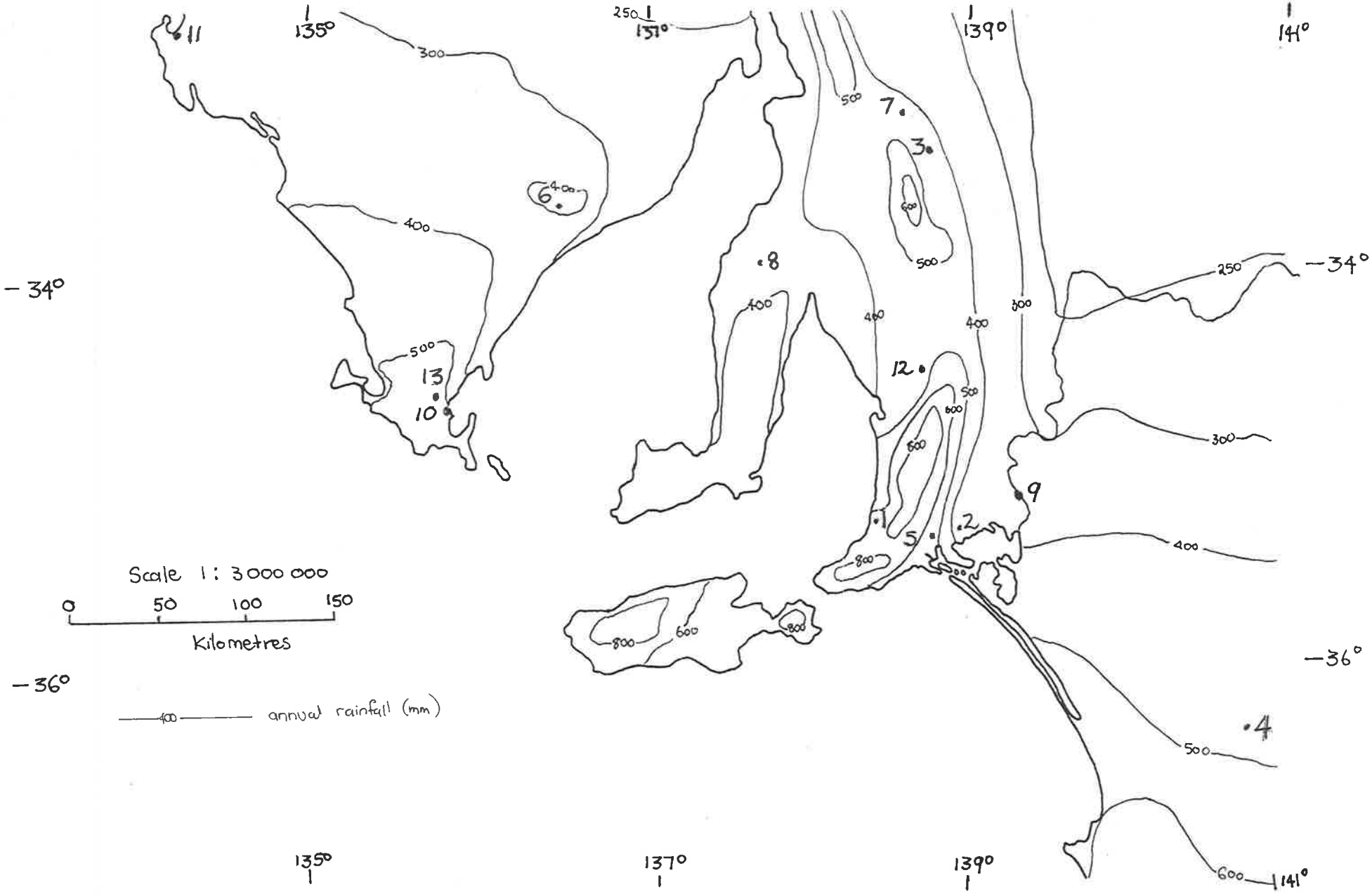
Mobile Nursery Sites- nurseries were placed for two weeks at the indicated sites in barley crops or barley stubble and then transferred to the glasshouse at the Waite Institute to await development of scald disease.

		Mobile nurseries placed in field during-		
		Autumn 1983	Spring 1983	1984*
Site no.	**Site**	**M.N.No.**	**M.N.No.**	**M.N.No.**
1	Aldinga	1	11	
2	Angas Plains	2	12	22,23
3	Booborowie		13	
4	Bordertown	3		
5	Charlick	4	15	24,25,26
6	Cleve	5	14	
7	Jamestown	6	16	
8	Kadina	7	17	27
9	Murray Bridge		18	
10	Port Lincoln	8	19	
11	Streaky Bay	9	20	
12	Turretfield	10		28
13	Wanilla		21	

*M.N.No.	Date Assessed	M.N.No.	Date Assessed
22	28/5/84	26	2/10/84
23	17/7/84	27	10/7/84
24	15/6/84	28	4/7/84
25	22/8/84		

M.N.No. is the Mobile Nursery Number

Map Reference-'Atlas of South Australia' ed. T. Griffin & M. McCaskill, S.A. Govt. Printing Division 1986


135°
137°
139°
141°
250
300
400
500
600
800
11
6
7
3
8
13
10
12
1
2
5
9
4
−34°
−36°
Scale 1 : 3 000 000
0 50 100 150
Kilometres
400 annual rainfall (mm)

Results

The results of each mobile nursery tray at each site is shown in Tables 2, 3 and 4 (Pages 7,9,10). In 1983 there was extensive development of scald disease, as indicated in these tables, while in 1984 severe wind and cold weather so adversely affected seedlings that few could be scored for disease. There was also less scald disease apparent in barley crops in this season, compared to the previous season.

Table 2 (page 7) is the first (autumn) set of 1983 mobile nurseries, that were put in the field during crop establishment. The number of diseased Clipper plants in a mobile nursery indicated the success rate of the test. Mobile nurseries from three sites showed no scald disease, and the Jamestown (M.N.No.6) nursery showed very little disease; Clipper was the only cultivar there with lesions. In nurseries from other sites, Clipper's disease incidence varied from 100% to 30% of plants with lesions. The range of cultivars in a mobile nursery that developed scald lesions varied between sites. Disregarding the mobile nurseries where there was no disease, Jamestown nursery had the narrowest range of diseased cultivars; Clipper was the only cultivar diseased. The Streaky Bay (M.N.No.9) nursery had the widest range of cultivars diseased with eleven cultivars showing lesions. A high disease incidence on Clipper was not always associated with a wide host range as can be seen by the Port Lincoln (M.N.No.8) nursery.

TABLE 2

CULTIVAR INFECTION RATIO IN MOBILE NURSERIES THAT WERE PLACED IN THE FIELD DURING CROP ESTABLISHMENT, 1983

M.N No.	1	2	3	4	5	6	7	8	9	10
Cultivar										
Abyssinian	Nil		Nil		Nil					
Atlas		7/10		3/10			2/10		6/10	
Atlas 46										
Brier		6/10		7/9			5/10		8/10	1/10
Clipper		29/29		22/25		6/27	15/27	25/28	29/30	9/30
Galleon		9/10		8/10			1/9	8/10	9/9	1/10
Gospeck		4/10		2/10			3/8		5/10	1/10
Hudson										
La Mesita							5/10		10/10	
Modoc[i]									10/10	
Nigrinudum									7/9	
Osiris										
Psaknon										
Sakigake									8/10	
Sultan									8/10	
Turk										
West China							2/10		5/7	1/10
WWxG*										
CI3515										
CI4364										
CI8618										
Total number of diseased cultivars in mobile nursery (maximum 21)	0	5	0	5	0	1	7	2	11	5
Total number of diseased plants in mobile nursery (maximum 230)	0	55	0	42	0	6	33	33	105	13

NB. Denominator indicates the number of surviving plants per cultivar

M.N.No. is the number assigned to the mobile nursery

* Wisconsin Winter X Glabron

[i]Modoc-California

Table 3 (Page 9) is the second set of mobile nurseries for 1983, that were put in the field when crops were at growth stage 30 (Zadoks scale). ie. stem elongation. This set had a higher level of disease than was seen in the earlier set. Only one nursery had no scald disease while three nurseries had 100% of Clipper plants with disease. Again the range of cultivars in a mobile nursery that developed scald lesions varied between sites. The narrowest range of diseased cultivars occurred on the Cleve (M.N.No.14) nursery, with two cultivars diseased, and the widest range of cultivars diseased occurred on Angas Plains (M.N.No.12) and Murray Bridge (M.N.No.18) nurseries. These had eight and seven cultivars diseased, respectively. Again disease incidence on Clipper and width of host range were not associated ie. in the three mobile nurseries where 100% of Clipper plants were diseased the number of cultivars diseased was three, four and seven.

Table 4 (Page 10) contains the few mobile nursery results for 1984. Many nurseries in this set were destroyed by adverse weather conditions, and could not be scored. Disease incidence on Clipper plants varied from 0-100%. The two nurseries (M.N.Nos. 22 and 24) that were put in the field prior to crop emergence (28/5/84 and 15/6/84) had no disease on Clipper but there was a low incidence of disease on the Atlas plants. The range of cultivars with scald disease varied with sites and time. This table shows the increasing disease incidence and increasing range in cultivar infection at two sites (Angas Plains (M.N.Nos.22,23) and Charlick (M.N.Nos.24,25,26)) during 1984 as the season progressed. A comparison between the mobile nursery results at Kadina (M.N.Nos.7,17,27) and Turretfield (M.N.Nos.10,28) in 1984 and 1983 shows that the range of diseased cultivars varies between seasons. Lesion type also varied. Many of the lesions on the 1984 Turretfield (M.N.No.28) mobile nursery were very restricted in size, which did not occur on the 1983 nursery at this site.

TABLE 3

CULTIVAR INFECTION RATIO IN MOBILE NURSERIES THAT WERE PLACED IN THE FIELD DURING SPRING, 1983.

M.N.No	11	12	13	14	15	16	17	18	19	20	21
Cultivar											
Abyssinian			Nil								
Atlas		5/10			1/10		6/10	1/10		4/10	
Atlas 46											
Clipper	26/26	27/28		4/20	1/29	19/28	22/25	30/30	23/25	25/27	20/20
Galleon	8/10	7/10		1/10	1/10	5/10	8/10	10/10	3/10	6/10	8/10
Gospeck	3/10	4/10				2/9	7/10	10/10	2/10		4/10
Hudson											
La Mesita										3/10	
Modoc[i]											2/10
Nigrinudum		4/10									
Osiris											
Psaknon											
Sakigake								7/10		10/10	
Sultan											
Turk											
WestChina		8/10					2/10	7/10	2/10		
WWxG*								5/10			
CI3515											
CI4364		3/10									
CI8618		1/10								2/10	
Total number of diseased cultivars(maximum 21)	3	8	0	2	3	3	5	7	4	6	4
Total number of diseased plants(maximum 230)	37	59	0	5	3	26	45	70	30	50	34

NB. Denominator indicates the number of surviving plants per cultivator

M.N. No. is the number assigned to the mobile nursery

*Wisconsin Winter X Glabron [i]Modoc-California

TABLE 4

CULTIVAR INFECTION RATIO IN MOBILE NURSERIES THAT WERE PLACED IN THE FIELD DURING 1984

M.N.No.	22	23	24	25	26	27	28
Cultivar							
Abyssinian					2/6		
Atlas	1/10		2/10		6/6	1/10	1/9(r)
Atlas 46							
Brier				5/5	6/6		10/10
Clipper		11/30		4/4	10/10	14/30	28/28
Galleon		3/10		4/4	6/6	4/10	10/10
Gospeck		2/10		3/4	6/6		10/10
Hudson		3/10					
La Mesita					4/5	1/10	
Modoc[i]				2/5	5/5		2/10(r)
Nigrinudum					4/5		2/10(r)
Osiris						1/10	
Psaknon		2/10					
Sakigake		2/10			5/5		4/9(r)
Sultan		1/10			2/4		
Turk							
West China				3/5	5/5		2/6(r)
WWxG*							
CI3515							
CI4364				2/3(r)	2/5		
CI8618					3/4		
Number of diseased cultivars (maximum 21)	1	7	1	7	14	5	9
Number of diseased plants (maximum 230)	1	24	2	23	66	21	69

NB. Denominator indicates the number of surviving plants per cultivar

(r)=lesions restricted in size

M.N.No. is the number assigned to the mobile nursery

*Wisconsin Winter X Glabron [i]Modoc-California

Table 5 (Page 12) summarises Tables 2-4 with respect to the disease incidence on cultivars in mobile nurseries over all the sites and times tested. The table is arranged in descending order of percentage plants with disease. It can be seen that Clipper had the highest disease incidence with 54.2% of all Clipper plants tested showing scald disease. Galleon was the second highest with 44.4% disease incidence, and Brier, Gospeck and Atlas were next with 30.0%, 25.2% and 19.0% respectively. No disease was seen on Atlas 46, Turk or CI 3515. The remaining cultivars ranged in disease incidence from 0.4% to 14.5%.

Table 6 (Page 13) presents the scald host range for mobile nurseries and sites from Tables 2-4. Again the cultivars are arranged in descending order of disease incidence. Clipper and Galleon were the cultivars that were diseased at most sites. They were scalded in eleven out of the thirteen sites tested, while Gospeck was diseased at only one less site. Sakigake, Atlas and Modoc-California were diseased at seven, six and five sites respectively out of thirteen sites, and Brier diseased in five out of ten sites, while the remaining cultivars were diseased at four or fewer sites. A similar order was reflected in the number of mobile nurseries in which cultivars were infected.

Rainfall records for the time the trays were in the field were kept at each site (Appendix 1 a-c) because it is known that raindrop splash is important in infection of *R.secalis*. No relationship was found between rainfall figures and disease incidence upon cultivars. (Table 7, Pages 14-15).

TABLE 5

THE NUMBER OF SEEDLINGS WITH SCALD LESIONS, AFTER THREE WEEKS OF INCUBATION TOTALLED OVER THE 28 MOBILE NURSERIES

Cultivars	a)Total number of plants diseased	Percentage of plants diseased
Clipper	399/736	54.2
Galleon	120	44.4
Brier	48/160	30.0
Gospeck	67/266	25.2
Atlas	51	19.0
WestChina	38/262	14.5
Sakigake	32	11.9
Modoc-California	29	10.7
La Mesita	23	9.0
Nigrinudum	17	6.3
Sultan	11	4.0
CI4364	7	2.6
WWxG*	4	2.0
CI8618	4	1.5
Hudson	3	1.1
Abyssinian	2	0.7
Psaknon	2	0.7
Osiris	1	0.4
Atlas 46	0	0.0
Turk	0	0.0
CI3515	0	0.0

a)Total number of plants in test is 270 unless shown otherwise

* Wisconsin Winter x Glabron

TABLE 6

CULTIVARS WITH SCALD LESIONS AFTER THREE WEEKS OF INCUBATION
TOTALLED OVER THE 28 MOBILE NURSERIES

Cultivar	a) Number of sites where cultivar was infected	b)Number of mobile nurseries in which cultivar was infected
Clipper	11	22
Galleon	11	21
Brier	5/10	8/17
Gospeck	10	16
West China	7	10
Atlas	6	14
Sakigake	5	6
Modoc-California	5	6
La Mesita	3	5
Nigrinudum	4	4
Sultan	3	3
CI 8618	3	2
CI 4364	2	3
WWxG*	1	1
Hudson	1	1
Abyssinian	1	1
Psaknon	1	1
Osiris	1	1
Atlas 46	0	0
Turk	0	0
CI 3515	0	0

a) Total number of sites tested is 13 unless shown otherwise

b)Total number of mobile nurseries is 28 unless shown otherwise

* Wisconsin Winter X Glabron

TABLE 7

RAINFALL AT MOBILE NURSERY SITES DURING EXPOSURE OF TRAYS

Mobile Nurseries Autumn 1983

M.N.No.	1	5	3	6	8	10	2	4	7	9
No. of infected cultivars (from Table 2)	0	0	0	1	2	5	5	5	7	11
No. of infected Clipper (Table 2)	0	0	0	6	25	9	29	22	15	29
mm. rain	26	10.8	26.8	20.4	36	14.8	14.4	33	13.3	5.4
Days of rain	6	5	7	4	5	7	4	5	3	5

M.N.No. is the Mobile Nursery Number

Mobile Nurseries Spring 1983

M.N.No.	13	14	11	15	16	19	21	17	20	18	12
No. of infected Cultivars (from Table 3)	0	2	3	3	3	4	4	5	6	7	8
No. of infected Clipper (from Table 3)	0	1	26	1	19	23	20	22	25	30	27
mm. rain	58	44.4	46	36.4	50.8	39.2	65	77	18.4	37.7	58
Days of rain	5	7	10	11	12	6	3	3	7	13	7

M.N.No. is the Mobile Nursery Number

Table 7 (continued)

Mobile Nurseries 1984

M.N.No.	22	24	27	23	25	28	26
No. of infected cultivars (From Table 4)	1	1	5	7	7	9	14
No. of infected Clipper (From Table 4)	0	0	14/30	11/30	4/4	28/28	10/10
mm. rain	23.4	21.6	32.9	17	28.8	38.2	24.2
Days of rain	4	5	6	4	7	7	7

M.N.No. is the Mobile Nursery Number

B. GLASSHOUSE TESTING

Materials and Methods

Isolates which were tested against the differential cultivars in the glasshouse came from three sources.

i) Isolates from the *R.secalis* collection in the Waite Institute. These had been collected through southern Australia by other workers, and cultures from single spore isolates had been preserved on porcelain beads (see Lange and Boyd, 1968, for method).

ii) Scald lesions were collected from the field and single spore isolates from these were tested for virulence against the differential cultivars. (See Appendix 2 for single spore isolation technique.)

iii) Cultures from lesions taken from mobile nursery plants were tested for virulence in the glasshouse to determine the similarity of results between the two tests.

Cultures of *R.secalis* were grown, from either porcelain beads or single spore isolations, on potato-sucrose-peptone agar (see Appendix 3), thus allowing for good mycelial growth and sporulation (Schein and Kerelo, 1956). When the cultures were 2-3 weeks old they were macerated in sterile distilled water and filtered through muslin. Spore suspensions were adjusted to $5x10^5$ spores per ml. for spray inoculation, and $1x10^6$ spores per ml. for single droplet inoculation.

Initially inoculation was carried out by spraying plants with a spore suspension from a handheld sprayer. However later experiments indicated that the single droplet technique was a more sensitive method. Any further testing of isolates was done by that method.

Preliminary experiments with the susceptible cultivar, Clipper, had shown that spray inoculation with $1x10^6$ spores per ml. resulted in wilting of

infected leaves without lesion development. Thus the lower concentration of $5x10^5$ spores per ml. was preferred. However, with single droplet inoculation an escape was possible when using $5x10^5$ spores per ml. and $1x10^6$ spores per ml. was preferred since it was known that this rate could be used for droplet inoculation without evoking a wilting reaction.

Seeds were treated and germinated as in the mobile nursery trials. Five seeds of each cultivar were planted, 2 cm deep, in 12.5 cm pots, and placed in a glasshouse where temperature was controlled between 15-25°C. At the two and a half leaf stage the plants were inoculated, either by spraying the second leaf until runoff, with a handheld pressure can, or by applying a 10 *ul* droplet to the axis between the second leaf and the partly emerged third leaf. The plants were allowed to dry for an hour and then placed in a high humidity chamber for 16 hours. This chamber, which was maintained at 17°C, enabled plants to be sprayed with distilled water for 2 seconds every 15 minutes. The plants were then placed in the glasshouse and lesions developed 7 to 21 days after inoculation.

The cultivars were scored as resistant or susceptible according to the manner in which they reacted to the isolate. Susceptible cultivars developed grey to light brown lesions with dark brown edges. Occasionally a cultivar x isolate combination would produce lesions much smaller in size than those of susceptible reactions and these were confined to the margins of the leaf. These reactions were placed in the resistant class, together with the cultivars that did not develop any symptoms. Frequently, not all the plants of a cultivar x isolate test showed the same reaction. A low frequency of plants without symptoms in an otherwise susceptible reaction was treated as a miss. However, when one or two plants showed susceptibility in an otherwise resistant reaction the results were not thought to be reliable.

Results

Table 8a-b (Pages 19-20) presents results from eleven isolates in the Waite collection. A high degree of variability was found as for example in isolate 44/29. The two tests (a and b) using spray inoculation with this isolate showed different results, with one being a subset of the other. The same occurred with the two tests using single droplet inoculation. Such variable results were obtained throughout the isolate testing programme in the glasshouse.

The cultivars that showed no susceptible rating to this set of eleven isolates were Abyssinian, Atlas 46, Osiris, Psaknon and CI4364. Clipper was rated as susceptible (S), to all the isolates, though not in all the tests. The remaining cultivars were susceptible to a range of isolates. Two types of lesions were seen on Sakigake and West China. These were the normal oval shaped lesions on the leaf blade, and also lesions which were confined to the edge of the leaf. When only the latter occurred, the plants were classified as resistant.

Table 9 (Page 21) summarises the reactions on the differentials of eight isolates from Western Australia. Again different results were produced by the different tests. Clipper and Gospeck were found to be consistently susceptible, while Galleon and Atlas were resistant to isolate 412. Brier and West China were resistant to three and four isolates respectively, while Modoc-California, Psaknon, Sakigake and CI4364 were each resistant to all but one of the isolates. No susceptible reactions occurred on the remaining cultivars.

TABLE 8a

REACTIONS OF *R.SECALIS* ISOLATES, FROM THE WAITE COLLECTION, ON TWENTY ONE BARLEY CULTIVARS

Spray Inoculation **Isolates**	44/29 a	 b	68/28
Cultivars			
Abyssinian			
Atlas	s		s
Atlas 46			
Brier	s	s	s
Clipper	s	s	s
Galleon	s	s	s
Gospeck	s	s	s
Hudson			
LaMesita			
Modoc-California	s		s
Nigrinudum	s		s
Osiris			
Psaknon			
Sakigake	s		s
Sultan			
Turk			
West China	s	s	s
WWxG*			
CI3515			
CI4364			
CI8618			

NB. Blank entry indicates resistance ie. no lesions were formed

S=susceptible reaction

Multiple entry , (a, b), shows results from repeated tests

See Appendix 4a for number of plants per cultivar with lesions.

*Wisconsin Winter x Glabron

TABLE 8b

REACTIONS OF ELEVEN *R.SECALIS* ISOLATES, FROM THE WAITE COLLECTION, ON TWENTY ONE BARLEY CULTIVARS

Isolates	Single Droplet Inoculation														
	44/29		68	9		12	166		172	173	183		187	217	219
	a	b	/28	a	b		a	b			a	b			
Cultivars															
Abyssinian															
Atlas		s	s			s								s	s
Atlas 46															
Brier		s		s		s		s			s	s	s	s	s
Clipper	s	s	s	s	s	s	s	s	s	s		s	s	s	s
Galleon	s	s	s		s	s			s	s	s	s	s	s	s
Gospeck		s	s		s	s	s	s	s	s	s	s		s	
Hudson					s										
La Mesita		s													
Modoc[i]											s		s		
Nigrinudum					s										
Osiris															
Psaknon															
Sakigake		s	s						s		s		s		
Sultan								s					s		
Turk					s										
West China		s	s	s					s		s		s	s	s
WWxG*					s								s		
CI3515								s							
CI4364															
CI8618								s							

NB. Blank entry indicates resistance ie. no lesions were formed

S=susceptible reaction

Multiple entry , (a, b), shows results from repeated tests

See Appendix 4a for number of plants per cultivar with lesions.

*Wisconsin Winter x Glabron [i] Modoc California

TABLE 9

REACTIONS OF EIGHT WESTERN AUSTRALIAN *R.SECALIS* ISOLATES ON TWENTY ONE BARLEY CULTIVARS

	Spray inoculation							Single droplet inoculation						
Isolates	342	350		354	405	408	409	342	350	405	406	408	412	
		a	b										a	b
Cultivars														
Abyssinian														
Atlas	S			S	S	S	S		S	S	S	S		
Atlas 46														
Brier	S			S		S	S				S	S		
Clipper	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Galleon	S	S	S	S		S	S		S	S	S	S		
Gospeck	S	S	S	S	S	S	S	S	S	S	S	S	S	
Hudson														
La Mesita														
Modoc[i]					S									
Nigrinudum														
Osiris														
Psaknon									S					
Sakigake											S			
Sultan														
Turk														
WestChina	S				S					S	S			
WWxG*														
CI3515														
CI4364									S					
CI8618														

NB. S=susceptible reaction

Blank entry indicates resistance ie. no lesions were formed.

Multiple entries (a,b) shows results from repeated tests

See Appendix 4b for numbers of plants per cultivar with lesions

*Wisconsin Winter x Glabron [i]Modoc-California

Table 10 (Page 23) presents the results of single droplet inoculation with single spore isolates made from lesions collected in the field. S-1 and S-2 were both isolated from a single lesion on the cultivar Sultan. Neither isolate was able to attack Sultan when tested in the glasshouse and they differed in their virulence on Sakigake. WI-1 to WI-5 were all isolated from a single lesion on the barley line WI-2477. When tested in the glasshouse these five isolates differed in virulence on Sakigake, La Mesita and Brier. The remaining isolates in Table 10 were not tested against Brier, due to an unforeseen lack of seed. They were all virulent on Clipper. One, FC5, was not able to attack Galleon or Gospeck and it also caused very small lesions (r) on Clipper. F1 and F4 were not virulent on Gospeck and F3 was not virulent on Galleon. The final isolate in the table was isolated from barley grass and was virulent on Clipper, Galleon and West China.

Table 11 (Page 24) presents results from single droplet inoculation with spores from mobile nursery lesions. The seven tests all found Clipper to be susceptible though one test resulted in very small lesions, (S(r)). The inoculum for this test had originated from a lesion on the cultivar Modoc-California, but was unable to infect Modoc-California in the glasshouse. The remaining three isolates in the table were collected from Turretfield. These differed in their ability to attack Atlas, while they were all virulent on Brier, Clipper, Galleon and Gospeck.

TABLE 10

REACTIONS OF FOURTEEN *R.SECALIS* ISOLATES ,FROM FIELD COLLECTIONS OF SCALD LESIONS, ON TWENTY ONE BARLEY CULTIVARS

Isolates	s-1	s-2	WI-1	WI-2	WI-3	WI-4	WI-5	F1	F2	F3	F4	F5	F6	BG
Cultivars														
Abyssinian														
Atlas														
Atlas 46														
Brier	s	s	s	s	s		s	-	-	-	-	-	-	-
Clipper	s	s	s	s	s	s	s	s	s	s	s	s(r)	s	s
Galleon	s	s	s	s	s	s	s	s	s		s		s	s
Gospeck	s	s	s	s	s	s	s		s	s			s	
Hudson														
La Mesita				s										
Modoc[i]														
Nigrinudum														
Osiris														
Psaknon														
Sakigake		s			s								s	
Sultan														
Turk														
West China	s	s	s	s	s	s	s		s				s	s
WWxG*														
CI3515														
CI4364														
CI8618														

NB. S=susceptible reaction

Blank entry indicates resistance ie. no lesions were formed

- =No test carried out

(r) = restricted development of lesions ie. very small

See Appendix 4c for numbers of plants per cultivar with lesions

* Wisconsin Winter x Glabron

i = Modoc California

TABLE 11

REACTIONS OF SEVEN *R.SECALIS* ISOLATES, ISOLATED FROM MOBILE NURSERY LESIONS, WHEN TESTED ON TWENTY ONE BARLEY CULTIVARS IN GLASSHOUSE TEST

Mobile Nursery from which isolate originated	7	10	10	18	28	28	28
Cultivar from which isolate originated	Clipper	Clipper	W.China	Modoc	Clipper	Brier	Brier
Cultivar							
Abyssinian							
Atlas					S	S	
Atlas 46							
Brier	-	-	-	-	S	S	S
Clipper	S	S	S	S(r)	S	S	S
Galleon	S	S	S		S	S	S
Gospeck	S	S	S		S	S	S
Hudson							
La Mesita							
Modoc-California							
Nigrinudum							
Osiris							
Psaknon							
Sakigake		S					
Sultan							
Turk							
WestChina	S	S	S				
WWxG*							
CI3515							
CI4364							
CI8618							

NB. S=susceptible reaction;

Blank entry indicates resistance ie. no lesions were formed

- =No test carried out

(r)=restricted development of lesions ie. very small lesions

See Appendix 4d for number of plants per cultivar with lesions

*Wisconsin Winter X Glabron

C. DISCUSSION

i) Field testing with mobile nurseries

A total of twenty eight mobile nurseries were placed in the field for two weeks during 1983 and 1984 and returned to the glasshouse for three weeks for incubation. Twenty four of these mobile nurseries were found to have scald lesions by the end of the three week incubation period. Clipper was diseased with scald in twenty two of these, and the two mobile nurseries without scalded Clipper had developed scald lesions only on Atlas.

Since Clipper has complete susceptibility to *R.secalis*, disease incidence on this cultivar is most likely to be associated with environmental factors. In those twenty two mobile nurseries, the percentage of Clipper plants per nursery that were diseased varied from 100% in seven nurseries down to 3.4% in one nursery. The disease incidence on susceptible cultivars (ie. those with scald disease) in the mobile nursery was related to the disease incidence on Clipper. The correlation between these two factors was r= 0.85*** (Table 12, Page 26) These results suggest that disease incidence is strongly affected by environmental factors.

The disease incidence on Clipper was also correlated with the range of diseased cultivars in the mobile nurseries. This is despite the fact that in the seven nurseries with 100% Clipper infection, the number of cultivars with scald disease ranged from fourteen down to three (Table 12). The correlation coefficient for percentage Clipper infection and number of cultivars infected per mobile nursery is r= 0.536**.

TABLE 12

INFECTION RATE OF CLIPPER PLANTS PER MOBILE NURSERY COMPARED TO AVERAGE INFECTION RATE OF OTHER SCALDED CULTIVARS

Mobile Nursery Number	(a) Infection rate of Clipper	(b) Infection rate of 'susceptible'(i) cultivars	(c) Number of cultivars with scald lesions
2	100.0	65.0 (1)	5
11	100.0	55.0	3
18	100.0	66.6	7
21	100.0	46.7	4
25	100.0	73.1	7
26	100.0	82.4	14
28	100.0	55.4	9
9	96.0	80.0	11
12	96.0	45.7	8
20	93.0	57.5	5
19	92.0	23.3	4
4	88.0	51.3	5
17	88.0	57.5	5
8	81.0	80.0	2
16	68.0	36.8	3
27	46.7	17.5	5
7	44.4	31.6	7
23	36.7	21.7	7
10	30.0	10.0	5
6	22.2	0.0	1
14	20.0	10.0	2
15	3.4	10.0	3
24	0.0	20.0	1
22	0.0	10.0	1

Correlation coefficient (r)

(a) and (b) $(r) = 0.8408^{**}$ $(P<0.001)$

(a) and (c) $(r) = 0.536^{**}$ $(P< 0.01)$

(b) and (c) $(r) = 0.5933^{***}$ $(P<0.001)$

(i) Susceptible' cultivars in this table are all the cultivars that have been classified as susceptible in the mobile nursery.

Therefore (1) is calculated from Table 2, mobile nursery number 2

$(7/10 + 6/10 + 9/10 + 4/10) \times 100 = 65.0\%$

There was a significant positive relationship (r=0.59***) between the disease incidence on susceptible cultivars and the range of cultivars infected with scald, ie. cultivar range infected was affected by environmental conditions. This suggests that environment is affecting genetic expression, suggesting that in environmental conditions favourable to the development of *R.secalis* a wider range of cultivars will be susceptible to the pathogen than in other conditions. This had been indicated in the studies by Ali and Boyd (1974) and Janakiram and Boyd (1980a).

The above results suggest that the range of cultivars diseased in a mobile nursery is affected by environmental factors, as well as by the range of virulence in the *R.secalis* population at the test site. However when attempting to identify pathotypes of *R.secalis* by mobile nurseries, the range of cultivars infected appears to be an important factor while numbers of plants infected is more likely to indicate environmentally favourable conditions.

The range in cultivar infection was seen to vary widely from site to site. Some sites had a very restricted cultivar host range for *R.secalis* with only Clipper or Atlas showing disease, while at other mobile nursery sites the number of cultivars diseased went as high as fourteen out of a possible twenty one.

Cultivar host range varied when the same site was tested at different times. This may suggest that individual pathotypes vary in their competitive ability according to environmental conditions, or that the cultivars react differently with environmental conditions, as suggested before.

Rainfall records for the time the trays were in the field did not explain the range of cultivars diseased in the mobile nurseries (Table 7, Pages 12-13. Streaky Bay, (M.N.No.9) showed the least total rainfall but the highest cultivar disease range, while the highest rainfall for that set of mobile nurseries had only two cultivars diseased. Similar results were seen in the other two sets of mobile nurseries. There appeared to be no relationship between rainfall and disease incidence in any of the mobile nursery series. It

is possible that rainfall figures do not give a reliable measure of the value of splash dispersal since a few short heavy showers may have more dispersive effect than many prolonged gentle falls. According to Stedman (1980) the success of infection is more reliant upon humidity and length of dew period but no data on this is available for these experiments.

Tables 5 and 6 (Pages 12 and 13) present the disease incidence and the cultivar host range for all the mobile nurseries. Clipper, Galleon, Brier, Gospeck and Atlas were diseased in more than a quarter of the nurseries. Also, these cultivars were diseased in a quarter or more of the total number of plants used in the nurseries. Therefore the virulent gene(s) required to overcome any resistance in these cultivars must be widely spread through the natural *R.secalis* population in South Australia. Virulent genes for overcoming resistance in other cultivars occur with less frequency according to the mobile nursery results. No virulent genes were able to overcome Atlas 46, Turk or CI3515 in the nurseries.

ii) Glasshouse testing

Forty isolates from various sources were tested against the twenty one cultivars in the glasshouse. Results were far from satisfactory as reactions were not consistent. The results on Clipper, Galleon and Gospeck were usually consistent, generally being susceptible. Other cultivar x isolate combinations often showed different results within and between tests. Variability was further indicated when different results were seen in five isolates from the one lesion. Further inconsistencies occurred when attempts were made to match mobile nursery results with glasshouse reactions. Some isolates were found to be unable to reinfect the cultivar from which they were isolated. Whether or not these results were real or were caused by deficiencies in the testing procedure was not able to be ascertained. It is possible that in some cases there is a complicated inheritance of resistance and/or an environmental interaction

causing some of the variable results. The mobile nursery results also pointed to the environment having an effect upon cultivar x pathogen interactions, as seen by the varying results when sites were tested several times, and the effect of the environment upon numbers of cultivars diseased.

An attempt was made to classify results as resistant or susceptible depending upon frequency of plants infected in the test, and the lesion types. This resulted in a tentative 28-32 pathotypes (depending upon the unknown reaction upon Brier) being identified from the 40 isolates tested (Table 13, Page 31). One of the aims set out in the beginning was to identify most common pathotypes occurring in the population. The two most frequently occurring in this study have been marked with an asterisk in Table 13. These are both virulent on the cultivars Brier, Clipper, Galleon and Gospeck, and are distinguished from each other by the ability to infect the cultivar Atlas and West China. However together they represent only 18.5% of the total number of isolates tested, and so it is obvious that a wide number of pathotypes exist at a low frequency in the pathogen population.

Comparisons between the mobile nursery and glasshouse tests were made by comparing the total disease incidence per cultivar. Table 14 (Page 32) shows the percentage of diseased plants per cultivar in mobile nurseries and the glasshouse tests, and the number of isolates rated as virulent upon the cultivar in glasshouse tests. A correlation coefficient of r=0.964*** (Figure 1a-b, Page 33), indicates that the two methods of testing achieved similar results with respect to percentage of plants with scald disease. The only obvious discrepancies are West China and Gospeck which showed a greater susceptibility in the glasshouse tests than with the mobile nurseries. This corresponding result is what would be expected if the glasshouse isolates were a good representation of the field population and if the test was working. Therefore while there are many individual discrepancies, in general the test matches field results. It may be that the interpretation of results, and attempting to put isolates into races,

which is an artificial process, is what causes the confusion. The organism is seen to be highly variable, changes (or loses) virulence if left in culture over numerous generations, and even appears to produce different pathotypes within a lesion. A similar situation has been noted with the organism *Pyricularia oryzae* which infects the rice plant (Ou *et. al.*, 1968). All these factors make it very difficult to pathotype *R.secalis* and select 'races' for screening purposes.

Figure 1 seems to indicate an important difference between genotypes. Rather than dividing cultivars into distinctly resistant and susceptible groups, which is a pre-requisite for classifying a pathogen into races, they appear to differ in their degree of susceptibility. Slow scalding types show a small level of disease incidence and the graph shows a gradual increase in disease rating in the remaining cultivars. This gradual increase in cultivar susceptibility is seen in both infection frequency and for the percentage of isolates that are virulent upon cultivars. This is pursued further in Chapter 2 where barley types are screened in the field.

TABLE 13

SUMMARY OF THE REACTIONS OF THE FORTY *R.SECALIS* ISOLATES USED IN THE GLASSHOUSE TESTS ON TWENTY ONE BARLEY CULTIVARS

	Isolate group																																
Cultivars																																	
Abyssinian																																	
Atlas															s		s	s	s				s			s	s			s	s		s
Atlas 46																																	
Brier			-			-	-		s	-	s		-	s	s		s			-	s		s	s	s		s		s	s	s	s
Clipper		s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s	s
Galleon					s	s		s		s	s	s	s	s	s	s	s		s	s	s	s	s		s	s	s	s	s	s	s	s
Gospeck	s			s			s	s			s	s	s	s	s	s		s	s	s	s	s	s	s	s	s	s	s	s	s		s
Hudson																												s					
La Mesita																										s			s				
Modoc-California																s							s							s	s	
Nigrinudum																											s						
Osiris																																	
Psaknon																									s								
Sakigake														s				s	s	s		s						s	s	s	s		
Sultan																					s									s			
Turk																											s						
West China								s	s		s	s	s		s	s	s	s	s	s	s		s	s		s		s	s	s	s		
WisconsinWinter x Glabron																											s			s			
CI3515																						s											
CI4364																									s								
CI8618																						s											
Number of isolates with indicated reaction (54 tests resulted in 32 pathotypes)	1	1	2	2	2	2	1	2	1	1	2	1	3	4*	6*	1	1	1	2	2	2	2	1	1	2	1	1	1	1	2	1	1
Number of susceptible cultivars	1	1	1	2	2	2	2	3	3	3	4	4	4	5	5	5	5	5	5	5	6	6	6	6	6	6	6	7	7	8	8	9	

NB. S=susceptible reaction Blank entry indicates resistance ie. no lesions were formed - = No test was carried out

(a) Although there were 40 isolates, isolates were tested more than one time and some gave more than one type of result. Thus 54 tests are shown in this table..

TABLE 14

COMPARISON OF INFECTION RATES IN GLASSHOUSE AND MOBILE NURSERY TESTS

	Percentage of plants infected in-		Number of isolates classified virulent in glasshouse	
	Glasshouse	Mobile Nurseries		
Cultivar				%
Abyssinian	3.7	0.7	0	0.0
Atlas	22.3	17.0	14	35.0
Atlas 46	1.4	0.0	0	0.0
Brier	45.9	29.8	(i) 23/29	79.3
Clipper	85.7	54.2	40	100.0
Galleon	64.2	44.0	35	87.5
Gospeck	67.9	25.4	33	82.5
Hudson	2.3	1.1	1	2.5
La Mesita	8.9	8.5	2	5.0
Modoc[i]	11.1	7.8	5	12.5
Osiris	3.3	0.4	0	12.5
Psaknon	5.6	0.7	1	2.5
Sakigake	27.6	13.4	10	25.0
Sultan	4.7	4.1	2	5.0
Turk	3.7	0.0	1	2.5
WestChina	45.5	14.1	24	60.0
WWxG*	4.7	1.8	2	5.0
CI3515	3.3	0.0	1	2.5
CI4364	5.1	2.6	1	2.5
CI8618	4.2	2.2	1	2.5

* Wisconsin Winter X Glabron [i]Modoc-California

(i) Only 29 of the 40 isolates were tested on Brier

Figure 1a

The percentage of plants per cultivar showing scald lesions in glasshouse tests compared to mobile nursery tests.

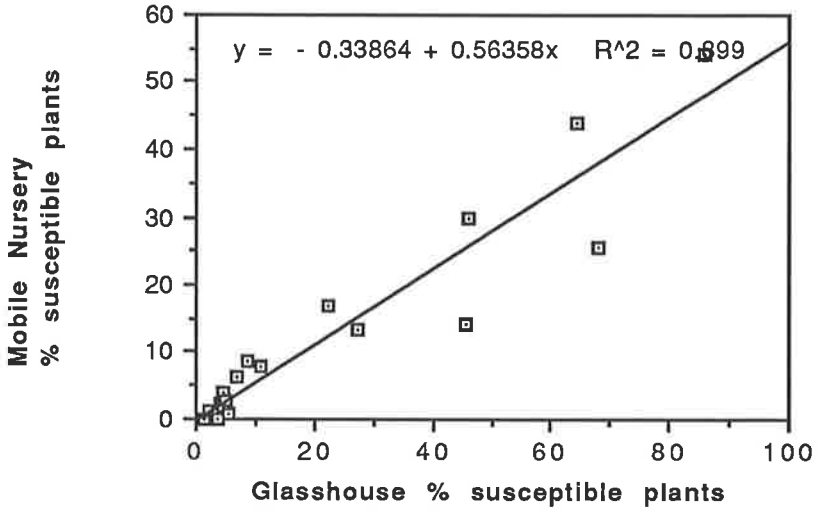
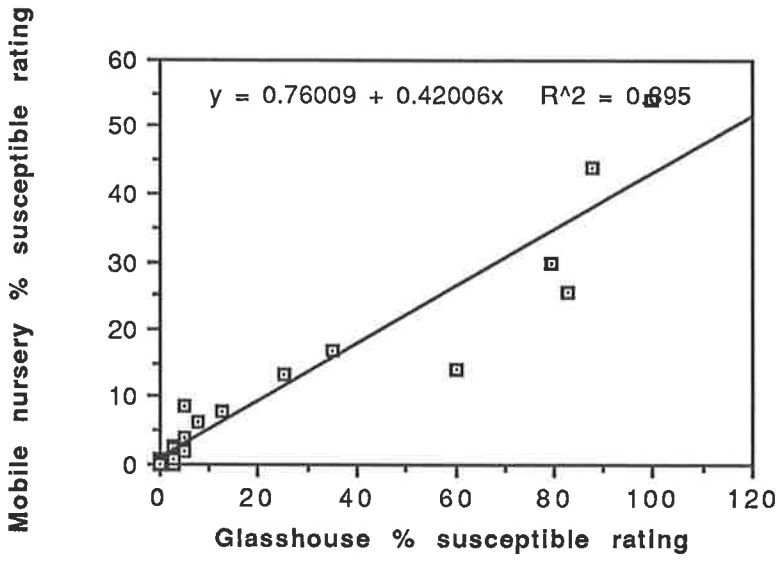


Figure 1b

The percentage of isolates that were virulent per cultivar compared to the percentage of plants showing scald lesions per cultivar



CHAPTER 2

MEASUREMENT OF RESISTANCE TO *R.SECALIS* IN BARLEY

A. FIELD SCREENING

B. GLASSHOUSE SCREENING

INTRODUCTION

In the past, resistance has been classified as either specific or nonspecific, controlled by monogenes or polygenes, respectively. Further resistance studies have shown these classifications to overlap, so that it is now recognised that nonspecific resistance may well be under monogenic as well as polygenic inheritance. Slow-scalding cultivars were thought to be due to nonspecific resistance, but Habgood (1974) concluded that one cultivar with this type of resistance had specific genes for its inheritance.

In the literature it can be seen that a range of scald symptoms occurs on cultivars, whether they are classified as resistant or susceptible to the disease. Germination of the *R.secalis* spores occurs on all hosts, regardless of their resistance (Fowler and Owen, 1971, Ayres and Owen, 1971, Ali, 1974). Penetration by the germ tube may be stopped by resistance, or merely reduced, compared to susceptible types (Fowler and Owen, 1971). Resistant types may have subcuticular hyphae without symptoms (Ayres and Owen, 1971, Ali, 1974), though generally proliferation of hyphae correlates with symptoms (Ayres and Owen, 1971). Mycelial growth rate is faster in susceptible cultivars (Ayres and Owen, 1971) and even a few abnormal conidia may be produced on resistant cultivars, without the presence of symptoms (Ali, 1974). Janakiram and Boyd (1980) suggest that all genotypes have symptoms but they differ in the extent and rate to which these symptoms develop.

It is possible therefore that the measurement of symptoms may be a measure of resistance, or the degree of susceptibility, as discussed in the previous chapter. This study investigates the components of disease expression in a series of barley lines, and the association between the components and the disease levels of these lines in the field. Components measured were the days to lesion development from inoculation (incubation period), leaf area diseased, infection rate and sporulation.

PART A. FIELD SCREENING

Screening of barley lines was carried out in the field for their resistance to barley leaf scald. The selected barley lines were grown in the field at two sites and the disease development was measured as the percentage leaf area diseased (%LAD), at five intervals during the growing season.

Materials and Methods

Barley lines used in this trial came from five sources-

i) Scald resistant differentials (Table 1, page 3)

ii) Commercial cultivars- Arivat, Bandulla, Cantala, Forrest, Golden Promise, Grimmett, Galleon, Ketch, Malebo, Proctor, Shannon, Stirling.

iii) Advanced breeding lines from the Waite Institute programme- WI2468 (later released as Schooner), WI2477, WI2594, WI2597, WI2598

iv) Selections made from crosses between Psaknon and Clipper, and Arivat and Clipper. Nine selections from each cross were screened in this trial.

(Psaknon x Clipper) x Clipper - selections	6, 13, 21, 22, 38, 41, 44, 58, 70.
(Arivat x Clipper) x Clipper - selections	19, 25, 78, 79, 89, 95, 96, 100, 104.

v) Material from a scald resistance breeding programme (source Ali,S.M.). Refer to Table 1, Chapter 1 for resistance genes.

Family	Cross	Selections
39	Atlas46 x Atlas57	87, 115, 116,117, 142, 207, 669
44	Atlas46 x Sakigake	493
47	Atlas57 x LaMesita	391
48	Atlas57 x Turk	467,468
49	Atlas57 x WestChina	106, 383, 391, 102, 104
51	Atlas57 x Hudson	215, 216, 217, 233, 328, 402, 517, 717, 742, 765, 778, 943
55	Psaknon x Sakigake	107, 560, 570
56	Psaknon x Hudson	30, 51, 95, 915, 926, 930, 931
70	CI 3515	69, 120, 162
71	Sultan x Nigrinudum	147, 383, 507, 515, 535

The seeds were sown, at two sites, Waite Institute and Charlick Experiment Farm, (situated approximately 60 km south east of Adelaide), in single rows, using 6.5 gm seed per row. Each row was 2.5m long with 1m pathways between the bays. Rows within a bay were 35 cm apart. The susceptible cultivar, Clipper, was sown in every fifth row as a check on disease development. Lines were randomised within each of the two replicates. Sowing was carried out early in the season, ie. the second week of May, to encourage the development of leaf scald. Irrigation was applied to both sites at regular intervals to encourage disease development through splash dispersal of spores and high humidity.

When the plants were at the 1-2 leaf stage (growth stage (g.s.) 12 Zadoks scale) they were inoculated with scald infected straw, collected from the previous season's crops. The straw was hammermilled and spread at a

rate of 20 gm per m^2. During late tillering (g.s. 29 Zadoks scale) the plots were again inoculated, this time with a spore suspension made from cultures of isolates in the Waite Institute collection. The suspension consisted of 14 different isolates, ten with known virulences according to testing by Ali *et al* (1976). The combined virulences of these isolates gave a suspension that in theory had the ability to attack all the differentials except Atlas 46, Psaknon, CI 3515, CI 8618 and Sultan. This method of inoculation enabled the barley lines to be subjected to a wide range of possible virulences and hence effectiveness of resistance could then be assessed.

Levels of disease were assessed at intervals of three weeks, beginning at early tillering (g.s. 22 Zadoks scale) and continuing until crop maturity (g.s. 87 Zadoks scale). The percentage of leaf area diseased (%LAD) on the lowest two green leaves of the most susceptible line (Clipper) was chosen for assessment. Leaves at the same position were assessed on the other barley lines. This usually resulted in the third and fourth leaves from the top of the plant being chosen. Disease severity was assessed on ten tillers selected at random from each row. Disease keys (James *et al*, 1968) were used to assist in estimating %LAD. Analysis of variance and cluster analysis were used to analyse the results.

Results

Barley leaf scald developed extensively in the trials early in crop growth (approximately g.s. 20 Zadoks scale). Near the end of tillering (g.s. 29 Zadoks scale), at Charlick, the assessments showed as much as 70 %LAD on the most susceptible lines. Disease did not develop to the same extent in later growth stages, mainly due to very dry weather in the latter part of the growing season. The %LAD at this time did not exceed 10% on the most susceptible lines. Scald levels in the Waite Institute trial were reduced compared to the Charlick site, with no more than 30% LAD on Clipper. A complete set of results is presented in Appendix 5a-e.

Analysis of variance was applied to the results at each assessment. The Least Significant Differences (LSD in Appendices 5a-e) indicated that there were significant differences in disease levels between the barley lines. The least significant differences (LSD) showed that there was a continual gradient of disease levels from resistant (no disease) to the susceptible lines ie. the analysis was unable to place the lines into discrete reaction groups.

In an attempt to classify the barley lines as either resistant or susceptible a cluster analysis was applied. First the data were transformed so that the highest recorded %LAD at each assessment time was 100%. This was done to prevent the second assessment at Charlick, in which the %LAD readings were much higher that at other assessments, from outweighing other readings. The cluster analysis was then able to divide the barley lines into homogeneous groups by minimising the within-group sums of squares. Five groups of lines were produced by the analysis, A-E, where A is the most resistant and E the most susceptible. The cluster groups are presented in Table 15 (pages 43-44) and in Appendix 5a-e with the % LAD results.

TABLE 15

RESULTS OF SCREENING OF BARLEY LINES IN THE FIELD FOR THEIR RESISTANCE TO BARLEY LEAF SCALD PRESENTED IN CLUSTER ANALYSIS GROUPS

Cluster groups	Differential cultivars	Commercial cultivars	Advanced breeding lines	(Psaknon x Clipper) x Clipper	(Arivat x Clipper) x Clipper	Resistant cultivar crosses	
A resistant at both sites)	Abyssinian	Arivat	WI2597			87/39	216/51
	Atlas 46	Cantala				115/39	217/51
	Hudson	Forrest				116/39	328/51
	La Mesita	Malebo				117/39	402/51
	Modoc					142/39	517/51
	Nigrinudum					669/39	717/51
	Osiris					493/44	742/51
	Psaknon					467/48	765/51
	Sultan					468/48	778/51
	Turk					383/49	943/51
	WWxG[i]					391/49	30/56
	CI3515					107/55	95/56
	CI4364					147/71	926/56
	CI8618					535/71	930/56
							107/55
A/B* resistant	Atlas	Shannon	WI2594	Selection22		102/49	560/55
	Sakigake		WI2598			104/49	51/56
	West China					106/49	915/56
						215/51	931/56
						233/51	69/70
						507/71	120/70
						515/71	162/70
B mod. resistant	Gospeck				Selection95 391/47	207/39	

[i]Wisconsin Winter x Glabron

* indicates that results from the two test sites put the barley line in different cluster groups

TABLE 15 (continued)

Cluster groups	Differential cultivars	Commercial cultivars	Advanced breeding lines	(Psaknon x Clipper) x Clipper	(Arivatx x Clipper) x Clipper	Resistant cultivar crosses
B/C* moderately resistant			WI2468			383/71
C moderately susceptible		Galleon		Selection13	Selection19	
C/D* moderately susceptible					Selection78 Selection89 Selection100	
D susceptible		Proctor				
D/E* susceptible				Selection44		
E Very susceptible		Bandulla Clipper Golden- -Promise Ketch Stirling		Selection38	Selection96	570/55
A/C*				Selection6		
B/E*				Selection21 Selection41 Selection70		
B/D*		Grimmett	WI2477	Selection58	Selection25 Selection79 Selection104	

* indicates that results from the two test sites put the barley line in different cluster groups.

The results can be summarised as follows:

i) Most of the scald resistant differentials showed only traces of the leaf scald, and were classified as resistant (class A). In class B (ie. moderately resistant) were Gospeck (at Charlick and Waite Institute), Sakigake (at Charlick), West China (at Charlick), and Atlas (at Waite Institute).

ii) Five of the commercial cultivars were put into class A, and three of these ie. Forrest, Malebo and Arivat, had no scald (0 %LAD). Galleon was found to be moderately susceptible (class C), while the remaining cultivars were placed in classes D and E, indicating their high degree of susceptibility.

iii) Advanced breeding lines- WI 2597 was placed in class A at both Charlick and Waite Institute, while WI 2598 and WI 2594 were found to be moderately resistant. WI 2468 was put into class B at Charlick, but into class C at Waite Institute so it was classified as moderately susceptible. WI 2477 was in class D (susceptible) at Waite Institute.

iv) Psaknon crosses- Selection 22 was placed in resistant and moderately resistant classes, while selection 6 was placed in the resistant class at Charlick and moderately susceptible class at Waite Institute. Selection 13 was moderately susceptible at both sites. The remaining six selections were found to be susceptible at both of the sites.

Arivat crosses- Selection 95 was placed in the moderately resistant class at both sites. Selections 19, 78 and 100 were put into the moderately susceptible class at one or both sites, while the remaining five selections were found to be susceptible.

v) Crosses between resistant cultivars-

The line 570/55 (Psaknon x Sakigake) was found to be very susceptible, being put into class E at both sites. Selection 383/71 (Sultan x Nigrinudum) scored moderately resistant at Charlick and moderately susceptible at Waite Institute. The remaining forty four lines were all moderately resistant or resistant at both sites.

PART B. GLASSHOUSE SCREENING

Materials and Methods

From the field trials, four barley lines were chosen for these experiments. Clipper was selected as the susceptible control. Galleon was used because, while it is moderately susceptible and without any known resistance genes, it develops less scald disease in the field than does Clipper. A technique which could detect this small amount of difference in disease development would be worthwhile. The barley line 207/39 is moderately resistant and so was chosen as a desirable level of resistance to aim for in a glasshouse screening test. The line 116/39 was also included since it was highly resistant until the end of tillering (g.s. 29 Zadoks scale), but was susceptible as an adult plant. A test that was able to detect this type of susceptibility would be useful.

Two methods of inoculating *R.secalis* are generally used. The most common of these is to spray the entire leaf (or leaves) of a seedling, with a spore solution ranging from 10^4 to 10^6 spores per ml. The seedlings are then kept humid for a minimum of ten hours. This results in extensive lesioning on the inoculated leaves. A much less widely used technique is to place a single droplet of inoculum, at about the same concentration as mentioned previously, either in the funnel of a newly developing leaf, or the axis of the newly developing leaf and the next oldest leaf. This results in a

single lesion on a leaf. Both these methods were first investigated for their suitability to study the components of disease development.

A) Comparison of Spray Inoculation and Single Droplet Inoculation

i) Spray Inoculation

Materials and Methods

Methods to produce seedlings, inoculum and inoculation procedures were as described in Chapter 1. Twenty four pots of Clipper and twenty four pots of Galleon, each with five seedlings were grown and the seedlings, when at the two and a half leaf stage were spray inoculated with a handheld sprayer. The following concentrations of *R.secalis* spores were used: $2x10^4$ spores per ml, $2x10^5$ spores per ml, $5x10^5$ spores per ml, and $2x10^6$ spores per ml.

The seedlings were assessed at 14, 21 and 28 days after inoculation. The percentage leaf area diseased (%LAD) and sporulation were recorded at each assessment. The latter was achieved by placing the second leaf in 5ml of sterile water at 15^o C for 48 hours. The leaf and water were shaken with a wrist action shaker for 5 mins. to liberate spores which were counted using a haemocytometer. Eight counts were recorded and averaged. Analysis of variance was used to statistically test the results.

Results

The second leaf, on which assessments of disease were to be made, had senesced by the 21st day from inoculation, thus results were available for the first assessment time only. The %LAD and spore counts differed significantly between Clipper and Galleon.(Figs. 2 & 3, page 49). The latter was found to be due to differences in %LAD since spores/lesion area did not differ between the cultivars.(Fig. 4, page 50). Increased inoculum concentration showed an increase in %LAD.

The interaction term (Table 16, page 51) shows that Galleon had less diseased area than Clipper when inoculum concentration was $5x10^5$ spores per ml. No significant difference occurred at the other concentrations, and both cultivars reached 100 %LAD at the highest concentration. An increased inoculum load resulted in an increased %LAD for both cultivars, except on Galleon when increasing concentration from $2x10^5$ to $5x10^5$ spores per ml. The Galleon reaction to $5x10^5$ spores per ml. was an atypical result and may have been due to error.

The spore count on Galleon was significantly less than on Clipper (Table 17, page 51) at the inoculum concentration of $5x10^5$. The spore count did not significantly increase with inoculum concentration.

The spore count per %LAD (Table 18, page 52) did not differ significantly between the cultivars or with inoculum concentrations. Therefore the difference in spore count per leaf was due to the difference in %LAD between the cultivars.

Figure 2

% Leaf Area Diseased (%LAD) on the second leaf of Clipper and Galleon 14 days after spray inoculation with 4 concentrations of *R. secalis* spores

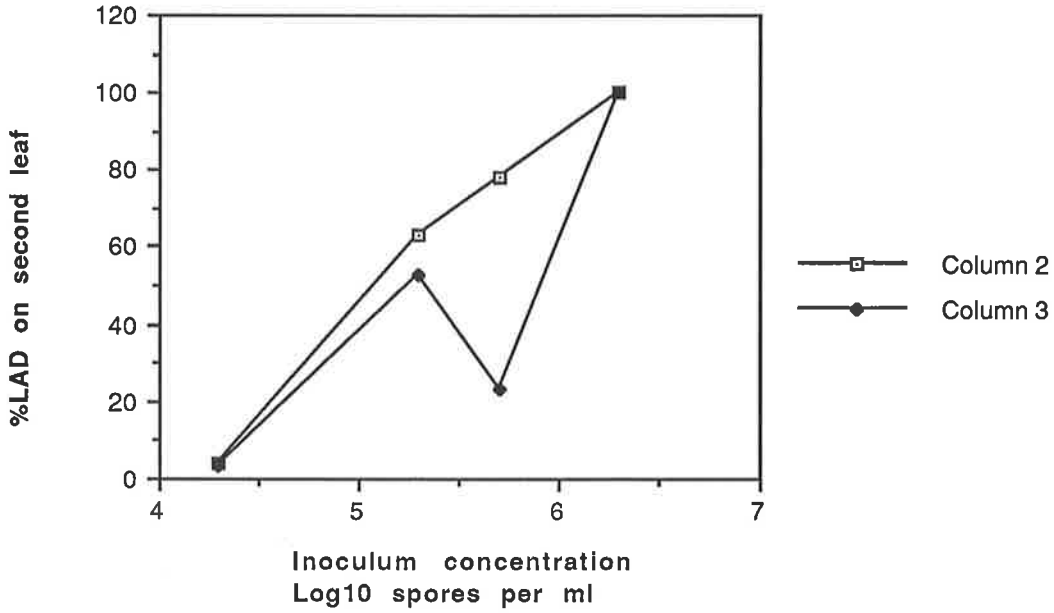
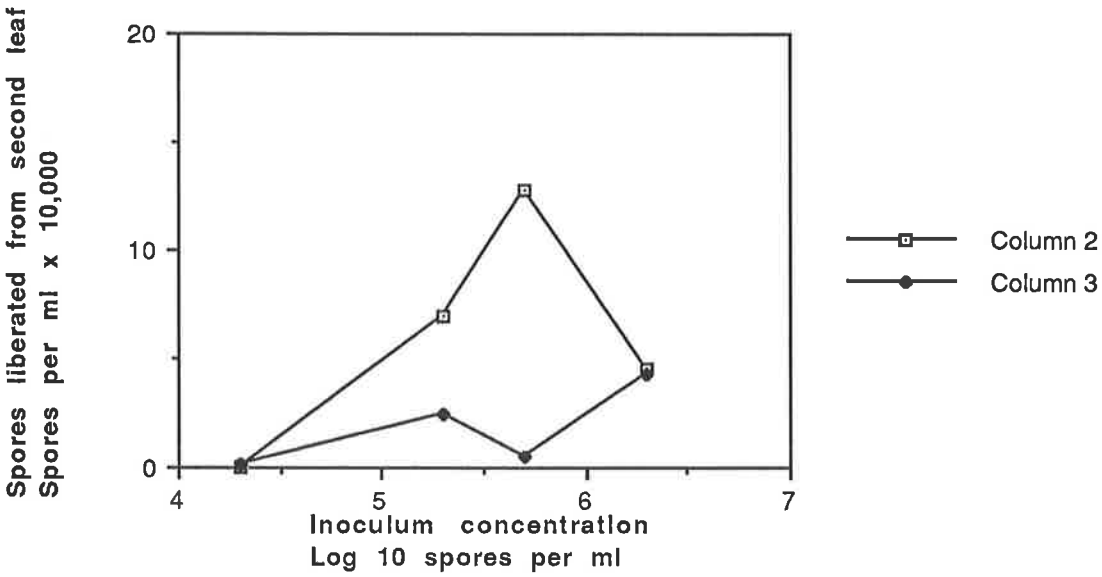


Figure 3

R. secalis spores harvested from second leaf of Clipper and Galleon 14 days after spray inoculation

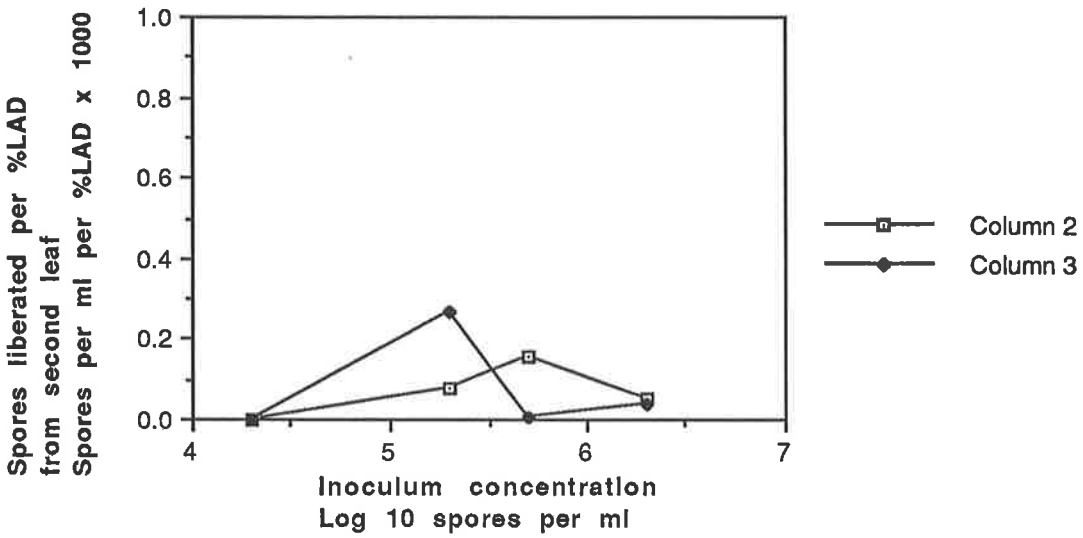


Column 2=Clipper

Column 3=Galleon

Figure 4

R.secalis spores per %LAD harvested from second leaf of Clipper and Galleon 14 days after inoculation



Column 2=Clipper

Column 3=Galleon

TABLE 16

Analysis of variance on %LAD measured on Clipper and Galleon
14 days after inoculation

	Inoculum Concentration spores per ml.				
	$2x10^4$	$2x10^5$	$5x10^5$	$2x10^6$	Mean
Cultivar					
Clipper	4.0	63.2	78.0	100.0	61.3
Galleon	3.2	52.5	23.0	100.0	44.6
Mean	3.6	57.7	50.5	100.0	53.0

$LSD_{treatment}$ = 34.0
$LSD_{cultivar}$ = 10.6
$LSD_{interaction}$ = 37.4

TABLE 17

Analysis of variance on Spore counts measured on Clipper and Galleon
14 days after inoculation

Spore counts $x10^4$ spores per ml.					
	Inoculum concentration spores per ml.				
	$2x10^4$	$2x10^5$	$5x10^5$	$2x10^6$	Mean
Cultivar					
Clipper	0.00	6.95	12.80	4.55	6.08
Galleon	0.13	2.38	0.43	4.22	1.79
Mean	0.06	4.66	6.61	4.39	3.93

$LSD_{treatment}$= Not Significant
$LSD_{cultivar}$ = 3.2
$LSD_{interaction}$ = 8.0

TABLE 18

Analysis of variance on spores per %LAD on Clipper and Galleon
14 days after inoculation

Spore counts x 10^4 spores per ml					
	Inoculum concentration spores per ml.				
	$2x10^4$	$2x10^5$	$5x10^5$	$2x10^6$	Mean
Cultivar					
Clipper	0.00	0.08	0.16	0.05	0.07
Galleon	0.00	0.27	0.004	0.04	0.08
Mean	0.00	0.18	0.08	0.04	0.076

$LSD_{treatment}$ = Not Significant
$LSD_{cultivar}$ = Not Significant
$LSD_{interaction}$ = Not Significant

ii) Single Droplet Inoculation

Materials and Methods

Twelve pots of Clipper and Galleon were grown to the two and a half leaf stage. Three concentrations of the inoculum were made up ; $1x10^5$ spores per ml, $5x10^5$ spores per ml, $1x10^6$ spores per ml. A droplet of inoculum, approximately 10 *u*l in size, was placed in the axis of the second and third leaves of the plants. Plants were treated as previously, and assessments were made at 14th, 21st and 28th day from inoculation. Analysis of variance was used to statistically test the results.

Results

Lesions developed at the base of the second leaf and midway along the third leaf. The latter was assessed for %LAD and sporulation. Assessments at the 21st and 28th day, as well as the 14th day, from this inoculation were possible since senescence was not apparent on the third leaf at these times. Galleon was found to have significantly less %LAD, spore count and spore count per %LAD than Clipper. Inoculum concentration had no effect upon results. (Tables 19, 20, 21, pages 54-56).

Galleon had significantly less %LAD than Clipper at the second and third assessments (Table 19, page 54). Inoculum concentration did not significantly vary the %LAD. While Galleon's spore count was less than Clipper at all times except at the highest concentration in the first assessment, this was significant in the second assessment only (Table 20, page 55). Inoculum concentrations did not significantly affect results. In the second assessment the spore count per %LAD was significantly different between the cultivars, with Galleon less than Clipper. (Table 21, page 56).

TABLE 19

Percentage Leaf Area Diseased on Clipper and Galleon Plants when Inoculated using the Single Droplet Technique

a) First Assessment (14 days after inoculation)

	Inoculum concentration (spores per ml)			
	$1x10^5$	$5x10^5$	$1x10^6$	Mean
Cultivar				
Clipper	15.0	10.0	9.2	11.4
Galleon	7.6	0.8	7.0	5.1
Mean	11.3	5.4	8.1	8.3

LSD was not significant

b) Second Assessment (21 days after inoculation)

	Inoculum concentration (spores per ml)			
	$1x10^5$	$5x10^5$	$1x10^6$	Mean
Cultivar				
Clipper	14.2	13.0	13.0	13.4
Galleon	2.5	3.2	4.2	3.3
Mean	8.3	8.1	8.6	8.3

LSD_{var} = 5.8* (P<0.05) LSD_{treat} is not significant LSD_{inter} is not significant

c) Third Assessment (28 days after inoculation)

	Inoculum concentration (spores per ml)			
	$1x10^5$	$5x10^5$	$1x10^6$	Mean
Cultivar				
Clipper	9.0	7.0	9.0	8.3
Galleon	3.4	2.0	6.0	3.8
Mean	6.2	4.5	7.5	6.1

LSD_{var} = 2.6* (P<0.05) LSD_{treat} is not significant LSD_{inter} is not significant

TABLE 20

Sporulation on Clipper and Galleon Plants when Inoculated using the Single Droplet Technique

a) First Assessment (14 days after inoculation)

Inoculum concentration (spores per ml x 10^4)				
	$1x10^5$	$5x10^5$	$1x10^6$	Mean
Cultivar				
Clipper	29.8	21.7	11.8	21.1
Galleon	4.6	0.4	17.6	7.6
Mean	17.2	11.1	14.7	14.3

LSD was not significant

b) Second Assessment (21 days after inoculation)

Inoculum concentration (spores per ml x 10^4)				
	$1x10^5$	$5x10^5$	$1x10^6$	Mean
Cultivar				
Clipper	10.3	9.8	8.8	9.6
Galleon	0.8	0.6	2.9	1.4
Mean	5.5	5.2	5.8	5.5

LSD_{var}= 4.4* ($P<0.05$) LSD_{treat} is not significant LSD_{inter} is not significant

c) Third Assessment (28 days after inoculation)

Inoculum concentration(spores per ml x 10^4)				
	$1x10^5$	$5x10^5$	$1x10^6$	Mean
Cultivar				
Clipper	7.9	10.2	15.8	11.3
Galleon	5.0	2.9	5.7	4.5
Mean	6.5	6.5	10.7	7.9

LSD was not significant

TABLE 21

Sporulation per percent of leaf area diseased on Clipper and Galleon Plants when Inoculated using the Single Droplet Technique

a) First Assessment (14 days after inoculation)

Inoculum concentration (spores per ml. x 10^4)				
	$1x10^5$	$5x10^5$	$1x10^6$	Mean
Cultivar				
Clipper	2.14	2.31	2.81	2.42
Galleon	0.47	0.42	2.05	0.98
Mean	1.31	1.37	2.43	1.70

LSD was not significant

b) Second Assessment (21 days after inoculation)

Inoculum concentration (spores per ml x 10^4)				
	$1x10^5$	$5x10^5$	$1x10^6$	Mean
Cultivar				
Clipper	0.95	0.93	0.82	0.90
Galleon	0.23	0.13	0.42	0.28
Mean	0.59	0.53	0.64	0.59

LSD_{var} = 0.4* (P<0.05) LSD_{treat} is not significant LSD_{inter} is not significant

c) Third Assessment (28 days after inoculation)

Inoculum concentration (spores per ml x 10^4)				
	$1x10^5$	$5x10^5$	$1x10^6$	Mean
Cultivar				
Clipper	0.80	1.45	1.60	1.28
Galleon	0.62	0.29	0.61	0.51
Mean	0.71	0.87	1.11	0.90

LSD was not significant

Discussion

Significant differences in %LAD and spore count were apparent between Galleon and Clipper, with both handheld spray inoculation and with single droplet inoculation. However with the former, differences in spore counts were attributed to the differences in %LAD. With the single droplet inoculation %LAD and spore count were not significantly affected by changes in inoculum concentrations. This means that different inoculation times would show less variable results if the single droplet technique were to be used. The variable nature of spray inoculation was demonstrated by the results on Galleon at $5x10^5$ spores per ml., further indicating that the more consistent results of single droplet technique are preferable. For further glasshouse experiments the single droplet technique was used.

B) MEASUREMENT OF DISEASE COMPONENTS

(i) Comparison of four isolates of *R.secalis*

Materials and Methods

The four barley lines chosen for these experiments were tested against four isolates of *R.secalis*. These isolates were thought to represent the greater proportion of pathotypes in the population in South Australia (from Chapter 1).

Isolate number	Virulent on
412	Clipper, Gospeck (test a); Clipper (test b) (narrowest virulent range)
44/29	Brier, Clipper, Gospeck, Galleon, Atlas, Modoc-California, Nigrinudum, Sakigake West China (also La Mesita in one test) (widest virulent range)
183	Brier, Gospeck, Galleon, Modoc-California, Sakigake, West China (only viable culture that showed no lesions on Clipper -test a)
409	Atlas, Brier, Clipper, Gospeck, Galleon (most common reaction type ie. six isolates showed this reaction- see Table 13)

Sixteen pots of each barley line, with five seedlings each, were grown to the two and a half leaf stage. Inoculum was prepared from culture plates and varied from $1x10^5$ to $2x10^6$ spores per ml. Isolate 412 was not available when testing Galleon. Each isolate was inoculated onto four pots of each barley line, using the single droplet technique. Seedlings were misted as described previously and incubated in a growth room at 17°C.

From the seventh day of inoculation the plants were checked each morning for lesion development. The average number of days required for symptoms to appear was recorded as the incubation period. On the fifteenth day from inoculation, infection rate per pot was recorded. The lesions on the third leaf were harvested and those from the one pot were bulked. Five mls. of sterile distilled water were added and lesions were shaken, with a wrist-action shaker, for five minutes. Eight spore counts were done, using a haemocytometer and averaged. The lesions were floated for 48 hours at 15°C to promote further sporulation. At the end of this period the leaves were shaken again and another eight spore counts were taken. These lesions were kept for another seven days and further spore counts were taken. Spore count was converted to spores per lesion to overcome the effects of different infection rates. When Clipper and Galleon were tested the size of lesions was also measured in mm^2.

Analysis of variance was used to test the significance of results. Where different infection rates resulted in varying replicate numbers for spore counts and incubation periods, a t-test was used.

Results

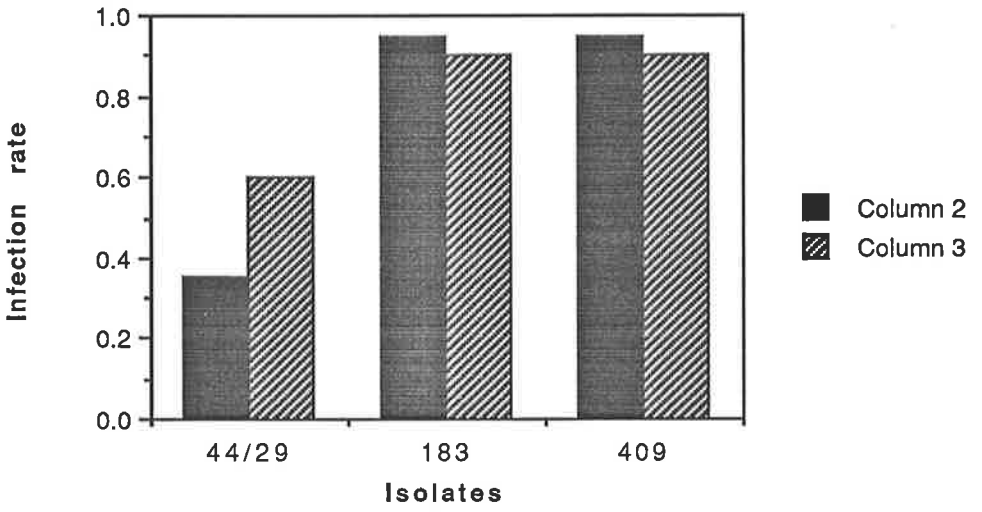
Infection rate of isolates was the only factor that varied between barley lines (Figs. 5,6,7, pages 61,62,63). Isolates varied in incubation period, infection rate and spore count.

Unlike the preliminary test no significant differences were found between Clipper and Galleon for any of the components measured (Table 22, pages 64-65) and this was attributed to the reduction in viability of the isolates. Isolate 44/29 had the longest incubation period, least infection rate and lowest spore count in the first two assessments.

The infection rate on line 207/39 was significantly less than that of Clipper, being approximately half the rate of Clipper (Table 23, page 66-67). The interaction term shows that the two barley lines had significantly different infection rates when inoculated with isolates 44/29 and 183. Isolate 44/29 again showed the lowest infection rate, and its spore count per lesion was equal lowest with isolate 412 at 24 days. Isolate 412 had the least spore count at 15 and 17 days. No other components were significantly different.

Figure 5

Infection rate (ratio of plants showing lesions out of total number inoculated) of three isolates of *R. secalis* inoculated by single droplet method on Clipper and Galleon.

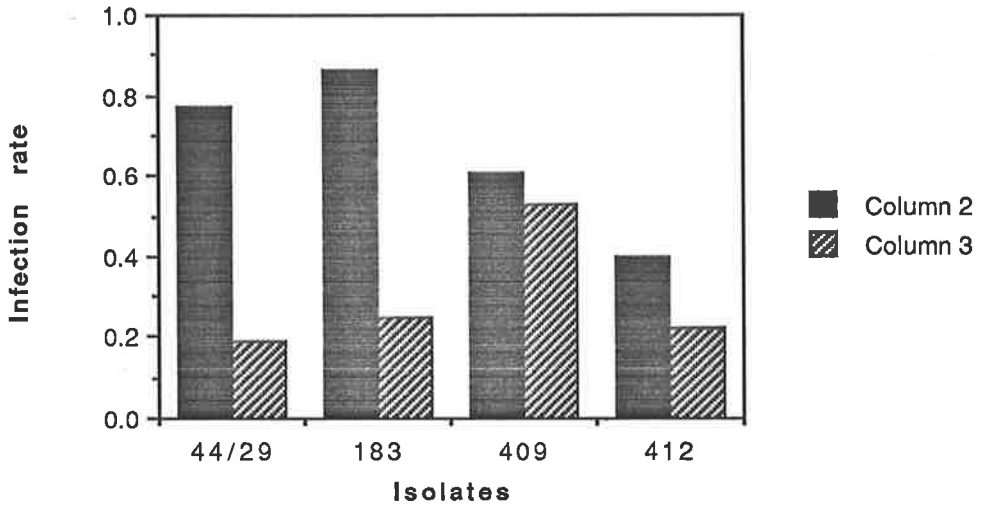


Column 2=Clipper

Column 3=Galleon

Figure 6

Infection rate (ratio of plants showing lesions out of total number inoculated) of four *R.secalis* isolates inoculated by single droplet method on Clipper and barley line 207/39

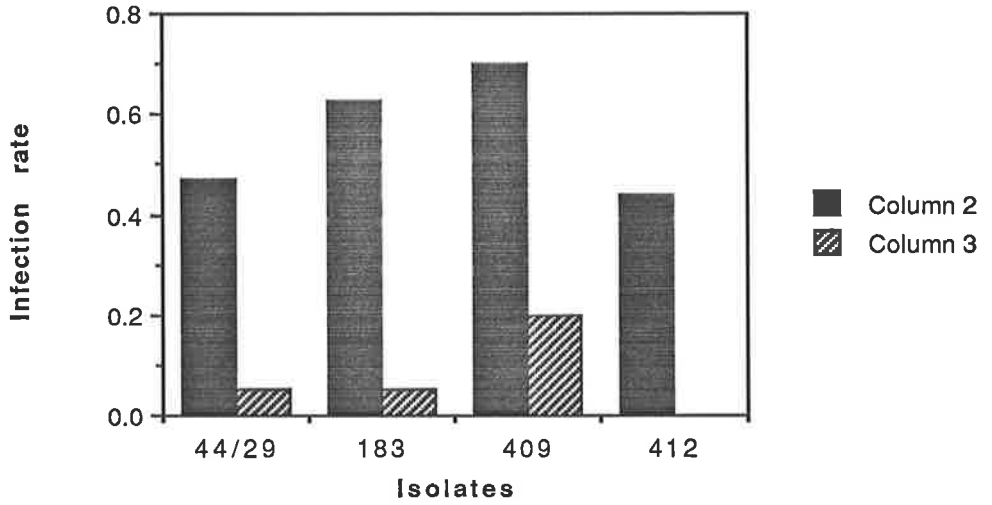


Column 2=Clipper

Column 3=207/39

Figure 7

Infection rate (ratio of plants showing lesions out of total number inoculated) of four *R.secalis* isolates inoculated by single droplet method on Clipper and barley line 116/39.



Column 2=Clipper

Column 3=116/39

TABLE 22

Analysis of variance on disease components measured on Clipper and Galleon when inoculated with three isolates of *R.secalis*.

a) Incubation period (days)

	Isolate			
	44/29	183	409	mean
Cultivar				
Clipper	14.0	10.4	9.2	11.2
Galleon	14.0	8.9	12.7	11.9
Mean	14.0	9.7	10.9	11.6

LSD_{treat} = 1.5* (P<0.05) LSD_{var}= Not Significant LSD_{inter}=Not Significant

b)Lesioned area mm^2

	Isolate			
	44/29	183	409	mean
Cultivar				
Clipper	27.3	76.9	13.6	39.3
Galleon	22.9	67.9	43.2	44.6
Mean	25.1	72.4	28.4	41.9

LSD_{treat} = 15.0* (P<0.05) LSD_{var}= Not Significant LSD_{inter}=Not Significant

c)Infection rate-i.e. ratio of inoculated plants that developed lesions

	Isolate			
	44/29	183	409	mean
Cultivar				
Clipper	0.35	0.95	0.95	0.75
Galleon	0.60	0.90	0.90	0.80
mean	0.48	0.93	0.93	0.78

LSD_{treat} = 0.18* (P<0.05) LSD_{var}= Not Significant LSD_{inter}= Not Significant

TABLE 22 (continued)

d) Spore count per lesion per ml. x 10^4

(i) 15 days after inoculation

	Isolate			
	44/29	183	409	Mean
Cultivar				
Clipper	0.06	0.29	1.86	0.74
Galleon	0.18	0.37	0.82	0.46
Mean	0.12	0.33	1.34	0.60

LSD_{treat} = 0.56* (P<0.05) LSD_{var}= Not Significant LSD_{inter}= Not Significant

(ii) 17 days after inoculation

	Isolate			
	44/29	183	409	Mean
Cultivar				
Clipper	4.89	4.69	10.70	6.76
Galleon	2.82	15.20	7.81	8.61
Mean	3.85	9.95	9.25	7.69

LSD_{treat}=NotSignificant LSD_{var}=NotSignificant LSD_{inter}=NotSignificant

(iii) 24 days after inoculation

	Isolate			
	44/29	183	409	Mean
Cultivar				
Clipper	7.88	8.07	7.00	7.68
Galleon	8.95	7.38	9.53	8.62
Mean	8.41	7.73	8.26	8.15

LSD_{treat}=NotSignificant LSD_{var}=NotSignificant LSD_{inter}=NotSignificant

TABLE 23

Measurement of disease components on Clipper and barley line 207/39 after inoculation with four separate isolate of *R.secalis*.

a) Incubation period (days)

	Isolate				
	412	44/29	183	409	Mean
Cultivar					
Clipper	14.9	14.5	14.0	14.8	14.5
207/39	14.5	15.3	14.3	14.6	14.7
Mean	14.7	14.9	14.2	14.7	14.6

LSD_{treat}=NotSignificant LSD_{var}=NotSignificant LSD_{inter}=NotSignificant

b) Infection rate- i.e. ratio of inoculated plants that developed lesions

	Isolate				
	412	44/29	183	409	Mean
Cultivar					
Clipper	0.40	0.77	0.86	0.61	0.66
207/39	0.22	0.19	0.25	0.53	0.30
Mean	0.50	0.30	0.56	0.57	0.48

LSD_{treat}=0.20* ($P<0.05$) LSD_{var}=0.14* ($P<0.05$) LSD_{inter}=0.28* ($P<0.05$)

c)Spore count per lesion per ml x 10^4

(i) 15 days after inoculation

	Isolate				
	412	44/29	183	409	Mean
Cultivar					
Clipper	0.4	3.7	5.7	4.3	3.5
207/39	0.6	3.8	11.1	5.2	5.2
Mean	0.5	3.8	8.4	4.8	4.4

t-test indicates that results for isolate 412 are significantly less than for isolate 183.

$t_{(P<0.05)}$=3.83

No other significant results were obtained.

TABLE 23 (continued)

(ii) 17 days after inoculation

	Isolate				
	412	44/29	183	409	Mean
Cultivar					
Clipper	1.8	4.9	9.8	6.6	5.8
207/39	2.6	9.9	20.6	11.8	11.2
Mean	2.2	7.4	15.2	9.2	8.5

t-tests indicate that results for isolate 412 are significantly less than for isolate 183

$t_{(P<0.05)}=3.26$

No other significant results were obtained

(iii) 24 days after inoculation

	Isolates				
	412	44/29	183	409	Mean
Cultivars					
Clipper	1.8	2.2	10.4	8.9	5.8
207/39	2.1	1.9	15.8	10.0	7.5
Mean	2.0	2.1	13.1	9.5	6.7

t-tests indicate that results for the isolates 44/29 and 412 are less than for the isolates 183 and 409

For isolates 412 and 183 $t_{(P<0.05)}=4.09$

For isolates 412 and 409 $t_{(P<0.05)}=2.68$

For isolates 44/29 and 409 $t_{(P<0.05)}=2.60$

The infection rate was significantly reduced on line 116/39 compared to Clipper (Table 24, page 69-70). This occurred across all four isolates even though a reduced infection rate was seen on Clipper compared to previous tests. This is thought to be due to reduced viability of inoculum brought about by continued transfers of cultures onto further plates.

While infection rate differences between Clipper and Galleon were masked by reduced viability, this was not the case with line 116/39 where resistance was much greater. Incubation period for the isolates that developed lesions on both barley lines did not differ significantly while spore counts per lesion, though not significantly different, varied widely (Fig. 8, page 71). The spore count for isolate 183 on barley line 116/39 was much higher than for Clipper. This was an unexpected result. It may be reflecting the 'adult susceptibility' that 116/39 showed in the field, which is otherwise a very resistant line.

TABLE 24

Measurement of disease components on Clipper and barley line 116/39 when inoculated with four separate isolates of *R.secalis*.

a)Incubation period (days)

	Isolates				
	412	44/29	183	409	Mean
Cultivar					
Clipper	14.0	14.3	15.1	14.1	14.4
116/39	-	16.0	14.0	15.5	15.0
Mean	14.0	15.2	14.6	14.8	14.8

LSD_{treat}=NotSignificant LSD_{var}=NotSignificant LSD_{inter}=NotSignificant

b)Infection rate-i.e. ratio of inoculated plants that developed lesions

	Isolates				
	412	44/29	183	409	Mean
Cultivar					
Clipper	0.46	0.50	0.64	0.70	0.58
116/39	0.00	0.05	0.05	0.20	0.08
Mean	0.23	0.28	0.35	0.45	0.33

LSD_{treat}=NotSignificant LSD_{var}=0.14*** ($P<0.001$) LSD_{inter}=NotSignificant

c)Spore count per ml per lesion $x10^4$

(i)15 days after inoculation

	Isolates				
	412	44/29	183	409	Mean
Cultivar					
Clipper	2.62	1.32	5.22	13.29	5.61
116/39	-	0.25	14.50	4.04	6.25
Mean	2.62	0.77	9.86	8.67	5.93

LSD_{treat}=NotSignificant LSD_{var}=NotSignificant LSD_{inter}=NotSignificant

TABLE 24 (continued)

(ii)17 days after inoculation

	Isolates				
	412	44/29	183	409	Mean
Cultivar					
Clipper	8.70	11.66	17.01	20.64	14.50
116/39	-	6.63	28.75	4.28	13.22
Mean	8.70	9.15	22.88	12.46	13.86

LSD_{treat}=NotSignificant LSD_{var}=NotSignificant LSD_{inter}=NotSignificant

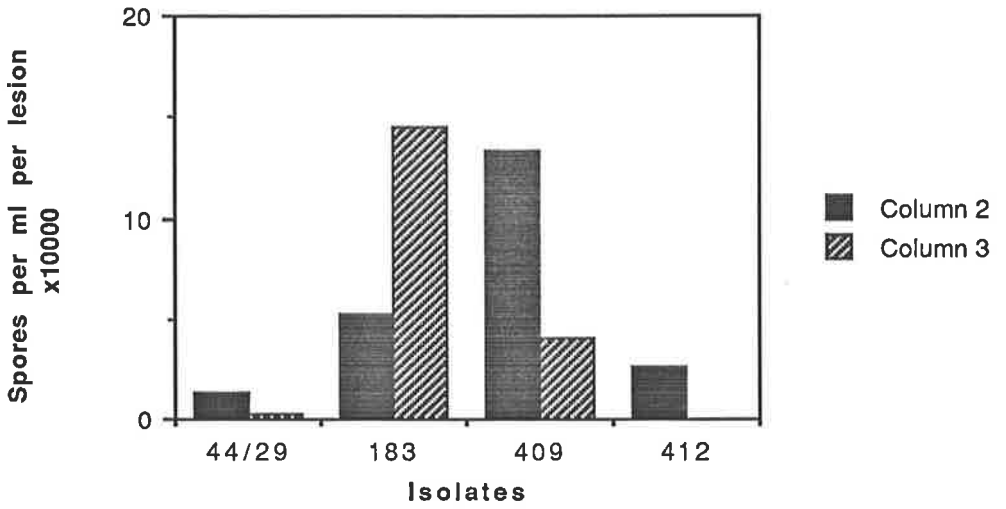
(iii)24 days after inoculation

	Isolates				
	412	44/29	183	409	Mean
Cultivar					
Clipper	2.27	3.34	1.08	3.49	2.55
116/39	-	1.38	2.63	7.66	3.89
Mean	2.27	2.36	1.86	5.58	3.22

LSD_{treat}=NotSignificant LSD_{var}=NotSignificant LSD_{inter}=NotSignificant

Figure 8

Spores per ml per lesion harvested from barley lines Clipper and 116/39 after inoculation with four isolates of *R.secalis*



Column 2=Clipper

Column 3=116/39

ii) Effect of inoculum concentration

To increase the sensitivity of the tests it may be necessary to use a specific concentration of spores. This was indicated in the preliminary test with spray inoculation where an effect was found on %LAD when inoculum concentration was altered. Inoculum concentrations did not alter results between Clipper and Galleon in the single droplet experiment, but it is possible that more resistant types will show differences, as was seen by their differing reactions to the low virulence isolates.

Materials and Methods

Seedlings were prepared as before. To return viability to isolates it was necessary to pass them through the plant host for at least one generation before returning to the culture medium. Therefore inoculum was prepared by first inoculating Clipper plants, at the two and half leaf stage. Resultant lesions were harvested, surface sterilised and floated in sterile distilled water at 15°C for 48 hours. The lesions were then shaken in the water, with a wrist action shaker, for five minutes to liberate the spores. Leaf material was removed from the solution and four separate concentrations were made up: $1x10^4$ spores per ml, $5x10^4$ spores per ml, $1x10^5$ spores per ml and $1x10^6$ spores per ml.

Six pots per cultivar, each with five seedlings, were inoculated with one of the spore suspensions using the single droplet technique. These were maintained , after 16 hours in a dew cabinet, in a 17°C controlled growth room. This made a more favourable post-inoculation environment than the glasshouse where temperatures were more variable.

Assessments and analyses were made as described previously.

Results

Infection rate was again the most significant factor between the different barley lines. It was also affected by the inoculum concentration.

Galleon had a lower infection rate than Clipper (Table 25, page 74-75), and infection rate increased with inoculum concentration, reaching a maximum at the second highest concentration (Fig. 9, page 76). Spore count per lesion was also significantly reduced on Galleon in comparison to Clipper, in the first assessment. The different concentrations resulted in the lowest inoculum load having significantly less sporulation than the other three concentrations. At the second and third assessment there were no significant differences in spore counts.

Infection rate between Clipper and line 207/39 differed significantly in three out of the four concentrations, with 207/39 having the lower rate (Fig. 10, page 77). Unlike the experiment with Galleon and Clipper, infection rate on Clipper did not significantly differ between concentrations. The two higher concentrations had a significantly greater infection rate on 207/39 than the two lower concentrations (Table 26, page 78-79). Incubation period showed no significant differences between barley lines or concentrations. Spore counts varied so that at the first assessment most spores were produced by the highest inoculum loading. No difference was seen between the barley lines. There was no significant difference in spore count for the remaining assessment times.

TABLE 25

Measurement of disease components on Clipper and Galleon when inoculated with four concentrations of *R.secalis* spores

a) Infection rate -ie. ratio of inoculated plants that develop lesions

	Inoculum concentration (spores per ml)				
	$1x10^4$	$5x10^4$	$1x10^5$	$1x10^6$	Mean
Cultivar					
Clipper	0.40	0.90	0.99	0.97	0.81
Galleon	0.24	0.63	0.83	0.83	0.63
Mean	0.32	0.77	0.92	0.90	0.73

LSD_{treat}=0.11* (P<0.05) LSD_{var}=0.08* (P<0.05) LSD_{inter}=NotSignificant

b) Spore count per ml per lesion $x10^4$

(i) 15 days after inoculation

	Inoculum concentration (spores per ml)				
	$1x10^4$	$5x10^4$	$1x10^5$	$1x10^6$	Mean
Cultivar					
Clipper	3.85	9.01	14.24	9.51	9.15
Galleon	3.56	5.24	4.67	6.41	4.97
Mean	3.71	7.12	9.45	7.96	7.06

LSD_{treat}=2.4* (P<0.05) LSD_{var}=3.4* (P<0.05) LSD_{inter}=NotSignificant

TABLE 25 (continued)

(ii) 17 days after inoculation

	Inoculum concentration (spores per ml)				
	$1x10^4$	$5x10^4$	$1x10^5$	$1x10^6$	Mean
Cultivar					
Clipper	4.87	4.44	3.87	5.56	4.71
Galleon	9.56	3.83	2.12	3.13	4.66
Mean	7.21	4.13	3.00	4.39	4.68

LSD_{treat}=NotSignificant LSD_{var}=NotSignificant LSD_{inter}=NotSignificant

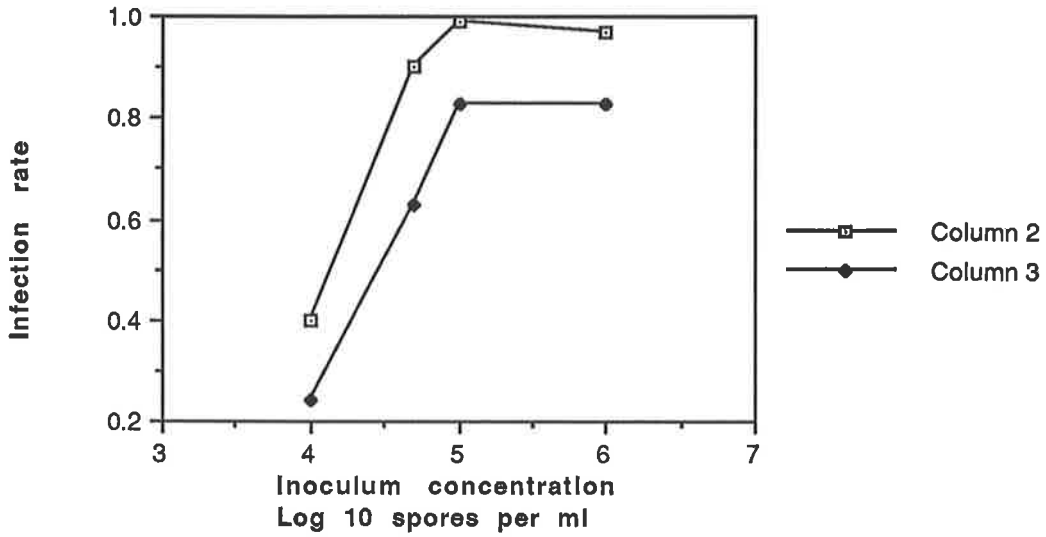
(iii)24 days after inoculation

	Inoculum concentration (spores per ml)				
	$1x10^4$	$5x10^4$	$1x10^5$	$1x10^6$	Mean
Cultivar					
Clipper	0.74	4.15	0.55	2.76	2.05
Galleon	3.84	0.56	0.23	1.35	1.50
Mean	2.29	2.36	0.39	2.06	1.78

LSD_{treat}=NotSignificant LSD_{var}=NotSignificant LSD_{inter}=NotSignificant

Figure 9

Infection rate (ratio of plants showing lesions out of total number inoculated) on Clipper and Galleon when inoculated with four concentrations of *R.secalis* spores using the single droplet method.

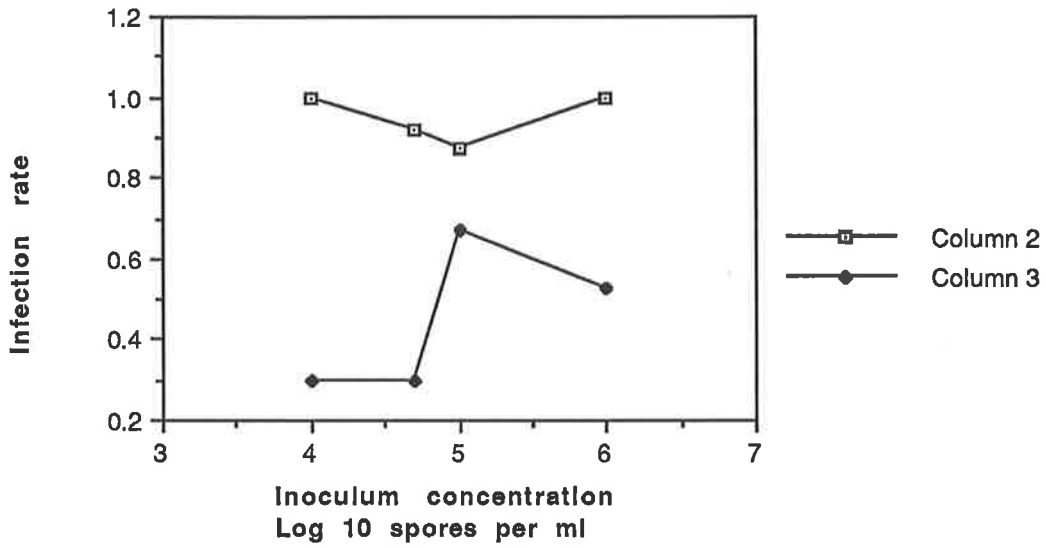


Column 2=Clipper

Column 3=Galleon

Figure 10

Infection rate (ratio of plants showing lesions out of total number inoculated) on Clipper and barley line 207/39 when inoculated with four concentrations of *R.secalis* spores using the single droplet method.



Column 2=Clipper

Column 3=207/39

TABLE 26

Measurement of disease components on Clipper and line 207/39 when inoculated with four concentrations of *R.secalis* spores.

a)Incubation period (days)

	Inoculum concentration (spores per ml)				
	$1x10^4$	$5x10^4$	$1x10^5$	$1x10^6$	Mean
Cultivar					
Clipper	12.4	12.0	11.7	11.7	12.0
Galleon	12.5	11.0	11.8	11.5	11.7
Mean	12.4	11.5	11.8	11.6	11.8

LSD_{treat}=NotSignificant LSD_{var}=NotSignificant LSD_{inter}=NotSignificant

b)Infection rate-ie. ratio of inoculated plants that develop lesions

	Inoculum concentration (spores per ml)				
	$1x10^4$	$5x10^4$	$1x10^5$	$1x10^6$	Mean
Cultivar					
Clipper	1.00	0.92	0.87	1.00	0.95
Galleon	0.30	0.30	0.67	0.53	0.45
Mean	0.65	0.61	0.77	0.77	0.70

LSD_{treat}=0.12* (P<0.05) LSD_{var}=0.24* (P<0.05) LSD_{inter}=NotSignificant

c)Spore count per ml per lesion $x10^4$

(i)15 days after inoculation

	Inoculum concentration (spores per ml)				
	$1x10^4$	$5x10^4$	$1x10^5$	$1x10^6$	Mean
Cultivar					
Clipper	16.5	9.4	13.8	25.4	16.3
207/39	8.7	8.7	13.9	24.2	13.9
Mean	12.6	9.0	13.8	24.8	15.1

LSD_{treat}=9.4* (P<0.05) LSD_{var}=NotSignificant LSD_{inter}=NotSignificant

TABLE 26 (continued)

(ii)17 days after inoculation

	Inoculum concentration (spores per ml)				
	$1x10^4$	$5x10^4$	$1x10^5$	$1x10^6$	Mean
Cultivar					
Clipper	16.7	12.5	15.1	25.6	17.5
207/39	10.1	11.3	16.4	25.1	16.5
Mean	13.4	11.3	16.4	25.1	16.5

LSD_{treat}=NotSignificant LSD_{var}=NotSignificant LSD_{inter}=NotSignificant

(iii)24 days after inoculation

	Inoculum concentration (spores per ml)				
	$1x10^4$	$1x10^5$	$5x10^5$	$1x10^6$	Mean
Cultivar					
Clipper	2.14	2.29	2.24	2.44	2.28
207/39	2.18	1.50	1.49	1.79	1.74
Mean	2.16	1.89	1.87	2.11	2.01

LSD_{treat}=NotSignificant LSD_{var}=NotSignificant LSD_{inter}=NotSignificant

(iii) Disease components on sixteen barley lines

Materials and Methods

Sixteen barley lines were chosen because they had shown a wide range of reactions to *R.secalis* when tested in the field. Selections from the same crosses were included in a single test and Clipper was always used as a susceptible control. The lines tested are listed below with their corresponding cluster group from field experiments.

SELECTION	CHARLICK CLUSTER	WARI CLUSTER
i) (Psaknon x Clipper) x Clipper		
6	A	C
21	B	E
22	A	C
44	E	E
70	B	E
ii) (Arivat x Clipper) x Clipper		
89	C	D
95	B	B
96	E	E
100	B	C
iii) Family 51		
215	B	A
943	A	A
iv) Family 39		
87	A	A
116	A	A
207	B	B
669	A	A

The four *R.secalis* isolates, 412, 44/29, 183, 409, thought to represent the range of pathotypes in natural population of the pathogen, were used in this experiment.

The seeds were surface sterilised, germinated and sown as described previously. Eight pots of each line were produced with five plants per pot. Two pots were inoculated with one isolate. The inoculum was prepared from lesions as described previously. Final concentrations were made up to $1\text{-}2x10^6$ spores per ml. The single droplet technique was used, seedlings were misted as before and incubated in a growth room at 17°C. Assessments were made of incubation period, infection rate and the spore count at 15, 17 and 24 days after inoculation.

Analysis of variance was used to test the significance of difference in results between isolates and barley lines. Linear regression and correlation were used to analyse the relationship between the disease components measured in the glasshouse and the %LAD recorded on the barley lines when screened in the field.

Results

Data from results are presented in Appendix 6. Analysis of variance of results showed that no significant differences occurred in incubation period and spore count across the isolates, or the sixteen barley lines under test. Infection rate was affected significantly by the isolate used in the inoculation and by the barley lines under test (Table 27, page 82).

TABLE 27

Analysis of variance of infection rate when sixteen barley lines are inoculated separately with four *R.secalis* isolates.

	Isolate				
	412	44/29	183	409	Mean
Barley line					
Clipper	0.70	1.00	1.00	1.00	0.93
Ps21(i)	0.75	1.00	1.00	1.00	0.94
Ps22	0.64	1.00	0.80	0.50	0.74
Ps44	0.84	0.50	0.60	0.60	0.63
Ps6	0.30	0.80	0.60	0.50	0.55
Ps70	0.50	0.60	0.70	0.35	0.54
Ar100(ii)	0.50	0.70	1.00	0.36	0.64
Ar95	0.17	0.67	1.00	0.74	0.64
Ar89	0.68	0.33	0.50	0.50	0.50
Ar96	0.33	0.50	0.50	0.23	0.39
207/39	0.30	0.67	0.33	0.20	0.38
116/39	0.10	0.40	0.30	0.33	0.28
87/39	0.00	0.00	0.10	0.00	0.03
669/39	0.00	0.00	0.00	0.00	0.00
943/51	0.00	0.00	0.00	0.00	0.00
215/51	0.00	0.00	0.00	0.00	0.00
Mean	0.36	0.51	0.53	0.39	0.45

LSD_{treat}=0.1* (P<0.05) LSD_{var}=0.2* (P<0.05) LSD_{inter}=NotSignificant

(i) Ps=(PsaknonxClipper)xClipper

(ii) Ar=(ArivatxClipper)xClipper

Isolate 409 and 412 had a reduced infection rate compared to isolates 183 and 44/29. No interaction was significant so that this was consistent across the barley lines. The lines 669/39, 943/51 and 215/51 were totally resistant to the four isolates ie. their infection rate was zero, and for 87/39 it was 0.03. The lines 116/39, 207/39 and ((Arivat x Clipper) x Clipper)/96 were the next least infected with average infection rates from 0.28 to 0.39. Highest infection rate was on Clipper (0.93) and ((Psaknon x Clipper) x Clipper)/21 (0.94).

Regressions were carried out between the glasshouse infection rates of each isolate and the corresponding %LAD on the barley lines, recorded when tested at Charlick and Waite Institute in 1982. There was no consistent relationship between glasshouse infection rate and field disease levels at a particular time in the crop growth as indicated by the regressions with individual assessment times (eg.Table 28, page 84) (Appendix 7a-d). The isolate infection rate significantly affects the relationship between the two factors. In all the assessments infection rate of isolate 412 (Table 28) had the highest correlation coefficient with %LAD, and highest percentage variation accounted for ie. 0.80 and 63.0% respectively, for assessment 3 at Waite Institute.

Usually epidemics are measured in terms of the rate of increase of the disease symptoms. However the epidemics used to screen the lines in the field were unusual in that the latter part of the growing season was very dry. Therefore disease levels measured at later growth stages were less than those at tillering (g.s. 29 Zadoks scale), making it difficult to use a rate as a measure of the epidemic. For this reason, the disease levels were

TABLE 28

Analysis of relationship between glasshouse infection rate and field %LAD for sixteen barley lines,at different assessment times.

Example: Isolate 412 (Analyses for other isolates presented in Appendix 7a-d)

Site	Assessment Number	Correlation Coefficient	Regression Coefficient	%Variation Accounted for
Charlick	1	0.42^{NS}	$0.0065^{*(P<0.05)}$	14.2
	2	0.42^{NS}	$0.0049^{*(P<0.05)}$	14.7
	3	0.52^{NS}	$0.0054^{**(P<0.01)}$	24.6
	4	$0.59^{*(P<0.05)}$	$0.0060^{**(P<0.01)}$	32.2
	5	0.55^{NS}	$0.0061^{**(P<0.01)}$	27.6
WARI	1	$0.71^{**(P<0.01)}$	$0.0064^{***(P<0.001)}$	47.9
	2	$0.60^{*(P<0.05)}$	$0.0055^{***(P<0.001)}$	33.6
	3	$0.80^{**(P<0.01)}$	$0.0234^{***(P<0.001)}$	63.0
	4	0.41^{NS}	$0.0193^{**(P<0.01)}$	13.6

Number of values =32

averaged over the growth stages, and this was used as a measure of the epidemic on individual barley lines. Regressions were carried out between this factor and the glasshouse infection rates.

Highest regression coefficients were seen between isolate 412 and the %LAD data (Table 29, page 86). The correlation coefficient for these two variables averaged over the two sites is 0.69 and 45.6% of the variation is accounted for by the regression line. The relationship between glasshouse infection rate and field disease recordings is reduced for isolates 183, then for 409 and isolate 44/29. The t-value for isolate 44/29 was not significant so that no regression line could be fitted. Thus this isolate showed no significant relationship between infection rate in the glasshouse and % LAD recordings from the field. Averaging the results of the four isolates produced a regression line that accounted for 19.9 % of the variation in the y-variate (Table 29). Figures 11-15 (pages 87-89) are the infection rates, on the sixteen barley lines tested, for each isolate and their average, plotted against the field disease levels. Many points vary widely from the line of best fit. ((Arivat x Clipper) x Clipper)/96 was consistently below the line, ie. had less disease in the glasshouse than in the field, while ((Psaknon x Clipper) x Clipper)/21 had a higher glasshouse disease rating than the field. Other barley lines were very variable in the glasshouse.

Data was available to allow infection rate for field results to be compared to infection rates in the glasshouse, for the sixteen barley lines tested. The mean infection rate of the four isolates on the sixteen barley lines was correlated with the mean infection rate of those lines in the field trials (Table 30, page 90). This resulted in a correlation coefficient of r = 0.741***. When correlated with the results from separate assessments at different growth stages, the correlations showed increasing significance occurring at the later growth stages.

TABLE 29

Correlation analyses between glasshouse infection rates of four *R.secalis* isolates and field %LAD for 16 barley lines

Isolate	Site	Correlation Coefficient	Regression Coefficient	%Variation Accounted for
412	Charlick	0.62*	0.0086***(P<0.001)	35.7
	WARI	0.70**	0.0110***(P<0.001)	47.5
	Mean	0.69**	0.0110***(P<0.001)	45.6
183	Charlick	0.45	0.0078*(P<0.05)	16.7
	WARI	0.48	0.0099*(P<0.05)	19.8
	Mean	0.48	0.0095*(P<0.05)	20.1
409	Charlick	0.24	0.0036^{NS}	2.0
	WARI	0.50	0.0091**(P<0.01)	21.5
	Mean	0.38	0.0067^{NS}	10.8
44/29	Charlick	0.23	0.0039^{NS}	1.4
	WARI	0.41	0.0086*(P<0.05)	13.8
	Mean	0.33	0.0064^{NS}	7.2
mean of four isolates		0.45	0.0084***(P<001)	19.9

Figure 11

Glasshouse infection rate of *R.secalis* isolate 412 on 16 barley lines compared to field disease ratings (%LAD) of same barley lines

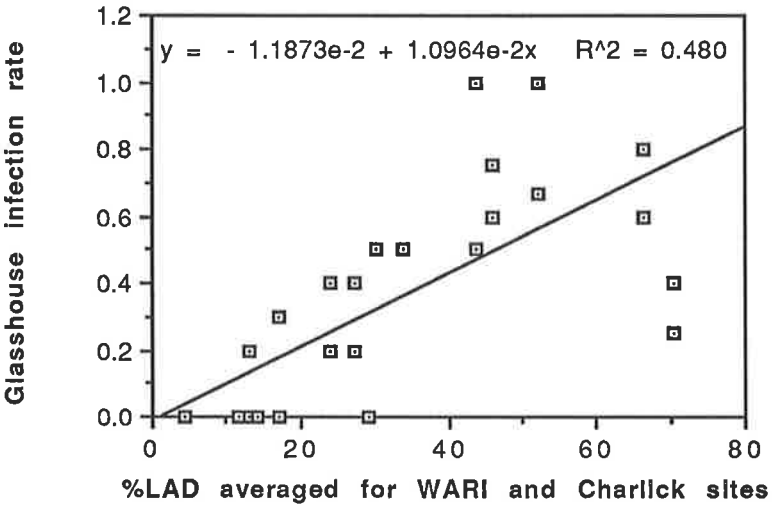


Figure 12

Glasshouse infection rate of *R.secalis* isolate 183 on 16 barley lines compared to field disease ratings (%LAD) of same barley lines

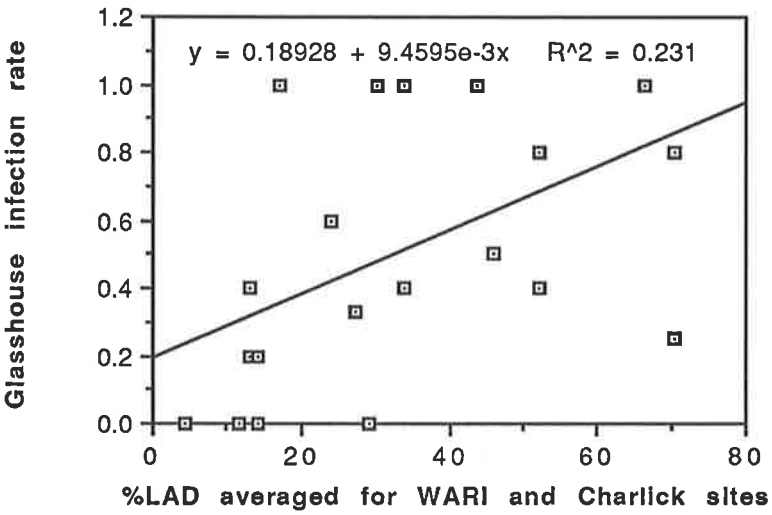


Figure 13

Glasshouse infection rate of *R.secalis* isolate 409 on 16 barley lines compared to field disease ratings (%LAD) of same barley lines

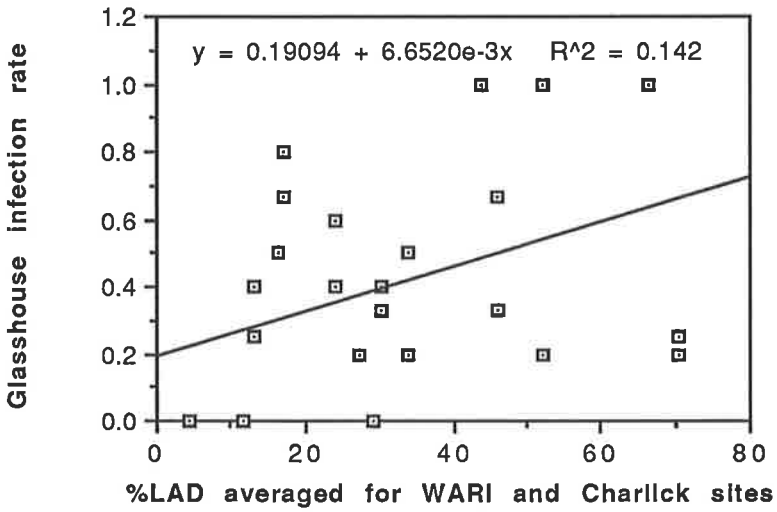


Figure 14

Glasshouse infection rate of *R.secalis* isolate 44/29 on 16 barley lines compared to field disease ratings (%LAD) of same barley lines

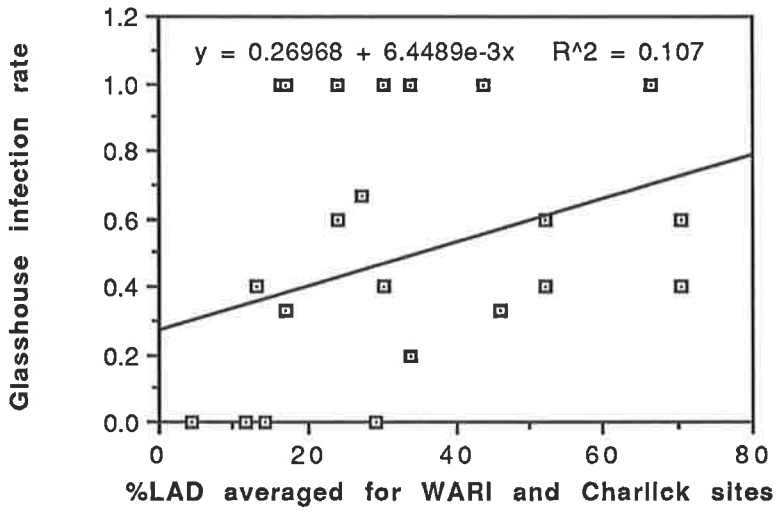


Figure 15

Mean glasshouse infection rate of four *R.secalis* isolates on 16 barley lines compared to field disease ratings (%LAD) of same barley lines

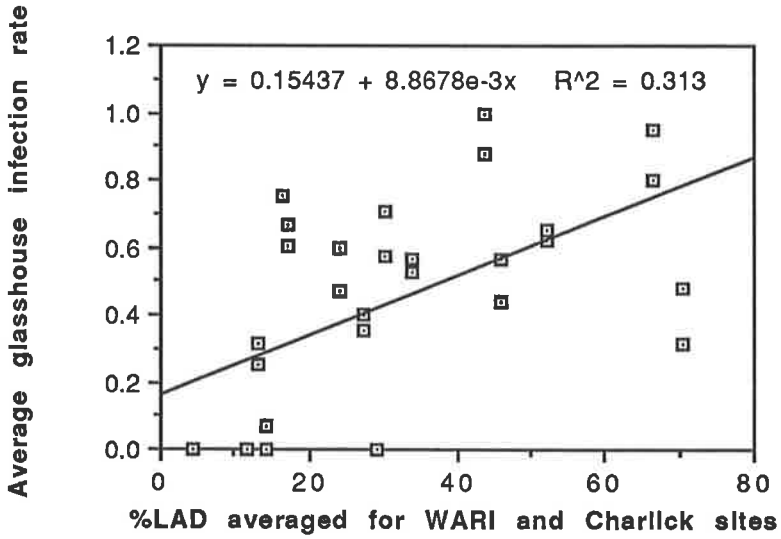


Table 30

Analysis of relationship between glasshouse infection rate and field infection rate at five assessment times, for sixteen barley lines.

Assessment number	Leaf number from top	Correlation Coefficient r^2	r
1	2	0.208	0.456^{ns}
	3	0.227	0.476^{ns}
2	2	0.181	0.425^{ns}
	3	0.256	$0.506^{*(P<0.05)}$
3	2	0.271	$0.521^{*(P<0.05)}$
	3	0.583	$0.764^{***(P<0.001)}$
4	2	0.290	$0.539^{*(P<0.05)}$
	3	0.424	$0.651^{**(P<0.01)}$
5	2	0.306	$0.553^{*(P<0.05)}$
	3	0.565	$0.752^{***(P<0.001)}$
mean	2^a	0.434	$0.659^{**(P<0.01)}$
	3^b	0.568	$0.754^{***(P<0.001)}$
	mean of a & b	0.549	$0.741^{**(P<0.01)}$

DISCUSSION

PART A. FIELD SCREENING

When tested in the field against a mixture of races, the barley lines showed varying degrees of resistance and susceptibility ie. a continuum of %LAD existed from 0% to 100%. As in Chapter 1, these results indicate that genotypes differed in degree of susceptibility rather than showing distinct resistant or susceptible groupings. This may have been why the analysis of variance showed no conclusive result and it is possible that the cluster analysis was not appropriate as it placed the barley lines into artificial resistant and susceptible groups, which in nature do not exist. Nevertheless the resistant genes tested in the field were found to be capable of significantly reducing disease levels when compared to susceptible cultivars, ie. those with no resistance genes. These genes were seen to be effective in a variety of backgrounds and in different combinations.

PART B. GLASSHOUSE SCREENING

Glasshouse tests had problems in maintaining consistency. The Clipper (control) infection rate varied from 0.58 to 1.00 in this set of experiments. In testing Clipper versus Galleon, infection rates were at first one hundred percent on both in the preliminary test, and were not found to be significantly different in the four pathotype test (b(i)), but were found to be significantly different in the concentration test (b(ii)).

The concentration of spore inoculum did not affect Clipper infection rate when Clipper and line 207/39 were tested. Nevertheless, the infection rate was significantly different between the cultivars, in both cases. Thus where resistance is high infection rate will still be detected. The discrepancies may have been due to the varying viability of the isolate

used to inoculate the cultivars eg. in test b(i) the isolate 44/29 had reduced infection rate so it was possibly losing viability.

It is important that glasshouse tests should have a control to measure the viability of the inoculum and to maintain consistent activity of the isolates, between tests. This can be partly overcome by passing the isolate through a generation on a susceptible host, such as Clipper, and harvesting the spores from the resultant lesions to be used as inoculum.

Spore count was seen to vary with resistance in only one instance, ie. with Clipper versus Galleon at the first spore count. Otherwise there was no significant difference in spore count associated with the host plant and its resistance to *R.secalis*.

From these tests it can be seen that different isolates will produce symptoms of *R.secalis* infection to varying degrees. A significant factor in incubation period, spore production and infection rate was the isolate itself. Therefore much of the variation seen in results may be due to the inherent differences between isolates, and individual viability levels of the isolates.

Experiments using the different isolates showed that infection rate is the one symptom, of those measured, that varies with scald susceptibility and resistance of the barley cultivar, in glasshouse tests. Inoculum spore concentration was seen to have an effect upon infection rate, emphasising the need to use constant inoculum concentrations. It also partly affected spore production, but not the incubation period.

Measuring infection rate of cultivars 207/39, 116/39 and Clipper in the glasshouse gave comparable results to those in the field. Where Clipper had an average field score of 66.7 %LAD, for the five field assessments, 207/39 had 27.5 %LAD and 116/39 had 13.0 %LAD ie. an approximate ratio of 5:2:1. The glasshouse infection rates were, respectively, 0.67,

0.30 and 0.08, or approximately 8:4:1. Thus the same order of susceptibility is maintained, though 116/39 had a lower relative glasshouse score than field score. This is indicative of the glasshouse test not reflecting the high disease levels seen in the adult plants of 116/39 in the field.

Infection rate was the only disease component, of those measured, that was significantly affected by the cultivar under test, when 16 barley lines were used. However different isolates produced varying relationships and one had no significant regression with field results. Isolate 412 had the highest correlation coefficient, 0.69, between infection rate and %LAD, and the regression analysis was able to account for 45.6% of the variation in the y-variate. Combining results merely averaged the coefficients and the % variation accounted for. Other variability may be due to differing viability of the inoculum, sensitivity of the pathogen to its environment and the wide variability occurring in the pathogen, demonstrated in this and the previous chapter.

Field tests emphasised that the degree of susceptibility of barley genotypes to *R.secalis* gave a clearer picture than attempting to classify genotypes as either resistant or susceptible. Comparing infection rates in the glasshouse and field, regardless of the isolate used, resulted in positive correlations (Table 30, page 90). This was first seen in the mobile nursery and glasshouse tests in Chapter 1 when the overall infection rates were found to be comparable if races were ignored. The final part of chapter 3 will indicate yet again that infection rate is an important measure of cultivar reaction to *R.secalis* again without the use of races.

CHAPTER 3

YIELD LOSSES IN BARLEY ASSOCIATED WITH LEAF SCALD

INTRODUCTION

With breakdown in major gene resistances occurring in many diseases, interest in resistance breeding has swung to using partial resistance, rather than complete resistance. This allows the disease to develop at a reduced rate, which has much less effect upon crop yield. It debatably reduces the selection pressure for more virulent and damaging forms of the pathogen. In order to make effective use of partial resistance it is of benefit to know what level of disease is tolerable, and which crop growth stages are most affected by the disease, in terms of grain yield. Previous chapters have indicated the presence of degrees of susceptibility rather than distinct resistant and susceptible types, and so the possibility of detecting slow-scalding types exists.

In a controlled environment, Mayfield (1982) showed that greatest losses occurred when plants were inoculated after stem elongation. However analysis in the field indicated that disease at early crop growth stages reduced grain yield more than at later growth stages, possibly due to effects on root growth. Closer to maturity there was a lower correlation between disease and yield. James *et al* (1968) in England have indicated the greater significance of scald disease later in the season. The above work suggests that in Australia the epidemic pattern differs from that reported elsewhere and so effect upon yield may differ accordingly. The following field experiment was designed to study the effects of different infection levels of scald disease on barley grain yield.

Yield components of barley that are reduced by the disease are to be identified and yield loss measured and correlated with disease levels at different crop growth stages. By studying different epidemics it should be possible to identify the crop growth stages at which the barley plant is damaged (in terms of final yield) by the disease. This information would enable the selection of barley types that may avoid disease (if at all

possible). The measurement of the epidemics will also give some indication of the amount of scald disease that is present before yield loss is measurable, and so provide a base measure of visible disease for the selection of barleys partially resistant to scald disease.

Materials and Methods

Four selections as described in Chapter 2, from family 39 (87, 116, 207 and 669 and three selections from family 51 (215, 765, 943 were chosen for the experiment.

Family 39= Atlas 46 (Rh2Rh3) x Atlas 57 (Rh3)

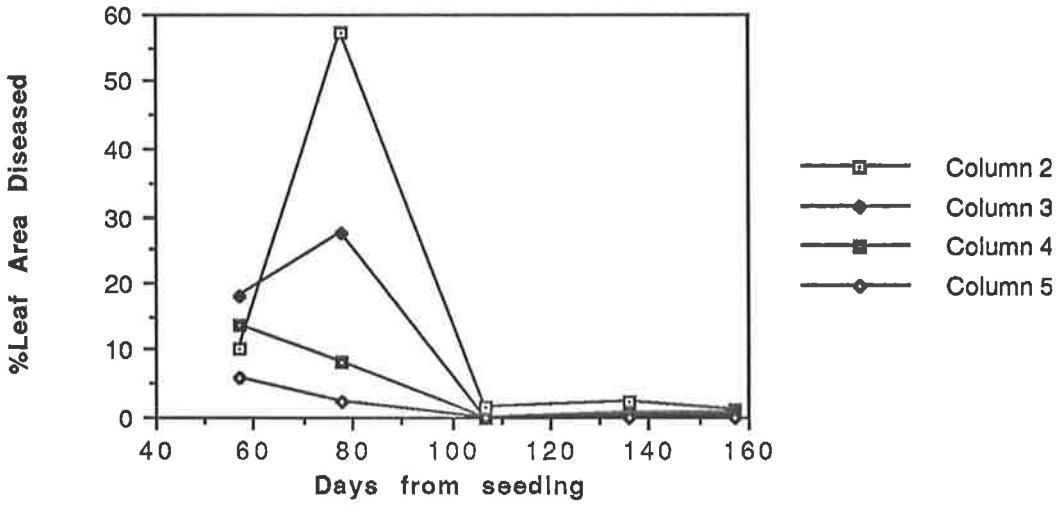
Family 51=Atlas 46 (Rh2Rh3) x. Hudson (Rh2Rh3)

Commercial cultivars = Clipper, Galleon and Schooner were also included.

Data obtained from screening trials (Chapter 2) was used to produce disease curves for these ten barley lines (Figs. 16-18, pages 96-98).

Figure 16

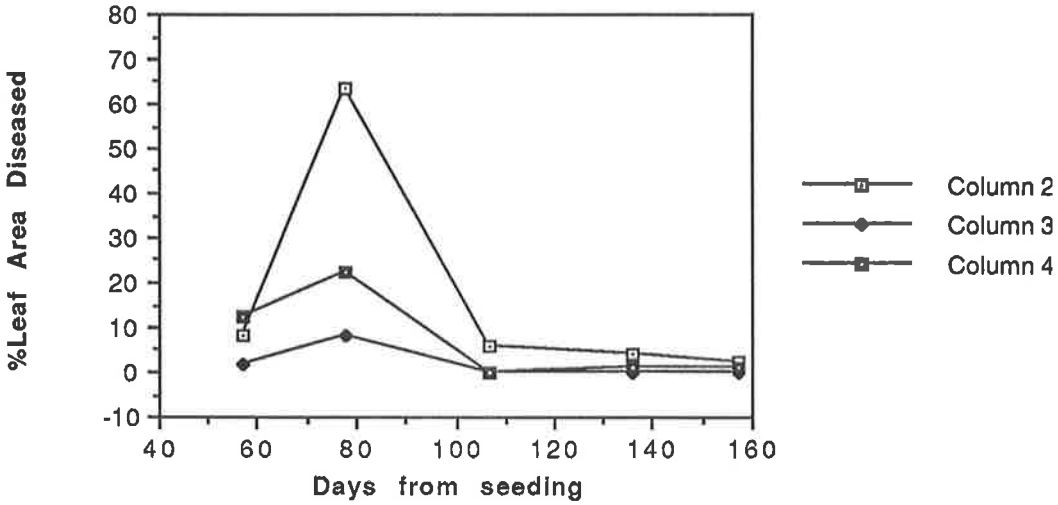
% Leaf Area Diseased (%LAD) measured in field screening trials (Chapter 2) on selected barley lines from family 39



Column 2=207/39
Column 3=87/39
Column 4=116/39
Column 5=669/39

Figure 17

% Leaf Area Diseased (%LAD) measured in field screening trials (Chapter 2) on selected barley lines from family 51



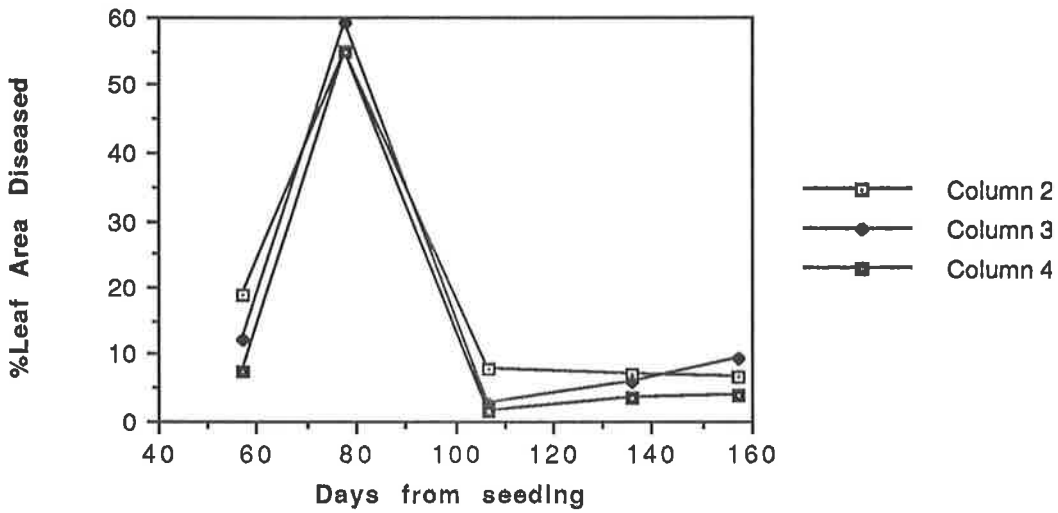
Column 2=215/51

Column 3=765/51

Column 4=943/51

Figure 18

% Leaf Area Diseased (%LAD) measured in field screening trials (Chapter 2) on commercial cultivars



Column 2=Clipper
Column 3=Galleon
Column 4=Schooner

There were four disease epidemic treatments (Table 31, page 100)

1) Treatment O was designed to have no disease development; these plots were sprayed with fungicide every 3 weeks

2) Treatment E (early) was designed to produce an epidemic at early crop growth stages only.

3) Treatment L (late) was designed to produce an epidemic at later growth stages only and,

4) Treatment D (disease) allowed early infection and continuation of the epidemic throughout the growth of the crop.

Irrigation was applied to the trial to encourage the development and spread of scald disease. There were only two replicates per treatment because of the wide range of epidemics likely and because effects would be assessed using the regression analysis, as well as the analysis of variance.

Plots were 4 rows wide, each row 0.25m apart, and 4m long and separated by 1m gaps. Wheat, which is immune to scald disease, was sown in alternate plots (also 4 rows wide x 4m long) to reduce interplot interference.

The trial was sprayed twice with Milgo (a.i. ethirimol) at the rate of 0.35 l a.i./ha to control powdery mildew (*Erysiphe graminis hordei)*. This chemical does not control scald. A low level of netblotch (*Pyrenophora teres*) developed very late in the crop growth and was not sprayed against.

TABLE 31

Crop growth stage at which experiment was sprayed with Benlate fungicide and inoculated with *R.secalis*, to obtain desired epidemics.

Treatment code	Growth stage of crop at inoculation	Spray time (c)
O	-	1-5
E	g.s. 12 (Zadoks scale) (a)	3-5
L	g.s. 31 (Zadoks scale) (b)	1-2
D	g.s. 12 (Zadoks scale) (a)	nil

a. Inoculation was achieved by spreading straw, infected with *R.secalis* , at a rate of 30 gm per m^2.

b. Inoculation was achieved by the spore suspension method described in Chapter 2.

c. Fungicide used was Benlate (50% a.i. benomyl) at 500 g a.i. per ha.

Spray timetable:

1=33 days after seeding

2=63 days after seeding

3=92 days after seeding

4=123 days after seeding

5=137 days after seeding

TABLE 32

Days after seeding and crop growth stage at which experiment was assessed for levels of scald disease.

Assessment number	Days after seeding	Decimal growth stage (Zadoks scale)
1	36	21
2	57	26
3	81	30-35
4	102	33-53
5	120	45-68
6	141	58-85
7	164	85

Disease assessments were carried out at intervals of approximately three weeks, as shown in Table 32 (above)

The growth stages at some of the assessment times differed for the different lines. The three commercial cultivars and selection 207/39 matured later than the other lines. The first growth stage listed for assessments 3-6 in Table 32 refers to these later maturing lines, and the second growth stage to the remainder. The final assessment refers only to the four later maturing lines as the others had matured at this stage and their final disease readings were taken at the previous assessment.

Disease severity was assessed on ten tillers selected at random from each plot, by estimating the percentage leaf area diseased (%LAD) on the top four fully expanded leaves. Disease keys (James *et al*, 1968) were used in estimating the %LAD. Heights of plants were also measured at each assessment.

The number of seeds per head and the number of undeveloped florets at the base of the heads were counted on twenty five heads per plot sampled at random. The remaining grain was harvested using a mechanical plot harvester. A sample of 100 grains per plot was weighed to calculate mean grain weight. The percentage screenings for Clipper, Galleon and Schooner were calculated by weighing 50 ml volume of grain from each plot and then shaking on a 2.5 mm sieve. The total weight of the grain that passed through the sieve was expressed as a percentage of the original weight.

Some of the plots were severely damaged by wind, causing a high proportion of heads to be blown to the ground. The number of heads per m^2 were counted within each plot after harvest, and after multiplication by the average head weight (ie. nos. seeds/head x seed weight) for respective plots, was added to the yield per plot.

The percentage protein content of grain was measured for the commercial cultivars using a near infrared (NIR) analyser technique.

Results

a) Disease development

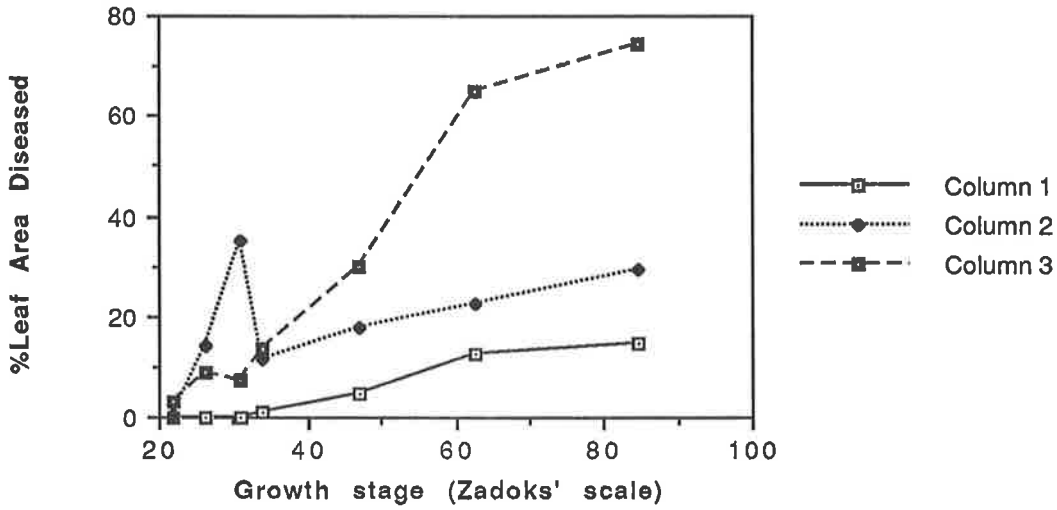
The disease development on each line is shown in Figs 19-28 (pages 104-113). A complete set of results is presented in Appendix 8. Development of scald disease in the L treatment was not significantly different from that in the O (no disease) treatment. It was concluded that the late inoculation was unsuccessful and this treatment could give little information about late development of scald disease, and hence is not included in the results.

Scald disease in treatment E appeared on the upper foliage of susceptible lines in all assessments, despite the fungicide applications after growth stage 31 (Zadoks scale). However the levels were significantly lower than on those in plots receiving treatment D. Generally, throughout the season the disease levels on the susceptible lines were higher in treatments E and D than in treatment O, while D increased beyond E as the crop matured.

The epidemic appeared to have two main peaks in disease severity, one much larger than the other. The larger peak was at the end of crop growth when as much as 65 %LAD was seen on Clipper with treatment D. The other peak occurred at the very end of tillering (g.s. 29 Zadoks scale). During stem elongation there was an apparent drop in disease levels, as the spread of disease lagged behind the production of new leaves.

Figure 19

% Leaf Area Diseased (%LAD) on Clipper under three spray regimes
(Treatments O, E and D)



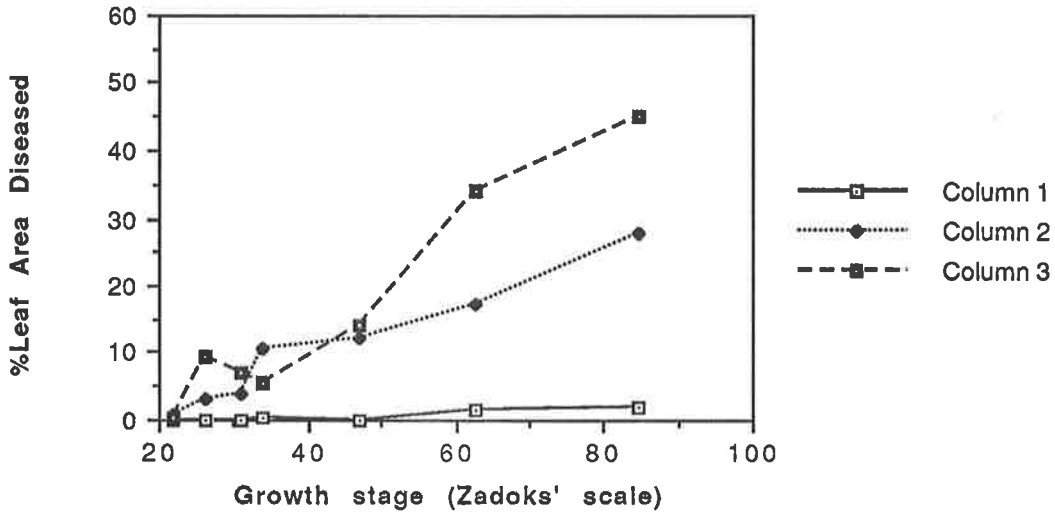
Column 1=Treatment O regular fungicide sprays to produce minimal levels of scald disease

Column 2=Treatment E fungicide sprays begin at 92 days after seeding to produce Early disease epidemic

Column 3=Treatment D no fungicide sprays applied, thus enabling epidemic to develop throughout the growing season.

Figure 20

% Leaf Area Diseased (%LAD) on Galleon under three spray regimes
(Treatments O, E and D)



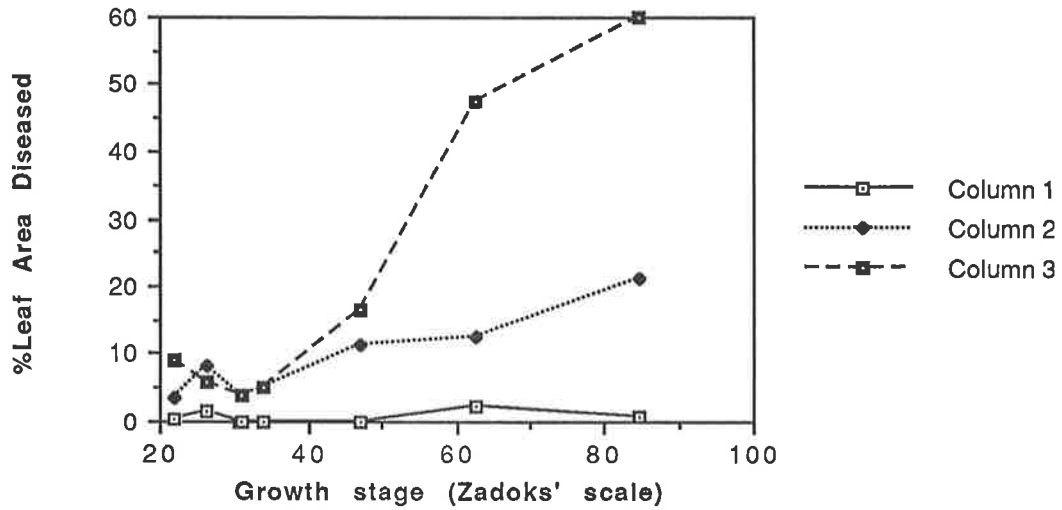
Column 1=Treatment O regular fungicide sprays to produce minimal levels of scald disease

Column 2=Treatment E fungicide sprays begin at 92 days after seeding to produce Early disease epidemic

Column 3=Treatment D no fungicide sprays applied, thus enabling epidemic to develop throughout the growing season.

Figure 21

% Leaf Area Diseased (%LAD) on Schooner under three spray regimes
(Treatments O, E and D)



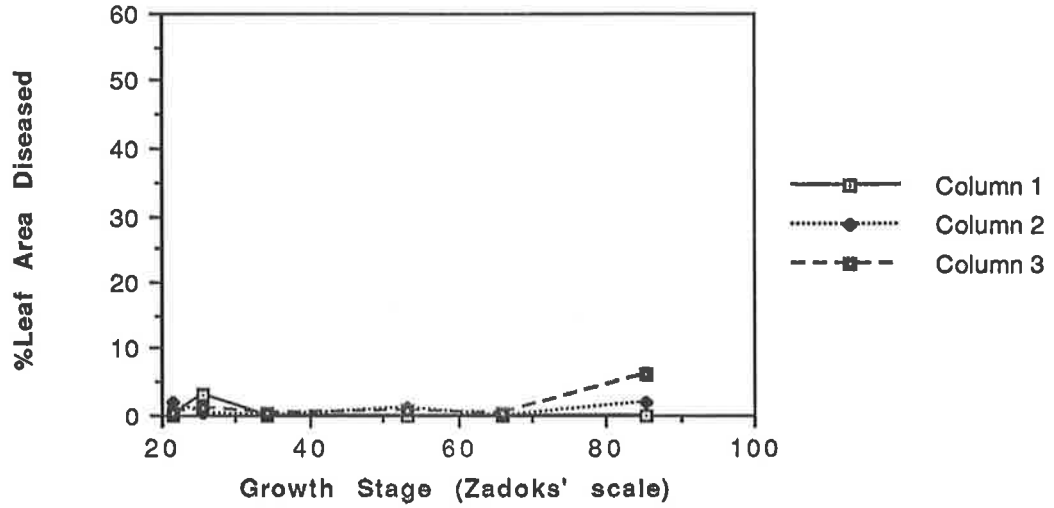
Column 1=Treatment O regular fungicide sprays to produce minimal levels of scald disease

Column 2=Treatment E fungicide sprays begin at 92 days after seeding to produce Early disease epidemic

Column 3=Treatment D no fungicide sprays applied, thus enabling epidemic to develop throughout the growing season.

Figure 22

% Leaf Area Diseased (%LAD) on 87/39 under three spray regimes
(Treatments O, E and D)



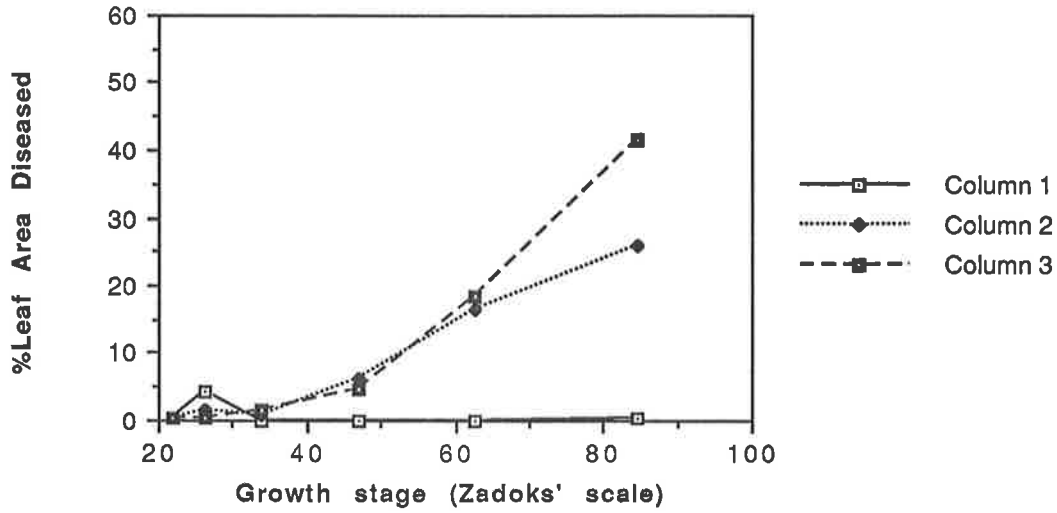
Column 1=Treatment O regular fungicide sprays to produce minimal levels of scald disease

Column 2=Treatment E fungicide sprays begin at 92 days after seeding to produce Early disease epidemic

Column 3=Treatment D no fungicide sprays applied, thus enabling epidemic to develop throughout the growing season.

Figure 23

% Leaf Area Diseased (%LAD) on 116/39 under three spray regimes
(Treatments O, E and D)

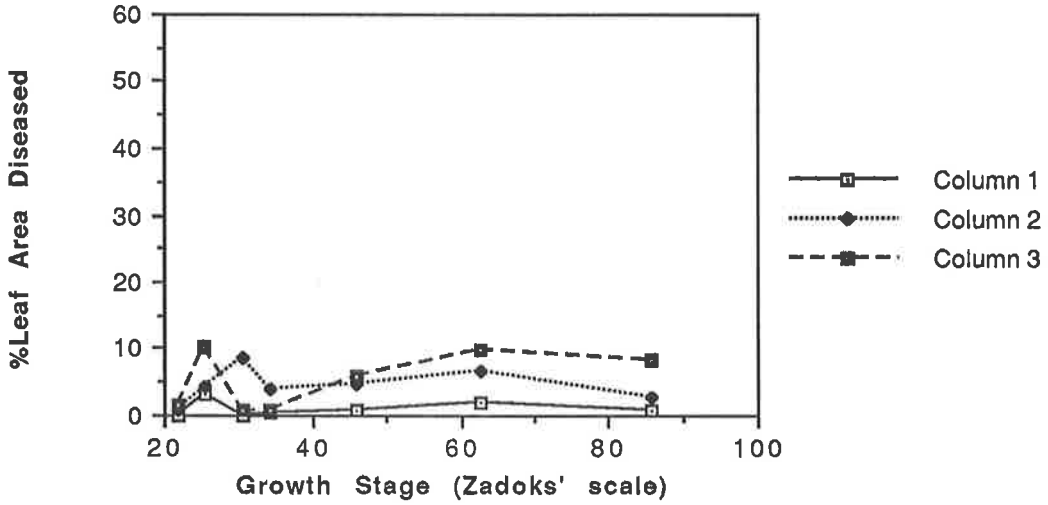


Column 1=Treatment O	-regular fungicide sprays to produce minimal levels of scald disease
Column 2=Treatment E	-fungicide sprays begin at 92 days after seeding to produce Early disease epidemic
Column 3=Treatment D	-no fungicide sprays applied, thus enabling epidemic to develop throughout the growing season.

Figure 24

% Leaf Area Diseased (%LAD) on 207/39 under three spray regimes

(Treatments O, E and D)



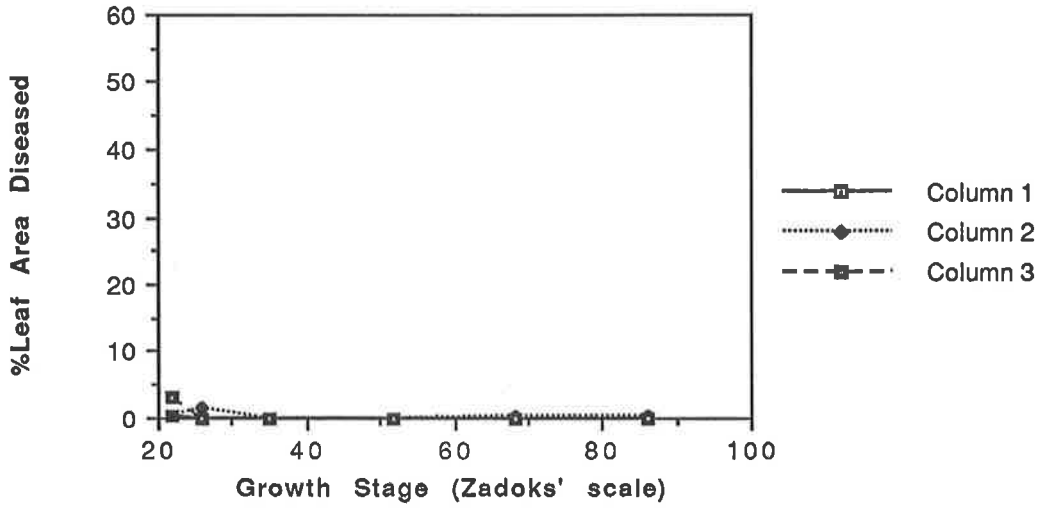
Column 1=Treatment O regular fungicide sprays to produce minimal levels of scald disease

Column 2=Treatment E fungicide sprays begin at 92 days after seeding to produce Early disease epidemic

Column 3=Treatment D no fungicide sprays applied, thus enabling epidemic to develop throughout the growing season.

Figure 25

% Leaf Area Diseased (%LAD) on 669/39 under three spray regimes
(Treatments O, E and D)



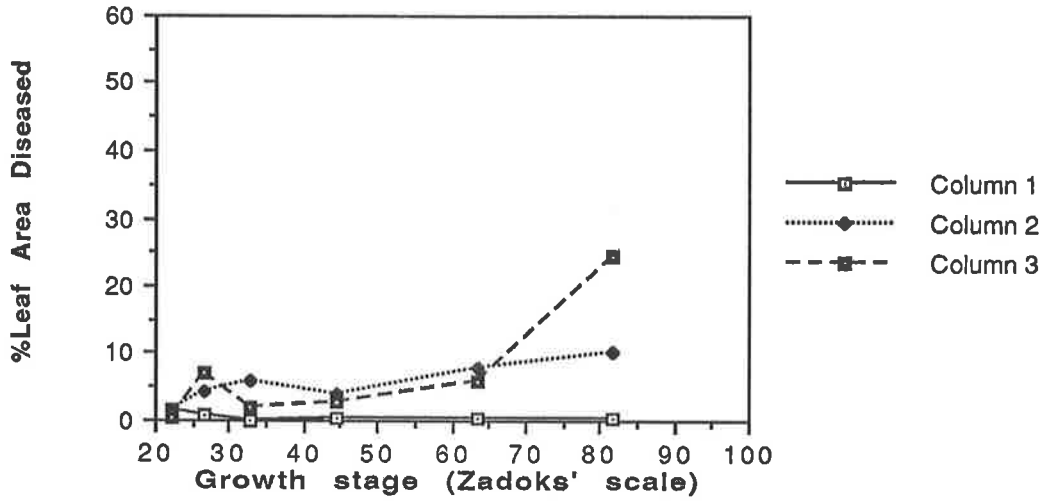
Column 1=Treatment O regular fungicide sprays to produce minimal levels of scald disease

Column 2=Treatment E fungicide sprays begin at 92 days after seeding to produce Early disease epidemic

Column 3=Treatment D no fungicide sprays applied, thus enabling epidemic to develop throughout the growing season.

Figure 26

% Leaf Area Diseased (%LAD) on 215/51 under three spray regimes
(Treatments O, E and D)



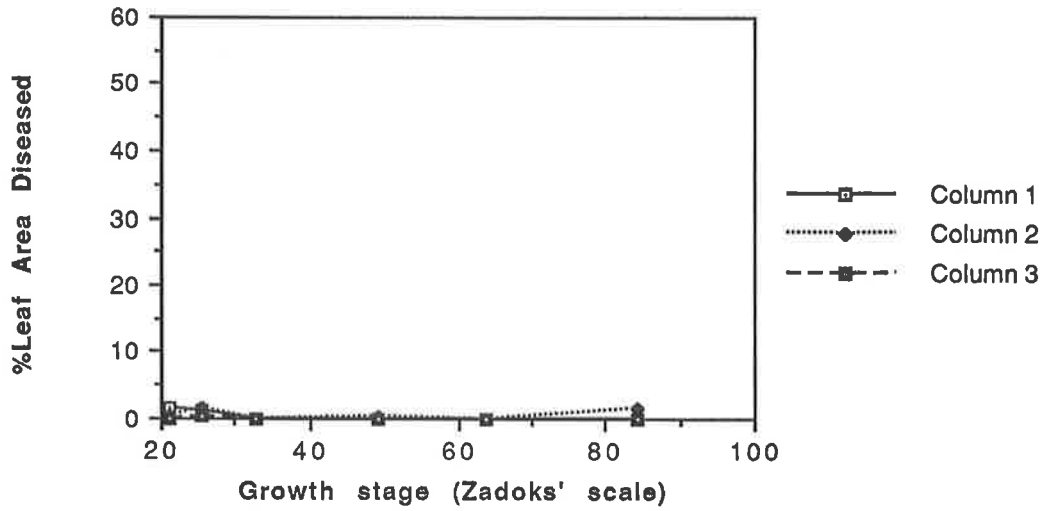
Column 1=Treatment O regular fungicide sprays to produce minimal levels of scald disease

Column 2=Treatment E fungicide sprays begin at 92 days after seeding to produce Early disease epidemic

Column 3=Treatment D no fungicide sprays applied, thus enabling epidemic to develop throughout the growing season.

Figure 27

% Leaf Area Diseased (%LAD) on 765/51 under three spray regimes
(Treatments O, E and D)



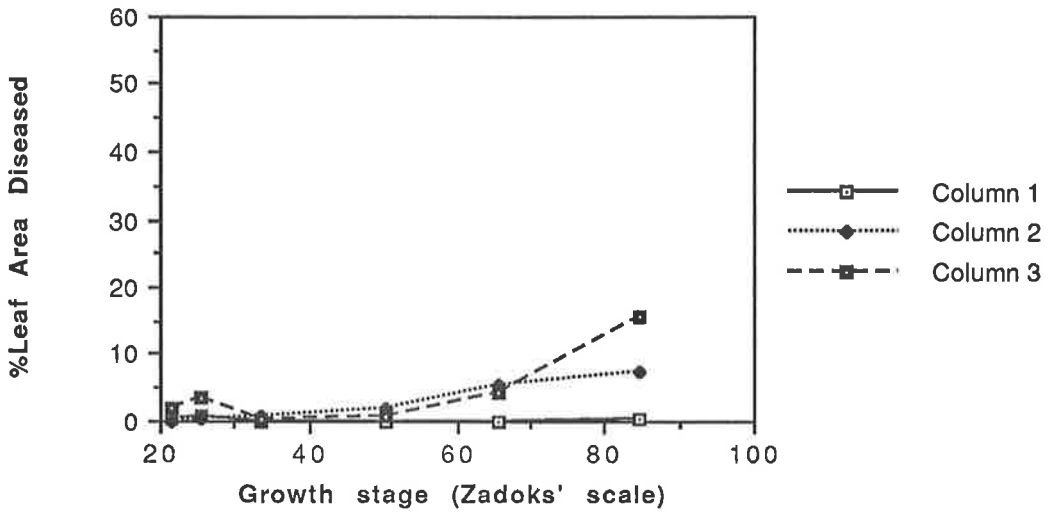
Column 1=Treatment O regular fungicide sprays to produce minimal levels of scald disease

Column 2=Treatment E fungicide sprays begin at 92 days after seeding to produce Early disease epidemic

Column 3=Treatment D no fungicide sprays applied, thus enabling epidemic to develop throughout the growing season.

Figure 28

% Leaf Area Diseased (%LAD) on 943/51 under three spray regimes
(Treatments O, E and D)



Column 1=Treatment O regular fungicide sprays to produce minimal levels of scald disease

Column 2=Treatment E fungicide sprays begin at 92 days after seeding to produce Early disease epidemic

Column 3=Treatment D no fungicide sprays applied, thus enabling epidemic to develop throughout the growing season.

Analysis of variance was carried out on each assessment period. There were no significant differences between lines and/or treatments for the first two assessments (at g.s. 21 and g.s. 25 Zadoks scale). However when the results of treatments E and D were combined and those of O and L combined for the second assessment and re-analysed, a significant difference in disease levels was found. This could be done since the inoculation and spray regimes at this time were the same for the combined treatments. Thus at assessment 2 significantly higher levels of scald were detected in the inoculated plots of Galleon, Clipper, Schooner and line 207/39 than in the untreated plots (Table 33, page 115). Lines 765/51, 87/39 and 669/39 showed only traces of scald disease throughout the season and so can be classified as being resistant. Lines 943/51 and 207/39 showed only low levels of scald and line 215/51 showed higher than 10 %LAD at the final assessment. Line 116/39 had very little scald disease early in the season but the upper foliage had developed 42 %LAD by the end of the season. This then is typical of a late epidemic. The commercial cultivars were the most susceptible in this experiment, with Clipper showing higher disease levels than Galleon and Schooner. From the third assessment when a difference in disease levels became significant, Clipper continuously showed higher levels of disease than all other lines.

TABLE 33

Percentage leaf area diseased (%LAD) on the ten barley lines at assessment 2 (57 days from seeding) (g.s.26.1 Zadoks decimal scale)

	Treatments[c]		
	[a]O,L mean	[b]E,D mean	mean
Barley line			
765/51	2.40	0.45	1.66
943/51	4.20	1.90	3.03
215/51	2.15	5.70	3.91
669/39	1.35	0.90	1.12
87/39	2.95	0.90	1.93
116/39	2.30	0.85	1.61
207/39	4.90	7.35	6.12
Schooner	1.15	7.10	4.10
Galleon	0.00	6.25	3.13
Clipper	0.20	11.70	5.95
mean	2.16	4.31	3.26

$LSD^{treatment}_{=0.16^*}$ ($P<0.05$) $LSD^{barley}_{=5.20^*}$ ($P<0.05$)

a O & L mean = mean of %LAD of zero (O) disease treatment and %LAD of late (L) disease treatment

b E & D mean = mean of %LAD of early(E) disease treatment and %LAD of continual disease (D) treatment

c O=Treatment O - no disease epidemic
E=Treatment E - disease epidemic early in growing season
L=Treatment L - disease epidemic late in growing season
D=Treatment D - disease epidemic throughout the growing season

b) Plant Growth

i) Growth stage results are presented in Appendix 9. At the first two assessments there were no differences in growth stage between any of the barley lines nor between any of the disease treatments. At the third assessment the commercial cultivars and line 207/39 had only reached growth stage 30 (Zadoks scale) whereas the remaining selections were at more advanced growth stages (33-35). At the fourth assessment the commercial cultivars and line 207/39 were at elongation (g.s. 32-35) while the other lines were from midway through booting (g.s. 44 Zadoks scale) to halfway through inflorescence emergence (g.s. 55 Zadoks scale) (Table 34, page 117). This variance in maturity continued for the remainder of the season. A final disease assessment consisting of only Clipper, Galleon, Schooner and 207/39 was necessary so that disease levels for the different growth stages were comparable across the different lines.

At the fourth assessment, plots receiving treatment O were significantly more mature than those receiving other treatments (Table 34). There were no significant interactions between treatments and lines. Thus both resistant and susceptible lines were affected to a similar extent even though there were significant differences in severity of scald. Hence it is not likely that disease was the cause of this effect. Later assessments did not show this difference in growth stage across the treatments.

TABLE 34

Effects of different scald disease epidemics on development of different barley lines (decimal growth stage, Zadoks, at the fourth assessment, 102 days from seeding)

	Treatments				
	O[A]	E	L	D	mean
Barley Line					
765/51	50.3	47.3	47.2	52.0	49.2
943/51	49.0	54.9	46.4	51.5	50.5
215/51	44.1	43.9	43.1	47.2	44.6
669/39	55.7	49.4	53.3	48.9	51.8
87/39	52.8	53.1	53.9	52.4	53.1
116/39	51.3	47.7	47.9	42.7	47.4
207/39	33.9	33.1	33.8	32.3	33.0
Schooner	35.2	32.8	33.6	33.6	33.8
Galleon	33.4	32.6	33.3	31.8	32.9
Clipper	33.1	32.1	35.0	31.8	33.0
mean	43.9	42.7	42.6	42.5	42.9

$LSD^{treatment} = 0.52^{**}$ (P<0.01) $LSD^{barley} = 2.80^{**}$ (P<0.01)

A O=Treatment O - no disease epidemic
E=Treatment E - disease epidemic early in growing season
L=Treatment L - disease epidemic late in growing season
D=Treatment D - disease epidemic throughout the growing season

ii) Plant height results are presented in Appendix 10. As with growth stage, barley lines did not develop significant differences in height until the third assessment. Clipper, Galleon, Schooner and line 207/39 were not significantly different from one another, but were shorter than the remaining lines. These showed significant differences from the shortest line (line 207/39) to the tallest (line 669/39). By the fifth assessment Clipper and Galleon were significantly shorter than Schooner and 207/39 while the remaining selections were taller than all four. In the final assessment 207/39 was the tallest of these four, and Schooner was taller than Clipper and Galleon.

From the third assessment a trend had appeared indicating that in the most susceptible lines, disease affected the height of plants. When Clipper, Galleon, Schooner and 207/39 were analysed separately from the other lines, this effect became significant in the sixth assessment (Table 35, page 119).

Thus the height of the most susceptible plants in treatment D was significantly lower than height in treatment E, which was in turn lower than in treatments L and O.

TABLE 35

Effects of different scald disease epidemics on growth of different barley lines
(Plant height, cms, at sixth assessment, 141 days from seeding)

	Treatments[A]				
	O	E	L	D	mean
Barley line					
Clipper	988.6	907.3	980.8	832.9	927.4
Galleon	947.2	921.5	984.4	898.9	938.0
Schooner	1021.8	1014.6	1047.5	967.6	1012.9
207/39	1080.7	1082.3	1108.7	1096.6	1092.0
mean	1009.6	981.4	1030.3	948.9	992.6

$LSD^{treatments}$=16.2**(P<0.01) LSD^{barley}=40.4**(P<0.01)

A O=Treatment O - no disease epidemic
E=Treatment E - disease epidemic early in growing season
L=Treatment L - disease epidemic late in growing season
D=Treatment D - disease epidemic throughout the growing season

c) Grain Yield Components

i) Grain Yield was least with treatment D, increased in treatments E and L and was greatest with treatment O (Table 36, page 121). Yield did not differ significantly between barley lines nor were there any significant interactions between barleys and disease treatments ($P<0.05$). This suggests that grain yield of all barley lines was affected similarly by the fungicide treatments. Therefore the barley lines were divided into three groups according to their genotypic relationships, and the groups analysed separately.

Group 1 (Clipper, Galleon and Schooner) Disease treatments had a significant effect upon grain yield of these cultivars (Table 37a, page 122). Individual cultivars did not differ significantly in yield, nor were there any interactions between the disease treatments and lines ($P<0.05$). Treatment D reduced yield by 42% below Treatment O, and treatment E was 15% below O.

Group 2 (Family 51) Grain yields did not differ significantly across treatments or barley lines(Table 37b, page 122). This is consistent with there being very little disease in any of these lines.

Group 3 (Family 39) As with family 51 grain yields did not differ significantly across treatments or barley lines (Table 37c, page 123).

TABLE 36

Effects of different scald disease epidemics upon the grain yield[A] (grams per plot) of different barley lines

	Treatment[B] O	E	L	D	mean
Barley line					
765/51	1330	1169	1144	1370	1253
943/51	1271	1185	1050	1052	1139
215/51	1188	989	1031	1118	1082
669/39	1247	947	1185	1282	1172
87/39	1243	1167	1113	1009	1158
116/39	1482	1257	1152	929	1205
207/37	1239	1022	1185	910	1089
Schooner	1326	1286	1363	827	1196
Galleon	1416	1219	1423	970	1257
Clipper	1371	1010	1177	583	1035
mean	1321	1126	1182	1005	1159

LSD ^{treatment}=130.2** (P<0.01) LSD ^{barley} is not significant

A. Mean grain weight calculated from a sample of 100 grains per plot

B O=Treatment O - no disease epidemic

E=Treatment E - disease epidemic early in growing season

L=Treatment L - disease epidemic late in growing season

D=Treatment D - disease epidemic throughout the growing season

TABLE 37

Effects of different scald disease epidemics on yield (grams per plot)[A] of different barley lines

a)commercial cultivars

	Treatments[B]				
	O	E	L	D	mean
Barley line					
Clipper	1371	1010	1171	583	1035
Galleon	1416	1219	1423	970	1257
Schooner	1326	1268	1363	827	1196
mean	1371	1166	1321	794	1163

$LSD^{treatment}$=198.4* (P<0.05) LSD^{barley} is Not Significant

b)Barley family 51

	Treatments[B]				
	O	E	L	D	mean
Barley line					
765/51	1330	1169	1144	1380	1256
943/51	1271	1185	1050	1052	1139
215/51	1188	989	1031	1118	1082
mean	1263	1115	1075	1183	1159

$LSD^{treatment}$ is not significant LSD^{barley} is not significant

TABLE 37(continued)

c)Barley family 39

	Treatments[B]				
	O	E	L	D	mean
Barley line					
669/39	1247	974	1185	1282	1172
87/39	1343	1167	1113	1009	1158
116/39	1474	1257	1152	929	1203
207/39	1239	1022	1185	910	1089
mean	1326	1105	1159	1032	1156

LSD ^{treatments} is not significant LSD ^{barley} is not significant

A Mean grain weight calculated from a sample of 100 grains per plot

B O=Treatment O - no disease epidemic
E=Treatment E - disease epidemic early in growing season
L=Treatment L - disease epidemic late in growing season
D=Treatment D - disease epidemic throughout the growing season

ii) Seeds per head- The commercial cultivars showed a significant difference in the number of seeds per head, and the disease epidemics significantly affected this factor. However there was no significant interaction (Table 38, page 126-127). Galleon had significantly fewer seeds per head than Clipper and Schooner, and treatment D significantly reduced the number of seeds per head in comparison with the other treatments. The number of seeds per head for the lines of families 51 and 39 differed significantly when averaged over all the disease epidemics.

iii) Undeveloped florets per head- For the commercial cultivars, Galleon had the highest number of undeveloped florets per head (Table 39, page 128-129). Treatment E had the least number while treatment D had the most. The interaction was brought about by Schooner not showing any increase in undeveloped florets in treatment D, but still a reduced number occurred in treatment E. Again family 39 showed only a significant genotypic effect and no significant disease epidemic effect occurred, while family 51 shoed no significant differences in results.

iv) Potential number of seeds per head- The number of seeds per head and the undeveloped florets per head were added to give the potential number of seeds per head. This was also analysed as described previously. The commercial cultivars showed both genotypic and treatment effects (Table 40, page 130-131). Galleon produced the least number of potential seeds per head, Schooner the most and Clipper was in between. Numbers in plots receiving treatment D were significantly less than in those receiving treatments O and L while treatment E was midway between D and O & L. Thus even though treatment E slightly reduced the potential number of seeds per head, a higher proportion of florets that were initiated produced seeds, as shown by the significantly fewer undeveloped florets at the base of the head. This compensatory

effect resulted in the number of seeds per head in treatment E not being significantly less than in treatment O or L. As with the other yield variables the families 39 and 51 showed a significant genotypic effect on potential number of seeds per head but no significant disease epidemic effect occurred.

v) Mean grain weight- The analysis of variance on commercial cultivars showed a significant effect of disease epidemic upon mean grain weight (Table 41, page 132-133). Treatment D produced the lightest grain, then E while O and L were not statistically different. Since disease in plots receiving treatment E was not entirely prevented during the latter part of the growing season, reduced grain weight can be seen in this treatment as well as in D. Family 51 showed only a genotypic effect on the grain weight while Family 39 showed no significant results. Line 116/39 did show a trend across treatments but due to the variation in the experiment (coefficient of variation =7.6% compared to 5.3% in the commercial cultivars) and due to there being only two replicates per treatment it was not found to be significant in the analysis.

vi) Percentage screenings- Grain size was measured for the commercial cultivars only. Treatment D had a large effect upon this component so that the percentage screenings was doubled under this treatment compared to the other epidemics (Table 42, page 134).

TABLE 38

Effects of different scald epidemics on number of seeds per head [A] of different barley lines

a)Commercial cultivars

	Treatments[B]				
	O	E	L	D	mean
Barley line					
Clipper	27.4	26.6	28.4	23.1	26.4
Galleon	24.5	24.1	26.0	23.0	24.4
Schooner	30.1	28.3	28.1	25.7	28.0
mean	27.3	26.3	27.5	23.9	26.3

$LSD^{treatment}_{=1.8**}$ (P<0.01) $LSD^{barley}_{=1.6**}$ (P<0.01)

b)Barley family 51

	Treatments[B]				
	O	E	L	D	mean
Barley line					
765/51	24.5	24.8	24.6	25.5	24.8
953/51	24.0	23.9	24.0	23.7	23.9
215/51	26.5	27.0	27.5	25.6	26.7
mean	25.0	25.2	25.4	24.9	25.1

$LSD^{treatment}$ is not significant LSD^{barley} = 1.2** (P< 0.01)

TABLE 38 (continued)

c)Barley family 39

	Treatments[B]				
	O	E	L	D	mean
Barley line					
669/39	23.9	23.8	23.3	24.2	23.8
87/39	25.9	25.6	25.9	25.8	25.8
116/39	26.6	24.3	24.7	25.2	25.2
207/39	29.5	31.0	32.2	29.6	30.6
mean	26.5	26.2	26.5	26.2	26.3

$LSD^{treatment}$ is not significant LSD^{barley} =1.5* (P<0.05)

A Seeds per head were counted on 25 heads per plot sampled at random

B O=Treatment O - no disease epidemic
E=Treatment E - disease epidemic early in growing season
L=Treatment L - disease epidemic late in growing season
D=Treatment D - disease epidemic throughout the growing season

TABLE 39

Effect of different scald disease epidemics upon number of undeveloped florets per head[A] on different barley lines

a)Commercial cultivars

	Treatments[B]				
	O	E	L	D	mean
Barley line					
Clipper	1.35	1.05	1.35	3.00	1.69
Galleon	2.20	1.40	2.50	2.60	2.18
Schooner	1.70	1.20	1.40	1.75	1.51
mean	1.75	1.22	1.75	2.45	1.79

$LSD^{treatment}$=0.34** (P<0.01) LSD^{barley}=0.30** (P<0.01)

$LSD^{interaction}$=0.60* (P<0.05)

b)Barley family 51

	Treatments[B]				
	O	E	L	D	mean
Barley line					
765/51	1.85	1.20	1.30	1.50	1.46
953/51	1.35	0.90	1.15	1.70	1.28
215/51	0.95	0.95	1.15	1.40	1.11
mean	1.38	1.02	1.20	1.53	1.28

$LSD^{treatment}$ is not significant LSD^{barley} is not significant

TABLE 39 (continued)

c)Barley family 39

	Treatments[B]				
	O	E	L	D	mean
Barley line					
669/39	0.70	0.55	1.05	1.15	0.8
87/39	0.85	1.45	1.15	1.60	1.26
116/39	1.25	1.85	1.60	1.90	1.65
207/39	1.13	1.10	1.35	2.25	1.60
mean	1.13	1.24	1.29	1.73	1.34

$LSD^{treatment}$ is not significant $LSD^{barley} = 0.4^{*}$ ($P<0.05$)

A Undeveloped florets per head were counted on 25 heads per plot sampled at random

B O=Treatment O - no disease epidemic
E=Treatment E - disease epidemic early in growing season
L=Treatment L - disease epidemic late in growing season
D=Treatment D - disease epidemic throughout the growing season

TABLE 40

Effect of different scald disease epidemics upon potential number of seeds per head[A] on different barley lines

a)Commercial cultivars

	Treatments[B]				
	O	E	L	D	mean
Barley line					
Clipper	28.75	27.60	29.75	26.05	28.04
Galleon	26.65	25.50	28.45	25.60	26.55
Schooner	31.75	29.40	29.50	27.45	29.52
mean	29.05	27.50	29.23	26.37	28.04

$LSD^{treatment}$=1.8*(P<0.05) LSD^{barley}=1.5**(P<0.01)

b)Barley family 51

	Treatments[B]				
	O	E	L	D	mean
Barley line					
765/51	26.04	26.00	25.85	26.95	26.21
953/51	25.30	24.80	25.15	25.40	25.16
215/51	27.45	27.95	28.65	27.00	27.76
mean	26.26	26.25	26.55	26.45	26.38

$LSD^{treatment}$ is not significant LSD^{barley}=1.2* (P<0.05)

TABLE 40 (continued)

c)Barley family 39

	Treatments[B]				
	O	E	L	D	mean
Barley line					
669/39	24.55	24.35	24.35	25.30	24.62
87/39	26.75	27.05	27.05	27.35	27.05
116/39	26.97	26.10	26.30	27.10	26.62
207/39	31.15	32.10	33.55	31.80	32.15
mean	27.35	27.40	27.80	27.89	27.16

$LSD^{treatment}$ is not significant LSD^{barley} = 1.6**(P<0.01)

A Seeds per head plus undeveloped florets per head were counted on 25 heads per plot sampled at random

B O=Treatment O - no disease epidemic
E=Treatment E - disease epidemic early in growing season
L=Treatment L - disease epidemic late in growing season
D=Treatment D - disease epidemic throughout the growing season

TABLE 41

Effect of different scald disease epidemics upon 100 grain weight on different barley lines

a)Commercial cultivars

	Treatments[A]				
	O	E	L	D	mean
Barley line					
Clipper	3.62	3.30	3.78	3.06	3.44
Galleon	3.67	3.38	3.60	3.07	3.43
Schooner	3.73	3.88	3.84	3.19	3.66
mean	3.68	3.52	3.74	3.12	3.51

$LSD^{treatment}$=0.20**(P<0.01) LSD^{barley} is not significant

b)Barley family 51

	Treatments[A]				
	O	E	L	D	mean
Barley line					
765/51	4.29	4.20	4.39	4.09	4.24
953/51	4.06	4.01	4.02	4.14	4.06
215/51	4.28	4.38	4.46	4.23	4.34
mean	4.21	4.20	4.29	4.15	4.21

$LSD^{treatment}$ is not significant LSD^{barley} =0.18* (P<0.05)

TABLE 41 (continued)

c)Barley family 39

	Treatments[A]				
	O	E	L	D	mean
Barley line					
669/39	4.12	4.00	4.19	4.12	4.11
87/39	3.95	4.12	4.06	3.84	3.99
116/39	4.50	4.12	3.80	3.50	3.98
207/39	3.80	3.69	3.88	3.59	3.74
mean	4.09	3.99	3.98	3.76	3.96

$LSD^{treatment}$ is not significant LSD^{barley} is not significant

A O=Treatment O - no disease epidemic
E=Treatment E - disease epidemic early in growing season
L=Treatment L - disease epidemic late in growing season
D=Treatment D - disease epidemic throughout the growing season

TABLE 42

Effect of different scald disease epidemics upon percentage screenings[A] on commercial cultivars

	Treatments[B]				
	O	E	L	D	mean
Barley line					
Clipper	30.0	37.0	32.5	62.5	40.5
Galleon	46.0	49.5	46.5	65.0	51.8
Schooner	34.5	29.0	25.5	66.5	38.9
mean	36.8	38.5	34.8	64.7	43.7

$LSD^{treatment}$= 9.8*** (P<0.001) LSD^{barley} is not significant

A Percentage screenings calculated from 50 ml volume sample from each plot on a 2.5 mm sieve

B O=Treatment O - no disease epidemic
E=Treatment E - disease epidemic early in growing season
L=Treatment L - disease epidemic late in growing season
D=Treatment D - disease epidemic throughout the growing season

vii) Heads per plot - An estimate of the heads per plot, for the commercial cultivars, was made by dividing the plot yield by the calculated mean head weight (ie. the number of seeds per head x seed weight). An analysis of variance showed that this figure was higher in Galleon than Clipper or Schooner (Table 43, page 136). No treatment effects were significant, possibly due to a high degree of variation (coefficient of variation = 14.1%) and due to number of replicates per treatment.

d) Percent protein content of grain

The percent protein content was measured for the commercial cultivars. This was not found to be affected significantly by the disease levels in this experiment.

TABLE 43

Effects of different scald epidemics upon the estimated number of heads per plot[A] on commercial cultivars

	Treatments[B]				
	O	E	L	D	mean
Barley Line					
Clipper	1596	1276	1177	931	1245
Galleon	1767	1697	1690	1532	1674
Schooner	1338	1270	1379	1153	1283
mean	1565	1414	1418	1205	1401

$LSD^{treatment}$ is not significant LSD^{barley} =197.4 **(P<0.01)

A Estimated number of heads was calculated by dividing the plot yield by the calculated mean head weight (i.e. the number of seeds per head x seed weight)

B O=Treatment O - no disease epidemic
E=Treatment E - disease epidemic early in growing season
L=Treatment L - disease epidemic late in growing season
D=Treatment D - disease epidemic throughout the growing season

e) Analysis of relationship between disease levels and yield loss

The relationship between scald disease levels and reduction in grain yield was assessed using linear and multiple regression analysis. Yield loss was used as the dependent variate and was calculated by subtracting the yield of undiseased plots (Treatment O) from that of the diseased plots (Treatments E, L and D).

The disease data was grouped according to growth stage rather than assessment period. Thus the third assessment results for the later maturing types were replaced with the results from the fourth assessment, so providing disease levels for the elongation growth stage. The results from the later assessments were modified in the same manner. The linear regressions on this set of data (Table 44, page 138) indicated that the later the growth stage the closer the correlation between disease levels and yield loss.

The disease levels on individual leaves at growth stage 87 (Zadoks scale) were also regressed against yield loss. The results in Table 45 (page 138) suggest that the disease levels on the uppermost leaf have the highest correlation with yield loss.

To further understand the effects of scald disease on yield reduction in barley, a multiple regression was carried out. The disease levels at each growth stage were regressed on yield loss. The correlation of each of these with yield loss and the respective t-values are shown in Table 46. (page 139) Again the disease levels at the dough stage were found to have a significant effect upon barley yield. No other disease recordings were seen to affect the yield in the multiple regression.

TABLE 44

Linear Regression of Disease Levels at Different Growth Stages with Yield Loss as the Dependent Variate

Growth Stage	Correlation Coefficient	t-value No. of values=40	% Variation in Yield Loss Accounted for
Early tillering	0.32	2.08*(P<0.05)	7.8
Late tillering	0.42	2.89*(P<0.05)	15.8
Elongation	0.55	4.02***(P<0.001)	28.0
Boot	0.67	5.60***(P<0.001)	43.8
Anthesis	0.72	6.41***(P<0.001)	50.7
Dough	0.76	7.31***(P<0.001)	57.3

TABLE 45

Linear regression of Leaves 1-4 at Dough Stage with Yield Loss as the Dependent Variate

Leaf number (1=top leaf)	Correlation with yield loss	t-value No. of values=40	% Variation in Yield Loss Accounted for
1	0.81	8.39***(P<0.001)	64.0
2	0.73	6.67***(P<0.001)	52.0
3	0.62	4.85***(P<0.001)	36.6
4	0.60	4.62***(P<0.001)	34.3

TABLE 46

Multiple Regression of Disease Levels at Different Crop Growth Stages with Yield Loss as the Dependent Variate

Growth stage	Correlation with yield loss	t-value No. of values=40
Early tillering	0.28	-0.83^{NS}
Late tillering	0.42	1.19^{NS}
Elongation	0.52	0.34^{NS}
Boot	0.66	-0.87^{NS}
Anthesis	0.71	0.02^{NS}
Dough	0.77	$2.66^{*(P<0.05)}$

Percentage variation in yield loss accounted for =56.2%

DISCUSSION

The results of the linear regression imply that while a critical point model involving disease levels on one or two leaves at a particular growth stage could be produced to describe the effects of scald disease on barley yield, analysis of the different yield components indicates that the disease has an effect on yield at more than one time or growth stage. For example, floret initiation, reduced in treatments E and D, occurs during growth stages 29-73, while grain filling, also affected by the disease, occurs after anthesis. A critical point model does not explain the effects of scald disease on all these yield parameters, which are developing at different crop growth stages.

The multiple regression indicates that, as with the linear regressions, disease levels at only the dough stage explain a significant proportion of the yield loss. However the second highest t-value, while not significant, does occur at late-tillering ie. it coincides with the peak in disease levels that occurs in susceptible genotypes near the end of tillering. Thus the two peaks of the disease cycle coincide with the two highest t-values in the multiple regression analysis. This suggests that the multiple regression better describes the epidemic than does the linear regression analysis. The non-significance of the t-value at late tillering may be due to the disease at this stage having less effect on yield than later in the season, due to a lower severity of disease than at the dough stage. The second cycle of disease, which peaks at the dough stage, affects seed set, grain weight and grain size, as well as having a correlation with plant height. The first cycle of disease, on the lower foliage, which peaked at the end of tillering, affected floret initiation, ie. the number of potential seeds; and right at the end of tillering the trend in reduction of plant height was appearing. Thus the contribution to yield loss at this stage

was much less than occurred later, and so was not high enough to be significant in the multiple regression.

This study has shown that scald disease has a continual effect upon yield by influencing the development of each component. The yield effects caused by the scald disease can be divided into-

a) A drop in the number of potential seeds per head, early in the season, as demonstrated by treatments E and D.

b) An effect on the number of florets which developed into seeds. Continual disease (treatment D) reduced the seed set whereas reducing the amount of disease later in the season produced a higher seed set (treatment E) suggesting a compensatory effect.

c) A reduction in the grain weight and size due to disease late in the season (treatment D).

d) A 42% reduction in the number of heads where scald was severe, as for Clipper plots receiving treatment D. This was brought about by a reduction in number of tillers and the failure of tillers to produce heads ie. a reduction in the vigour of plants.

e) Overall development of the plant was also affected as observed by the effect upon plant height.

To understand an epidemic it is necessary to take several disease assessments, at least during the two peaks of the epidemic. The importance of early development of scald disease can be seen by comparing the epidemic (D) of 116/39 and Galleon. Line 116/39 had only a late epidemic which affected grain weight. Galleon had a similar final disease level, but higher disease levels earlier in the season. This was reflected by reductions in seeds per head, potential seeds per head and plant height, as well as the grain weight and size.

For partial resistance to be effective the above results indicate that disease should be suppressed by the resistance at all growth stages, to prevent an effect upon yield. Early disease development can be compensated for if the upper foliage is free of disease, but compensation does not occur for a late epidemic, as seen in selection 116/39. However, a late epidemic of the level in 215/51, which did not exceed 25% LAD at dough stage, can be tolerated. In general it would appear that if disease can be reduced to no more than the levels seen in line 207/39, then significant yield reductions are unlikely to occur.

Relationship between field infection ratio and %LAD in the field

Evidence in glasshouse experiments and mobile nurseries pointed to infection rate as an important factor in resistance measurement. Data were available to analyse the infection rate in the field and corresponding %LAD from this experiment. Incidence of infection rate is given in Appendix 11. These two variables were regressed against each other and the results are shown in Table 47 (page 144). It can be seen that correlation coefficients are high and % variation accounted for is also high, peaking at 87.3 % at assessment 4 (g.s. 33-53 Zadoks scale). The regression line for assessment 4 is shown in Fig. 29 (page 144). Three outliers show a lower %LAD than the line of best fit would indicate for their infection rate. Two of these are Schooner and one is Galleon. These outliers may be showing a form of resistance that is not associated with infection, but with a subsequent symptom such as mycelial growth or sporulation. This indicates that there are two aspects of an epidemic. The first is the initial infection of the plants, which has been looked at in this study, and the second is the subsequent spread of the disease on the plants, an aspect of a scald epidemic that can usually only be studied in the field. Nevertheless, glasshouse and

field experiments indicate the importance of infection rate as a key factor in determining resistance.

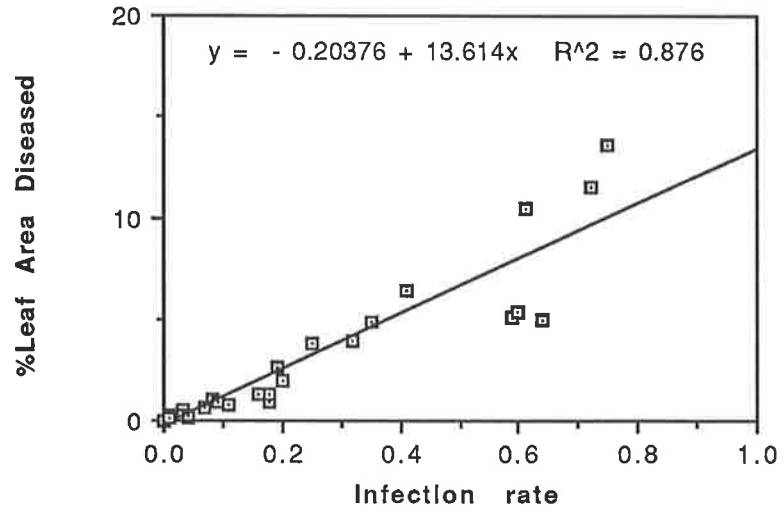
TABLE 47

Regression analysis between plant infection rate and %LAD (Leaf Area Diseased) in the field

Assessment	Correlation Coefficient	Regression Coefficient	Constant	t-value	%Variation Accounted for
1	0.55	0.015	0.0377	4.05***(P<0.001)	28.3
2	0.84	0.029	0.0253	9.63***(P<0.001)	70.2
3	0.76	0.029	0.0688	7.20***(P<0.001)	56.6
4	0.94	0.064	0.0339	16.40***(P<0.001)	87.3
5	0.91	0.036	0.0650	13.58***(P<0.001)	82.5
6	0.84	0.020	0.1494	9.42***(P<0.001)	69.2

Figure 29

Relationship between Field infection rate and % Leaf Area Diseased (%LAD) at Assessment 4 (g.s. 35-53 Zadoks scale)



CHAPTER 4

FINAL DISCUSSION

The results of this study indicate the widespread nature of *R.secalis* in South Australia. Of the twenty-eight mobile nurseries placed in the field over two years, only four did not become infected with scald, to some degree. In the field trial the cultivar Clipper showed a 50% drop in yield when infected with *R.secalis* and the cultivars Galleon and Schooner showed a 30% drop in yield due to the scald infection. In 1985 Galleon and Schooner made up 40% and 18%, respectively, of the barley crop in South Australia. The cultivar, Forrest, which had total resistance to the disease, made up 26% of the barley crop while the remaining 14% consisted of three susceptible cultivars, Clipper, Weeah and Dampier. Thus at the time of this study only 26% of the South Australian barley crop was protected, through resistance, against scald. Since then Forrest has become infected with scald disease over much of the South Australian barley growing area. Combined with the widespread nature of the disease, the potential losses in the barley crop can be very high.

In the introduction, aims were set out for this study to identify races of *R.secalis* in South Australia, to discover the effectiveness of resistant genes against these races, and to develop a screening test for resistance in the glasshouse. This included the possibility of identifying and screening for partial resistance. The damage to barley crops from scald in South Australia was also to be studied.

As in other studies (Ali *et al,* 1976) the population of *R.secalis* in South Australia was found to be highly variable, and pathotyping of isolates was difficult as inconsistent results occurred. Clear identification of *R.secalis* pathotypes was not successful. The inconsistency of results may be due to a high degree of variability in the viability of inoculum, as well as a wide range in virulence. It is believed that this wide-ranging virulence may be partly produced by passage of the pathogen through the the alternative host, barley grass (*Hordeum*

leporinum), which is a widespread weed in the barley growing areas of South Australia (Ali, 1981). A similarly variable situation is seen with *Pyricularia oryzae* (rice blast) on rice. One study (Latterell and Rossi, 1986) identified 50 races of *P.oryzae* while Oud and Ayad (1968) found as many as 14 races from one lesion. It was suggested that mutation and asexual, sexual, or even somatic recombination occurred which may explain some variability, especially when spores from a single lesion vary in virulence. However it was thought this aspect had been overestimated and further explanations for the great diversity of races was as follows-

1) The effects of environment and nutrition on host reaction

2) Seed impurity

3) Variation in reaction of different cultivars under different growing conditions

4) Deficiencies in testing procedures and materials

5) Differences in individual evaluation standards.

It is highly probable that these explanations, including mutation and recombination, would also explain a great deal of the *R.secalis* virulent diversity found in this study and described in the literature. While the second and fifth factors listed above can be eliminated within a single study, further work is required to overcome the other confounding factors. Studies of the environmental effect on *R.secalis*, its host and disease expression (ie. genetic x environment component) may resolve some of the confusion, so that glasshouse tests, under strictly defined environmental conditions, may produce consistent and clear results when testing for virulence.

Most studies on *R.secalis* have emphasised the major genes of disease resistance for this pathogen and its associated disease, barley

scald. Thus it was expected that a resistance study would place barley lines into resistant or susceptible groups, but this did not happen.

The mobile nursery results and glasshouse reactions were very inconsistent, though less problems occurred when cultures were maintained in such a way as to maintain viability, droplet inoculation was used, and a growth room was used instead of the glasshouse. The seedling infection frequency agreed with the infection frequency in mobile nurseries. This did not occur when attempting to use races, but only when information from all the 'race' testing was combined. A straight line relationship occurred so that the degree of susceptibility was found to be important, rather than being able to classify a barley line as resistant or susceptible. Thus the race theory does not fit the results of this study.

Glasshouse testing using four isolates again found difficulty in maintaining consistency of results. The only factor associated with the field screening results was the seedling infection rate. As with the glasshouse screening tests this matching of field results occurred if results were combined, not when analysed for separate 'races' or isolates.

The infection rate was also the only factor, of those measured in glasshouse experiments, that was significantly affected by the cultivar under test. This suggests that this factor reflects the resistance level in the barley genotype. Infection rate was also affected, to some degree, by the isolate used and the inoculum concentration. Field infection rates compared well with the field %LAD in the field trial, (Figure 29, Chapter 3), gaving a straight line relationship. Infection rate only measures one aspect of the epidemic ie. the initial infection of a plant or spread from plant to plant. Spread of the disease on a plant is not measured in glasshouse testing. This is probably why field (%LAD) and glasshouse

(infection rate) disease ratings are not always comparable (Figs. 11-16, Chapter 2). However a good relationship was expected between the glasshouse infection rate and field ratings if field assessments were taken during the early part of an epidemic when plant infection is the major part of the epidemic. This was seen to occur when mobile nursery results (infection rates) and glasshouse infection rates were compared but not for the field study in Chapter 3 (Table 47). Instead the correlation was highest for disease levels measured during the middle of the season. As the epidemic became established other factors, such as incubation time, spore production, etc. would be expected to have a greater effect upon the epidemic. However, when measured, spore production and incubation period did not vary with the barley lines and so could not be associated with cultivar field disease levels. This was unexpected as they are considered to be important factors in epidemic growth rate (Van der Planck, 1963) and were expected to be a measure of the ongoing epidemic after initial infection. Unlike this study, Habgood (1977) had found spore production to place five cultivars in the same ranking as their field disease ranking. In this study incubation period varied only with the isolate under test and also with its viability ie. the less viable an isolate became, the longer was the incubation period. These results suggest that infection rate plays a major role in the epidemic and its development, and influences it for a length of time.

All the resistance genes tested in this study, in the field, were found to suppress development of scald disease to some degree in a variety of backgrounds against a wide range of pathotypes of *R.secalis*. The most effective gene was Rh3, which allowed only very restricted development of scald on barley lines containing this gene. Isolate testing in this study and that by Ali *et al* (1976) found no isolates of *R.secalis* in southern Australia that were able to overcome this gene.

However the subsequent breakdown of Forrest resistance suggests that this is no longer correct.

Field testing did not produce distinct resistant or susceptible groups but instead there was a continuum of disease levels from 0% to 100% Leaf Area Diseased (%LAD) so that again the race theory was not supported. This suggests that the cluster analysis groupings are artificial groups, and it is not possible to place barley lines into resistant or susceptible groupings. It is postulated that the major genes that are known to exert some control over the inheritance of scald resistance, are also affected by gene modifiers. Further to that the modifiers, or the major genes, may be affected by the environment, thus blending results even more.

The study of scald epidemics showed that barley yield can be affected by scald disease at any crop growth stage, making it necessary to protect the crop for the entire growing season. Scald disease levels of 10% LAD and below did not cause any measurable yield loss. Scald epidemics were studied with the aim of identifying the crop growth stages at which the disease will cause yield loss, and the levels of disease that are damaging to yield. The disease affected growth rate and height of plants, and the effect on yield was reflected in a reduction in the overall yield per plot, seeds per head, seed initiation, grain weight and grain size. As these components develop at varying stages throughout the crop growth it would appear that scald disease can be damaging at any crop growth stage. Compensating effects are possible if the epidemic is controlled in the latter part of the crop growth. Mayfield(1982) found that greatest losses were associated with inoculation after stem elongation, due to a reduction in the number of heads developing to maturity, and that earlier inoculation resulted in fewer spikelet primordia per head. In this study, the results of

regression analyses combined with the interpretation of the effects upon yield components found that a multiple regression using disease levels from two points in the epidemic, ie. end of tillering (g.s 29 Zadoks scale) and mature plants (g.s. 85 Zadoks scale) was best able to describe the effect of the scald epidemic. This is in contrast to other workers who found a relationship between disease levels at a single growth stage and yield reduction. Their results were generally supported by a major part of the yield reduction being caused by a drop in seed weight and size (James *et al*, 1967, Jenkins *et al*, 1972, Khan and Portmann, 1980, Barr and Mayfield, 1981, Mercer et al, 1982). However, Schaller (1951) and Jackson and Webster (1981) had noted that yield reduction was brought about by a drop in grain number as well as grain weight, and Ozoe (1956) noted that scald disease on barley caused a drop in plant height, tillers, number of heads, seed number and delayed maturation. These results enforce the importance of understanding that the effect of epidemics may differ with environment, and it is necessary to study disease and its effects in different environments before attempting to describe the relationship between disease levels and yield loss.

At this stage it appears that all scald resistant genes are likely to be useful in barley breeding programmes in South Australia. However the wide range of virulence that was apparent may allow for the selection of 'resistance-breaking' pathotypes should resistant genes be used in commercial cultivars. This occurred in California when the cultivar, Atlas 46 was released in 1947 with the resistant gene Rh3. By 1956 the gene was no longer effective in protecting the cultivar from the scald disease (Webster *et al* 1980). A similar situation has occurred with the cultivar Forrest, which also contains the resistance gene Rh3. This was completely resistant on release but is now only moderately resistant. To reduce this problem it may be necessary for several

resistance genes to be incorporated into one barley cultivar. However the selection of races for screening specific genes is limited to those that are stable and show a consistent reaction on the barley plants. This means only a gene such as Rh3 would be easiest to incorporate into barley lines. None of the other known genes appear to be as easy to manipulate, and to be as consistent in results, possibly due to the effects of gene modifiers. Because of this it may be necessary to disregard 'races' and aim for a general resistance against *R.secalis*. This would mean using a mixture of isolates as inoculum and selecting plants that are partially or wholly resistant to this mixture.

Yield losses in barley can occur from a scald infection at any crop growth stage so resistance must be effective for the entire life of the plant. Since the glasshouse tests screen for resistance at the infection level, and does not take into account the further spread of the epidemic, it is necessary to use field screening in conjunction with glasshouse tests in breeding programs. Artificial inoculation with infected straw and irrigation, to enable spore spread and germination, can provide a successful field screening test. With careful manipulation of isolates, and a degree of environmental control, the glasshouse screening method enables a large number of barley genotypes to be screened for resistance, in a relatively short period of time. Used in conjunction with the field tests, this is an extremely useful tool. Further work is required to overcome some of the deficiencies of the glasshouse test. To be able to simulate the effects of an ongoing scald epidemic within the glasshouse, rather than just the initial infection, would greatly enhance the test.

Further work is necessary to discover what causes the great variability in virulence and its apparent instability in many cases. Studies of the genetic x environment effect may uncover the effects of the environment upon disease expression in the host and the *R.secalis*

pathogen, which are possibly confusing many glasshouse tests. There may be many more examples of environmentally affected Rh genes than that discovered by Ali (1975b). Ali & Boyd (1974) noted that under favourable conditions *R.secalis* can develop in the tissue of many host genera. It is possible that this environmental effect extends to overcoming 'resistance' within *Hordeum* species as well. Janakiram and Boyd (1980a) postulated that all genotypes can exhibit symptoms of scald infection and the variability in expression is subject to a number of influences such as environmental and genetic effects.

Studies of the genetic inheritance of scald resistance, with respect to the degree of susceptibility, and the effect of modifiers is one aspect which must be looked at more closely. Whilst major genes are known to play an important role in the inheritance of scald resistance in barley genetics, the results of this study show that they do not have complete control.

The inability to place barley lines into specific resistant or susceptible groups, combined with the field results of a continuum of disease levels, suggests that the major genes are influenced by the effect of modifier genes. These genes result in less clear inheritance of resistance, and are possibly highly affected by environmental conditions as indicated by the inconsistency of results in glasshouse conditions, reduced when placed in a growth room. Identifying modifiers and their effect upon disease expression may uncover the culprit of variability in scald disease expression, and so with greater understanding of the genetic system, lead to better resistance breeding results.

APPENDICES

APPENDIX 1a

Rainfall (mm) at mobile nursery sites during autumn (May-June 1983)
placement of nurseries

	Mobile Nursery Number									
	10	6	2	8	4	7	9	1	9	3
Day										
1	0.0	0.0	0.0	8.0	0.0	0.0	0.6	0.0	0.6	6.0
2	4.0	2.6	0.0	1.0	0.0	0.0	0.0	13.0	0.0	3.0
3	8.0	4.0	0.0	0.0	0.0	11.0	0.0	5.0	0.0	0.6
4	3.0	9.8	0.0	0.0	0.0	0.0	0.0	4.0	0.0	12.0
5	2.0	4.0	0.0	0.0	0.0	2.0	0.0	1.0	0.0	0.0
6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.3	0.0	1.0	1.0	0.0
8	0.0	0.0	0.8	5.0	0.6	0.0	1.0	0.0	0.4	4.0
9	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0	1.0
10	0.0	0.0	0.0	0.0	0.8	0.0	0.2	0.0	8.4	0.0
11	2.0	0.0	0.0	17.5	8.0	0.0	1.4	0.0	0.0	0.2
12	3.0	0.0	9.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0
13	0.0	0.0	3.0	4.5	3.0	0.0	0.0	0.0	0.0	0.0
14	11.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0
Total mm	33.0	20.4	14.8	36.0	14.4	13.3	5.4	26.0	10.8	26.8
Total days of rain	7	4	4	5	5	3	5	6	5	7

Mobile Nursery Number	Site
1	Aldinga
2	Angas Plains
3	Bordertown
4	Charlick
5	Cleve
6	Jamestown
7	Kadina
8	Port Lincoln
9	Streaky Bay
10	Turretfield

Appendix 1b

Rainfall (mm) at mobile nursery sites during spring (Sep-Oct 1983) placement of nurseries

	Mobile Nursery Number										
	11	12	14	15	16	17	18	19	20	21	13
Day											
1	0.0	18.0	15.0	7.0	0.1	0.0	6.0	2.0	0.0	0.0	0.6
2	11.0	9.0	0.0	0.0	5.4	0.0	0.2	2.0	5.6	0.0	0.0
3	4.0	0.0	0.0	0.0	4.4	0.0	0.0	0.0	0.6	0.0	0.0
4	4.0	0.0	0.0	4.0	13.8	0.0	4.0	0.0	0.0	40.0	2.0
5	12.0	3.0	3.8	4.0	0.8	0.0	0.4	8.5	0.0	0.0	0.0
6	1.0	8.0	2.2	1.0	5.8	35.0	9.0	0.0	0.0	0.0	0.0
7	4.0	0.0	0.0	8.0	12.4	0.0	1.0	0.0	3.0	0.0	23.0
8	3.0	0.0	0.0	3.0	0.2	0.0	4.0	0.0	0.0	2.0	0.0
9	3.0	0.0	8.6	0.6	0.3	0.0	9.0	7.0	5.6	0.0	0.0
10	3.0	0.0	0.0	3.0	1.0	0.0	2.0	0.0	0.0	23.0	13.0
11	1.0	14.0	0.0	5.0	2.6	0.0	0.2	1.5	1.2	0.0	14.0
12	0.0	0.0	5.8	0.0	4.0	0.0	0.4	20.0	1.0	0.0	0.0
13	0.0	2.0	0.6	0.6	0.0	14.0	1.0	0.0	1.4	0.0	0.0
14	0.0	4.0	8.4	0.2	0.0	19.0	5.0	0.0	0.0	0.0	0.0
Total mm	46.0	58.0	44.4	36.4	50.8	77.0	37.7	39.2	18.4	65.0	58.0
Total days of rain	10	7	7	11	12	3	13	6	7	3	5

M. N. Number	Site	M. N. Number	Site
11	Aldinga	17	Kadina
12	Angas Plains	18	Murray Bridge
13	Booborowie	19	Port Lincoln
14	Cleve	20	Streaky Bay
15	Charlick	21	Wanilla
16	Jamestown		

Appendix 1c

Rainfall (mm) at mobile nursery sites during 1984 placement of nurseries

	Mobile Nursery Numbers						
	22	23	24	25	26	27	28
Day							
1	0.4	3.0	1.0	0.0	0.0	0.0	0.0
2	0.0	0.0	1.0	0.0	0.0	9.3	14.2
3	0.0	0.0	0.0	0.0	0.0	17.0	5.0
4	7.0	6.0	0.0	3.0	0.0	0.0	0.2
5	0.0	0.0	11.0	0.0	4.0	1.0	0.0
6	0.0	0.0	8.0	0.0	0.2	0.0	0.0
7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8	1.0	0.0	0.0	0.0	0.0	0.0	2.0
9	0.0	0.0	0.0	0.8	0.0	0.0	15.4
10	0.0	0.0	0.0	7.0	3.0	0.0	0.0
11	15.0	5.0	0.0	1.0	10.0	2.3	1.2
12	0.0	0.0	0.0	0.2	5.0	2.0	0.0
13	0.0	0.0	0.0	0.8	0.0	01.3	0.2
14	0.0	3.0	0.6	15.0	1.0	0.0	0.0
Total mm	23.4	17.0	21.6	28.8	24.2	38.2	32.9
Total days of rain	4	4	5	7	7	6	7

Mobile Nursery Number	**Site**
22,23	Angas Plains
24,25,26	Charlick
27	Kadina
28	Turretfield

Appendix 2

SINGLE SPORE ISOLATION

Method A

Float surface sterilised diseased leaf in sterile distilled water (SDW) for 48 hours at 15°C. Shake leaves in SDW, for five minutes to liberate spores. Dilute suspension to $5x10^4$ spores per ml. and spread a droplet of the suspension on DWA plate. Twenty four hours later the germinated single spores can be transferred to Potato Sucrose Peptone (PSP) or Lima Bean Agar Plates.

Method B

Surface sterilise the diseased leaf piece and place on PSP agar plate. When fungus has grown out from the leaf make a spore suspension from the culture and isolate as above.

Appendix 3

POTATO SUCROSE PEPTONE (PSP) AGAR

1 litre distilled water
10 grams agar
10 grams Deb potato flakes
10 grams sucrose
10 grams peptone

Melt agar in water. Add the remaining ingredients. Sterilise agar solution. When it is cool add streptomycin at 1ml per 100 ml agar.

Appendix 4a

Reactions of *R. secalis* isolates, from Waite collection, on 21 barley cultivars when spray inoculated

Number of barley seedlings with susceptible reaction

	Isolate		
	44/29		68/28
	a	b*	
Cultivar			
Abyssinian			1
Atlas	4		5
Atlas 46			1
Brier	5		4
Clipper	3		5
Galleon	5		5
Gospeck	5		5
Hudson			1
La Mesita			1
ModocCalifornia	3		5
Nigrinudum	5		3
Osiris			1
Psaknon			1
Sakigake	5		5
Sultan			1
Turk	1		1
WestChina	3		5
WWxG^{i}			1
CI3515			1
CI4364			1
CI8618	2		1

Denominator is five unless stated otherwise.

* Data not available

i = Wisconsin Winter x Glabron

Appendix 4a (continued)

Reactions of eleven *R. secalis* isolates, from Waite collection, on 21 barley cultivars when inoculated using the single droplet inoculation

	Isolate																
	44/29		68	9		12	166		172	173	183		187	217	219	
	a	b*	/28	a	b*	*	a	b*		*	a	b		*	a	b
	Number of barley seedlings with susceptible reaction															
Cultivar																
Abyssinian											1	1	1			
Atlas	1		5								1	1	1		3	2
Atlas 46			1												1	
Brier	1		2	3							5	5	5		2	2
Clipper	3		4	5			2		5			5	5		4	1
Galleon	4		5	1					5		5	5	5		1	3
Gospeck	2		5	1			4		5		5	1			5	2
Hudson			1													
La Mesita			1								1	1	1			
Modoc**			1								3	2	3			
Nigrinudum			1								1	1	1			
Osiris			1										1			
Psaknon			1										1			
Sakigake			5	1					3		4	2	5			
Sultan			1				2				1	1	1			
Turk			1													
WestChina	1		5	4					3		5	4[A]	4[A]			
WWxG[i]			1	1									4			
CI3515			1				1						1			
CI4364			1				1					1	1			
CI8618			1								1		1			

Denominator is five except at [A] where it is four

* Data not available

i = Wisconsin Winter x Glabron ** = Modoc California

Appendix 4b

Reactions of eight Western Australian *R. secalis* isolates on twenty one barley cultivars

Isolates	350 a	350 b	354	409	342	350	402	405	408	412 a	412 b
	Spray inoculation				Single droplet inoculation						
	Number of barley seedlings with susceptible reaction										
Cultivars											
Abyssinian						1		1			
Atlas			1	5		4		5	1		
Atlas 46											
Brier				4			4	1			
Clipper	5	5	5	12/12	3	4	5	5	5	4	4
Galleon	5	5		4/4		5	5	5			2
Gospeck	5	5		3/4	5	4	4	5		5y*	2
Hudson			1					1	1		
La Mesita			1			1	5				1
Modoc**				1	1		2			1	
Nigrinudum			1			1		1			
Osiris			1					1	1		
Psaknon			1			3		1			
Sakigake						1		1			
Sultan			1			1		1			
Turk			1			1		1			1
WestChina						2		4			2
WWxG^{i}						1		1			
CI3515			1			1		1			
CI4364						3		1			
CI8618								1			

Denominator is five unless shown otherwise

* y indicates yellowing of leaves rather than distinct lesioning

i = Wisconsin Winter x Glabron ** = Modoc California

Appendix 4c

Reactions of fourteen *R.secalis* isolates, isolated from field collections of scald lesions

	Isolates													
	S1	S2	WI1	WI2	WI3	WI4	WI5	FC1	FC2	FC3	FC4	FC5	FC6	BG
	Number of barley seedlings with susceptible reaction													
Cultivars														
Abyssinian				1										
Atlas		1											1	
Atlas 46														
Brier	1	5	4	4	5		5	-	-	-	-	-	-	-
Clipper	3	5	5	5	5	5	5	5	5	5	5	3r*	4	5
Galleon	3	5	5		5	3	3	3	3	1	3		3	3
Gospeck	5	5	5	5	5	5	5	1	3	3	2		3	1
Hudson														
La Mesita				3/4										
Modoc**					1									
Nigrinudum														
Osiris														
Psaknon	1					1								
Sakigake		5	1/3	2	5	1				1			5	
Sultan														
Turk														
WestChina	3	5	3	4	5	5	5	1		4	2		3	3/4
WWxG^{i}														1/4
CI3515														
CI4364				1										1/4
CI8618	1													

Denominator is five unless stated otherwise

* r indicates that lesions were restricted (ie. small) in size

i = Wisconsin Winter x Glabron ** = Modoc California

Appendix 4d

Reactions of seven *R.secalis* isolates , from mobile nurseries, on twenty one barley cultivars.

M.N.No.**	7	10	10	18	28	28	28
cultivar*	Clipper	Clipper	WestChina	Modoc[ii]	Clipper	Brier	Brier
	Number of barley seedlings with susceptible reaction						
Cultivar							
Abyssinian			1				nil
Atlas	1		1		3	1	
Atlas 46							
Brier	-	-	-	-	4	3/6	
Clipper	4/4	4	4	3(r)	5	6/8	
Galleon	5	5	3	1(r)	5	4/6	
Gospeck	3	4	3	2(r)	5	3/6	
Hudson							
La Mesita		2	1				
Modoc[ii]		1					
Nigrinudum							
Osiris	1						
Psaknon			2				
Sakigake	1	5	1				
Sultan							
Turk		1					
WestChina	4	3/4	4	1			
WWxG[i]							
CI3515							
CI4364							
CI8618		1					

* Cultivar in mobile nursery from which isolate was cultured **Mobile Nursery Number

Denominator is five unless stated otherwise

i = Wisconsin Winter X Glabron ii = Modoc California

Appendix 5a

Percentage Leaf Area Diseased (%LAD) on differential barley lines in field screening trials at Waite and Charlick trial sites

Trial site	Waite					Charlick					
Assessment number	1	2	3	4	Cluster	1	2	3	4	5	Cluster
Cultivar											
Abyssinian	1.6	0.4	0.5	0.1	A	13.1	27.3	0.8	0.0	0.1	A
Atlas	0.8	2.4	8.8	4.6	B	3.0	13.3	1.6	3.1	2.0	A
Atlas 46	0.6	0.1	0.0	0.9	A	0.8	0.0	0.0	0.0	0.0	A
Gospeck	8.8	4.4	6.6	3.4	B	8.3	27.6	3.7	2.5	1.5	B
Hudson	1.8	0.0	1.0	0.0	A	2.8	0.0	0.0	0.0	0.0	A
La Mesita	0.0	2.6	1.0	0.7	A	6.8	8.6	0.0	1.5	0.4	A
Modoc[ii]	0.0	0.0	0.0	0.0	A	4.9	22.7	0.0	0.3	0.0	A
Nigrinudum	1.4	0.2	0.5	0.8	A	3.9	9.7	0.0	0.3	0.3	A
Osiris	0.1	0.2	0.3	0.6	A	16.8	0.0	0.0	0.0	0.0	A
Psaknon	0.0	0.0	0.0	0.0	A	0.0	12.5	0.0	0.0	0.0	A
Sakigake	0.7	2.2	1.9	2.5	A	5.1	32.6	5.6	5.8	4.3	B
Sultan	0.0	0.6	0.0	0.0	A	1.5	20.6	0.0	0.0	0.0	A
Turk	0.3	0.6	0.3	0.0	A	1.0	0.0	0.0	0.0	0.0	A
WestChina	1.8	3.9	0.6	2.3	A	2.5	56.9	2.3	0.9	0.6	B
WWxG[i]	0.2	0.0	0.0	0.0	A	6.9	0.0	0.0	0.0	0.0	A
CI3515	0.0	0.0	1.8	0.0	A	5.2	37.3	0.0	0.0	0.0	A
CI4364	0.0	0.0	0.0	1.3	A	7.8	36.1	0.0	0.0	0.0	A
CI8618	0.4	0.9	0.0	0.0	A	1.6	0.0	0.0	0.0	0.4	A
LSD=	3.9	12.4	5.7	4.1		11.8	37.0	6.0	5.0	4.2	

i = Wisconsin Winter X Glabron

ii = Modoc California

Appendix 5b

Percentage Leaf Area Diseased (%LAD) on commercial cultivars and advanced breeding lines in field screening trials at Waite and Charlick trial sites

Trial site	Waite					Charlick					
Assessment number	1	2	3	4	Cluster	1	2	3	4	5	Cluster
Cultivar											
1982											
Cantala	2.4	0.6	0.5	1.1	A	7.2	40.1	0.3	0.5	0.3	A
Clipper	15.5	28.9	10.5	4.9	E	19.0	55.0	8.0	7.0	6.7	E
Forrest	0.1	0.3	2.3	0.0	A	1.6	0.0	0.0	0.0	0.0	A
Galleon	6.0	2.8	9.4	7.4	C	12.3	59.4	2.9	5.7	9.3	C
Grimmett	9.1	8.1	10.1	5.7	D	12.7	32.6	2.0	4.6	4.5	B
Shannon	2.6	1.0	1.2	4.0	A	12.8	38.1	1.3	1.0	0.5	B
WI2468	7.9	6.7	6.3	9.1	C	7.4	54.8	1.4	3.7	3.8	B
WI2477	10.2	17.8	14.5	6.6	D	7.4	54.8	1.4	3.7	3.8	B
WI2594	2.7	4.8	7.6	6.0	B	12.5	15.6	0.3	0.5	0.8	A
WI2597	0.1	1.8	0.6	0.3	A	0.9	11.7	0.8	0.8	0.3	A
WI2598	2.8	5.3	2.0	1.9	A	9.4	43.3	1.4	1.3	0.6	B
1983											
Arivat	0.2	0.4	2.3	2.3	A						
Bandulla	1.5	8.0	51.3	24.9	E						
Clipper	1.3	7.6	15.3	24.2	E						
GoldenPromise	1.5	6.1	13.2	27.8	E						
Ketch	3.2	15.3	33.5	50.3	E						
Malebo	0.0	0.0	0.0	0.2	A						
Proctor	0.7	3.6	9.3	17.3	D						
Stirling	2.1	6.3	20.5	35.8	E						
LSD=	3.9	12.4	5.7	4.1		11.8	37.0	6.0	5.0	4.2	

Appendix 5c

Percentage Leaf Area Diseased (%LAD) on Psaknon and Arivat cross breeding lines in field screening trials at Waite and Charlick trial sites

Trial site	Waite					Charlick					
Assessment number	1	2	3	4	Cluster	1	2	3	4	5	Cluster
Cultivar											
(PsaknonxClipper)xClipper											
Selection 6	7.7	7.7	0.9	9.0	C	13.6	11.4	1.4	1.3	2.3	A
Selection 13	7.3	2.2	6.3	6.7	C	10.1	63.8	8.9	3.6	6.0	C
Selection 21	12.8	22.0	9.1	5.5	E	10.0	39.9	5.0	2.0	4.2	B
Selection 22	3.5	3.8	5.6	1.1	B	5.2	33.1	0.6	1.2	1.7	A
Selection 38	11.2	4.4	10.4	6.4	E	20.5	59.5	5.6	5.4	7.3	E
Selection 41	10.7	23.0	8.4	3.4	E	11.3	50.6	0.9	3.2	3.5	B
Selection 44	8.7	18.9	16.9	5.8	D	16.5	49.1	4.7	8.6	7.6	E
Selection 58	13.1	8.0	13.0	5.6	D	5.9	43.1	5.9	5.9	6.6	B
Selection 70	11.2	32.8	13.0	6.9	E	9.6	75.5	2.3	4.5	3.9	B
(ArivatxClipper)xClipper											
Selection 19	8.1	0.7	2.1	8.3	C	15.8	8.4	8.4	5.6	6.3	C
Selection 25	3.3	9.8	9.9	6.6	D	10.9	49.5	2.7	3.7	6.6	B
Selection 78	5.5	4.9	3.6	5.2	C	13.6	29.4	2.8	5.6	3.8	B
Selection 79	2.4	13.9	6.7	6.8	D	9.9	67.8	0.4	2.0	1.5	B
Selection 89	8.7	11.4	16.3	7.4	D	17.2	50.3	4.1	3.5	5.9	C
Selection 95	1.8	0.8	4.4	4.1	B	7.8	46.9	1.3	1.3	0.5	B
Selection 96	14.4	35.8	6.5	13.3	E	17.0	47.3	7.4	7.3	9.3	E
Selection 100	2.5	3.2	3.4	4.5	C	9.0	54.1	2.1	5.0	5.8	B
Selection 104	3.5	6.7	12.1	5.6	D	7.6	29.6	0.2	2.1	2.5	B
LSD=	3.9	12.4	5.7	4.1		11.8	37.0	6.0	5.0	4.2	

Appendix 5d

Percentage Leaf Area Diseased (%LAD) on crosses between breeding lines in field screening trials at Waite and Charlick trial sites

Trial site	Waite					Charlick					
Assessment number	1	2	3	4	Cluster	1	2	3	4	5	Cluster
Cultivar											
Family 39											
Selection 87	0.8	0.1	0.1	0.6	A	18.0	27.5	0.0	0.5	0.4	A
Selection 115	1.8	2.0	0.1	1.6	A	6.7	20.8	0.1	0.3	0.8	A
Selection 116	1.1	2.4	3.7	4.2	A	13.8	8.1	0.1	0.8	0.6	A
Selection 117	0.3	0.0	0.0	1.1	A	2.6	14.4	0.0	0.0	0.2	A
Selection 142	0.4	0.2	0.0	0.5	A	10.3	36.3	0.1	0.3	0.0	A
Selection 207	7.6	2.0	6.6	2.4	B	10.1	57.3	1.6	2.5	1.3	B
Selection 669	1.2	0.0	0.6	0.3	A	5.7	2.3	0.0	0.0	0.0	A
Family 44											
Selection 493	0.1	0.4	0.3	0.5	A	4.8	23.5	0.1	0.3	0.0	A
Family 47											
Selection 391	0.2	1.0	5.3	2.5	B	10.7	49.8	1.1	0.3	0.0	B
Family 48											
Selection 467	1.7	3.6	0.9	2.3	A	10.4	9.9	0.3	0.0	0.0	A
Selection 468	1.2	0.4	0.0	1.1	A	6.5	11.1	0.1	0.0	0.0	A
Family 49											
Selection 102	0.2	9.6	0.3	0.3	A	7.0	51.0	0.0	0.0	0.0	B
Selection 104	1.0	2.2	9.5	3.0	B	4.0	15.4	0.0	0.0	0.0	A
Selection 106	0.1	1.2	0.0	0.3	A	3.7	45.3	0.6	0.0	0.5	B
Selection 383	2.7	0.7	1.6	4.6	A	3.9	8.5	0.1	0.6	0.7	A
Selection 391	0.9	0.6	2.5	4.0	A	5.7	5.6	0.3	0.8	1.8	A
LSD=	3.9	12.4	5.7	4.1		11.8	37.0	6.0	5.0	4.2	

Appendix 5e

Percentage Leaf Area Diseased (%LAD) on crosses between breeding lines in field screening trials at Waite and Charlick trial sites

Trial site	Waite					Charlick					
Assessment number	1	2	3	4	Cluster	1	2	3	4	5	Cluster
Cultivar											
Family 51											
Selection 215	0.6	2.1	1.4	3.6	A	8.1	63.8	6.1	4.3	2.5	B
Selection 216	0.2	0.1	0.3	1.1	A	9.5	24.4	0.1	0.3	0.0	A
Selection 217	4.3	0.2	1.3	1.0	A	8.4	9.1	0.0	0.0	0.0	A
Selection 233	0.1	0.4	0.0	1.5	A	10.5	42.6	0.2	1.5	2.3	B
Selection 328	0.2	0.5	0.8	1.2	A	5.8	17.9	0.0	0.3	0.5	A
Selection 402	0.7	0.1	0.8	0.7	A	8.8	32.8	0.4	2.4	2.1	A
Selection 517	0.1	0.2	0.3	0.6	A	10.4	21.9	0.5	0.8	0.6	A
Selection 717	0.8	1.5	2.3	2.2	A	12.5	21.1	0.1	0.4	0.0	A
Selection 742	0.1	1.1	0.0	1.4	A	7.0	40.3	0.0	0.0	0.0	A
Selection 765	0.0	0.1	0.0	0.1	A	1.5	8.0	0.0	0.0	0.0	A
Selection 778	0.0	0.0	0.0	0.0	A	3.1	5.8	0.3	0.0	0.3	A
Selection 943	0.1	0.2	0.3	0.6	A	12.6	22.3	0.0	1.4	0.9	A
Family 55											
Selection 107	0.2	0.3	0.0	2.0	A	12.1	30.3	0.7	2.5	2.6	A
Selection 560	0.0	0.4	1.4	3.0	A	6.3	33.6	0.6	5.6	2.3	B
Selection 570	6.6	16.7	3.7	5.4	E	12.9	48.3	2.6	7.1	8.3	E
Family 56											
Selection 30	0.2	0.6	0.3	0.6	A	3.5	12.8	0.2	2.1	0.7	A
Selection 51	0.2	0.4	0.0	0.7	A	9.9	44.6	0.1	2.5	0.1	B
Selection 95	0.4	0.5	0.3	3.1	A	9.3	14.6	0.7	0.7	0.1	A
Selection 915	0.1	0.0	0.3	1.7	A	11.9	28.3	0.4	2.2	3.8	B
Selection 926	0.2	0.8	1.1	1.1	A	5.5	4.1	0.0	1.8	0.3	A
Selection 930	0.0	0.3	0.0	2.3	A	6.8	8.8	0.4	1.5	0.5	A
Selection 931	0.4	0.2	0.1	3.1	A	12.3	45.3	0.9	2.1	1.4	B
Family 70											
Selection 69	0.0	0.0	0.1	0.5	A	10.9	52.5	0.0	1.0	1.2	B
Selection 120	0.1	0.3	0.0	3.0	A	13.3	27.8	0.5	0.4	0.3	B
Selection 162	0.1	1.4	0.3	0.3	A	12.1	35.0	0.1	0.4	1.2	B
Family 71											
Selection 147	0.6	0.8	0.0	2.0	A	11.4	27.0	0.4	1.5	0.3	A
Selection 383	2.8	4.1	4.3	4.5	C	11.8	52.2	0.0	0.8	0.0	B
Selection 507	0.1	1.3	0.8	2.0	A	11.6	27.8	0.4	2.4	1.1	B
Selection 515	0.1	1.0	0.3	0.4	A	15.1	37.3	0.1	0.0	0.0	B
Selection 535	0.1	1.0	1.1	1.8	A	7.1	8.8	0.8	1.7	1.0	A
LSD=	3.9	12.4	5.7	4.1		11.8	37.0	6.0	5.0	4.2	

Appendix 6

Measurement of disease components on sixteen barley lines

Barley	Isolate Inoculum	Inoculum Conc'n. (Spores/ml)	Infection rate	Sporulation count (spores/ml/lesion) Day 15	17	24	Incubation period (days)
(PsaknonxClipper)xClipper							
Selection 6	412	$2x10^6$	0.30	4.53	27.81	22.07	11.5
	44/29	$1x10^6$	0.80	5.71	37.00	10.67	13.0
	409	$2x10^6$	0.50	1.12	8.90	4.29	14.0
	183	$3x10^5$	0.60	3.87	9.46	5.42	13.0
Selection 21	412	$2x10^6$	0.75	12.01	26.69	3.26	12.5
	44/29	$1x10^6$	1.00	33.33	34.08	5.21	11.0
	409	$2x10^6$	1.00	29.32	34.36	5.66	11.0
	183	$3x10^5$	1.00	10.88	56.88	5.13	14.0
Selection 22	412	$2x10^6$	0.00	*	*	*	*
	44/29	$1x10^6$	1.00	1.38	19.13	3.63	13.0
	409	$2x10^6$	0.50	30.38	82.38	1.88	14.0
	183	*	*	*	*	*	*
Selection 44	412	$2x10^6$	0.75	7.88	23.47	4.91	12.5
	44/29	$1x10^6$	0.50	29.01	35.22	4.61	12.0
	409	$2x10^6$	0.60	22.67	43.86	12.34	11.5
	183	$3x10^5$	0.60	5.13	16.33	2.44	12.0
Selection 70	412	$2x10^6$	0.50	0.81	3.50	1.25	12.0
	44/29	$1x10^6$	0.60	2.25	5.85	1.27	12.0
	409	$2x10^6$	0.35	21.38	36.88	2.07	11.0
	185	$3x10^5$	0.25	9.10	19.75	5.85	12.0
(ArivatxClipper)xClipper							
Selection 89	412	$3.5x10^5$	0.67	6.30	21.17	2.48	15.0
	409	$2x10^6$	0.50	6.00	20.50	4.19	12.0
	44/29	$5x10^5$	0.33	19.00	63.50	2.94	13.0
	183	$1.5x10^5$	0.50	34.00	53.75	6.10	11.0
Selection 95	412	$3.5x10^5$	0.15	2000.00	66.00	4.00	15.0
	409	$2x10^6$	0.75	5.75	6.50	2.39	15.0
	44/29	$5x10^5$	0.60	19.25	44.25	4.16	15.0
	183	$1.5x10^5$	1.00	19.65	19.83	16.27	11.6
Selection 96	412	$3.5x10^5$	0.33	4.75	18.5	6.94	14.0
	409	$2x10^6$	0.22	1.25	8.50	3.83	12.0
	44/29	$5x10^5$	0.50	4.83	8.67	3.42	13.6
	183	$1.5x10^5$	0.56	7.19	14.00	2.43	11.0
Selection 100	412	$3.5x10^5$	0.50	8.00	12.50	10.26	11.0
	409	$2x10^6$	0.38	12.50	32.00	7.81	14.0
	44/29	$5x10^5$	0.50	8.75	37.00	3.49	12.0
	183	$1.5x10^5$	1.00	27.42	35.83	3.26	11.0

Appendix 6 (continued)

Measurement of disease components on sixteen barley lines

Barley	Isolate Inoculum	Inoculum Conc'n. (Spores/ml)	Infection rate	Sporulation count Day 15 17 24 (spores/ml/lesion)			Incubation (Days)
Family 39							
Line 87	412	$3.5x10^5$	0.00	*	*	*	*
	409	$2x10^6$	0.00	*	*	*	*
	44/29	$5x10^5$	0.00	*	*	*	*
	183	$1.5x10^5$	0.10	1.00	5.00	2.00	11.0
Line 116	412	$3.5x10^5$	0.15	0.00	0.00	0.00	11.0
	409	$2x10^6$	0.33	4.50	9.00	3.38	14.0
	44/29	$5x10^5$	0.40	0.00	9.75	1.72	12.0
	183	$1.5x10^5$	0.30	0.88	5.25	2.33	15.0
Line 207	412	$3.5x10^5$	0.30	2.75	4.75	2.19	14.0
	409	$2x10^6$	0.20	1.00	19.00	4.13	11.0
	44/29	$5x10^5$	0.67	4.00	9.00	0.38	13.0
	183	$1.5x10^5$	0.33	0.25	4.00	1.88	15.0
Line 669	no disease		0.00	*	*	*	*
Family 51							
Line 215	no disease		0.00	*	*	*	*
Line 943	no disease		0.00	*	*	*	*
Clipper	412	$3.5x10^5$	0.70	7.00	15.17	6.38	12.0
	409	$2x10^6$	1.00	12.00	9.00	3.40	14.2
	44/29	$5x10^5$	1.00	6.40	31.00	4.10	11.0
	183	$1.5x10^5$	1.00	12.25	17.60	8.29	12.6

Appendix 7a

Correlation between glasshouse infection rates of isolate 183, and field infection rates of barley lines

Site	Assessment	Correlation Coefficient	Constant	Regression Coefficient	t-value	%variation
Charlick	1	0.08^{NS}	0.402	0.0015	0.40^{NS}	-
	2	0.44^{NS}	0.152	0.0063	2.47*	16.4
	3	0.35^{NS}	0.348	0.0044	1.84^{NS}	8.5
	4	0.42^{NS}	0.283	0.0053	2.29*	14.0
	5	0.46^{NS}	0.292	0.0063	2.56*	17.7
Waite	1	0.53^{NS}	0.266	0.0060	3.09**	24.8
	2	0.44^{NS}	0.348	0.0048	2.42*	15.7
	3	0.45^{NS}	0.296	0.0160	2.53*	17.2
	4	0.42^{NS}	0.258	0.0251	2.35*	14.8
Average	1	0.41^{NS}	0.158	0.0067	2.27*	13.7
	2	0.50^{NS}	0.194	0.0071	2.89**	22.0
	3	0.42^{NS}	0.296	0.0087	2.29*	14.0
	4	0.44^{NS}	0.251	0.010	2.48*	16.5

*=P<0.05 **=P<0.01 ***=P<0.001

Appendix 7b

Correlation between glasshouse infection rates of isolate 409, and field infection rates of barley lines

Site	Assessment	Correlation Coefficient	Constant	Regression Coefficient	t-value	%variation
Charlick	1	0.22^{NS}	0.188	0.0038	1.14^{NS}	1.2
	2	0.16^{NS}	0.301	0.0019	0.83^{NS}	-
	3	0.27^{NS}	0.299	0.0032	1.42^{NS}	3.7
	4	0.14^{NS}	0.344	0.0016	0.70^{NS}	-
	5	0.19^{NS}	0.326	0.0024	0.99^{NS}	-
Waite	1	0.50^{NS}	0.200	0.0051	2.91**	22.2
	2	0.32^{NS}	0.305	0.0031	1.72^{NS}	7.0
	3	0.45^{NS}	0.226	0.0139	2.52*	17.1
	4	0.24^{NS}	0.282	0.0119	1.23^{NS}	2.0
Average	1	0.45^{NS}	0.094	0.0064	2.50*	16.8
	2	0.29^{NS}	0.251	0.0036	1.53^{NS}	4.9
	3	0.36^{NS}	0.246	0.0069	1.95^{NS}	9.7
	4	0.17^{NS}	0.322	0.0033	0.86^{NS}	-

*=P<0.05 **=P<0.01

Appendix 7c

Correlation between glasshouse infection rates of isolate 44/29, and field infection rates of barley lines

Site	Assessment	Correlation Coefficient	Constant	Regression Coefficient	t-value	%variation
Charlick	1	0.03^{NS}	0.440	0.0005	0.14^{NS}	-
	2	0.21^{NS}	0.327	0.0027	1.05^{NS}	0.4
	3	0.17^{NS}	0.401	0.0022	0.86^{NS}	-
	4	0.20^{NS}	0.376	0.0024	1.01^{NS}	0.1
	5	0.29^{NS}	0.349	0.0038	1.49^{NS}	4.5
Waite	1	0.50^{NS}	0.256	0.0057	2.86**	21.6
	2	0.34^{NS}	0.362	0.0036	1.79^{NS}	7.8
	3	0.29^{NS}	0.350	0.0098	1.50^{NS}	4.6
	4	0.35^{NS}	0.282	0.0191	1.87^{NS}	8.8
Average	1	0.36^{NS}	0.1897	0.0058	1.93^{NS}	9.5
	2	0.32^{NS}	0.2895	0.0044	1.69^{NS}	6.6
	3	0.23^{NS}	0.3664	0.0048	1.16^{NS}	1.3
	4	0.24^{NS}	0.3434	0.0052	1.25^{NS}	2.1

**=P<0.01

Appendix 7d

Correlation between glasshouse infection rates (average of the four isolates), and field infection rates of barley lines

Site	Assessment	Correlation Coefficient	Constant	Regression Coefficient	t-value	%variation
Charlick	1	0.36^{NS}	0.098	0.0053	1.78^{NS}	9.0
	2	0.38^{NS}	0.198	0.0040	1.91^{NS}	10.7
	3	0.34^{NS}	0.298	0.0033	1.65^{NS}	7.3
	4	0.42^{NS}	0.252	0.0039	2.09*	13.3
	5	0.42^{NS}	0.271	0.0042	2.14*	14.0
Waite	1	0.58^{NS}	0.237	0.0050	3.23**	29.9
	2	0.46^{NS}	0.301	0.0039	2.35*	17.1
	3	0.57^{NS}	0.226	0.0149	3.15**	28.8
	4	0.42^{NS}	0.234	0.0185	2.10*	13.4
Average	1	0.55^{NS}	0.101	0.0066	3.01**	26.8
	2	0.50^{NS}	0.186	0.0055	2.64*	21.4
	3	0.45^{NS}	0.243	0.0073	2.32*	16.6
	4	0.44^{NS}	0.230	0.0072	2.24*	15.4

*=P<0.05 **=P<0.01

Appendix 8

Average Percentage Leaf Area Diseased (%LAD) on four leaves assessed, in yield assessment trials

Treatment	Barley line 765/51	943/51	215/51	669/39	87/39	116/39	207/39	Sch*	Galleon	Clipper
Assessment 1										
O	1.6	0.2	1.4	0.5	0.1	0.2	0.1	0.4	0.0	0.0
E	0.4	0.1	1.5	0.5	1.8	0.5	0.7	3.4	0.6	0.7
L	1.8	2.1	1.5	0.6	0.1	0.0	0.0	0.8	0.4	0.2
D	0.1	2.0	0.2	3.1	0.2	0.3	1.5	9.0	0.2	3.3
LSD was not significant										
Assessment 2										
O	1.0	0.8	0.9	0.0	3.0	4.3	3.1	1.5	0.0	0.0
E	1.7	0.3	4.4	1.7	0.5	1.7	4.5	8.4	3.0	14.3
L	3.8	7.6	3.4	2.7	2.9	0.3	6.7	0.8	0.0	0.4
D	0.2	3.5	7.0	0.1	1.3	0.2	10.2	5.8	9.5	9.1
LSD was not significant										
Assessment 3										
O	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
E	0.1	0.7	5.7	0.0	0.0	0.8	8.8	4.0	3.9	34.8
L	0.4	0.0	1.3	0.0	0.0	0.0	3.0	0.0	0.1	0.0
D	0.0	0.3	1.9	0.0	0.3	1.4	0.8	4.1	7.2	7.1
LSD = 3.6										
Assessment 4										
O	0.0	0.0	0.3	0.0	0.0	0.0	0.2	0.1	0.5	1.3
E	0.3	2.0	3.9	0.0	1.0	6.4	3.8	5.1	10.5	11.5
L	0.0	0.0	0.6	0.0	0.1	0.0	0.1	0.1	0.2	1.3
D	0.0	0.8	2.6	0.0	0.9	4.8	0.9	5.0	5.3	13.6
LSD = 3.6										
Assessment 5										
O	0.0	0.0	0.4	0.0	0.0	0.1	0.8	0.1	0.0	4.9
E	0.0	5.4	7.8	0.2	0.0	16.5	4.6	11.4	12.1	17.9
L	0.0	0.9	2.8	0.0	0.1	0.2	0.4	0.9	2.0	2.6
D	0.1	4.3	5.9	0.0	0.2	18.5	5.7	16.5	14.3	29.9
LSD = 12.0										
Assessment 6										
O	0.0	0.2	0.2	0.0	0.0	0.3	2.1	2.3	1.6	12.7
E	1.6	7.5	10.2	0.5	2.1	25.7	6.6	12.4	17.1	22.5
L	0.0	1.4	6.6	1.6	0.3	7.1	0.3	5.8	11.3	14.1
D	0.0	15.8	24.3	0.0	6.2	41.5	9.9	47.5	34.1	64.7
LSD = 14.9										
Assessment 7										
O							0.9	0.8	2.1	14.4
E							2.9	21.2	27.9	29.3
L							1.8	8.1	14.6	20.9
D							8.1	59.9	45.0	74.2
LSD = 15.2										

* Schooner

Appendix 9

Average growth stage (Zadoks decimal scale) of barley lines, at each assessment, in yield assessment trial.

	Assessment number						
	1	2	3	4	5	6	7
Barley line							
765/51	21.1	25.4	32.9	49.2	63.8	84.2	
943/51	21.5	25.6	33.5	50.5	65.5	84.7	
215/51	22.1	26.5	32.8	44.6	63.3	81.9	
669/39	22.0	26.0	35.0	51.8	68.1	86.3	
87/39	21.5	25.5	34.1	53.1	65.9	85.3	
116/39	21.9	26.2	32.9	47.4	64.9	82.8	
207/39	21.8	25.6	30.7	34.1	46.1	62.6	85.8
Schooner	20.8	26.3	30.9	35.4	46.4	58.8	85.2
Galleon	22.7	27.3	31.2	32.9	45.6	58.6	85.7
Clipper	21.6	26.7	30.8	33.0	44.8	59.7	84.4
LSD=	21.6	26.7	30.8	33.0	44.8	59.7	84.4

Appendix 10

Average height of plants (cms) at each assessment, in yield assessment trials

	Barley line									
	765/51	943/51	215/51	669/39	87/39	116/39	207/39	Sch*	Galleon	Clipper
Treatment										
Assessment 1										
O	198.2	207.0	219.7	227.8	214.5	219.9	239.9	222.0	188.9	205.2
E	210.5	229.3	203.0	214.3	215.6	225.9	211.9	202.6	169.6	192.1
L	223.5	199.1	201.5	211.3	234.3	211.2	231.2	207.1	169.8	188.3
D	233.8	173.5	223.9	199.4	232.5	178.4	205.3	182.6	179.4	183.5
LSD was not significant										
Assessment 2										
O	407.5	429.1	435.7	476.8	419.3	421.1	446.9	401.8	380.8	393.0
E	416.2	465.6	408.8	454.6	434.8	464.1	420.0	345.5	324.6	343.3
L	419.3	396.5	420.4	428.9	437.7	423.4	393.3	403.2	335.3	355.5
D	396.3	361.8	418.5	377.5	436.3	382.3	375.3	357.1	350.7	336.4
LSD was not significant										
Assessment 3										
O	600.6	633.0	565.5	705.9	580.6	581.0	528.9	567.0	531.2	538.7
E	619.3	696.8	575.2	692.2	666.3	658.9	517.5	487.1	491.9	473.0
L	632.1	574.5	569.4	712.1	637.7	620.1	511.0	574.0	510.5	521.7
D	635.6	611.7	592.0	629.1	691.2	585.5	526.8	540.1	478.0	510.1
LSD was not significant										
Assessment 4										
O	909.5	870.6	838.9	936.1	862.0	866.1	695.1	707.6	630.9	679.9
E	913.8	873.0	758.0	855.9	877.4	818.5	673.1	616.3	586.5	612.0
L	893.7	826.3	808.2	943.4	908.8	866.2	680.8	741.9	630.9	679.9
D	889.0	898.4	846.0	897.9	900.0	837.2	680.1	668.5	603.3	619.7
LSD was not significant										
Assessment 5										
O	1124.2	1070.8	1079.8	1115.2	1055.6	1061.0	972.4	958.3	869.8	898.7
E	1136.1	1046.3	1095.4	1090.9	1118.5	1065.0	966.5	889.0	801.8	793.8
L	1123.3	1079.9	1059.2	1133.1	1114.8	1026.9	936.9	975.6	909.4	868.5
D	1136.3	1085.9	1088.4	1146.3	1134.4	1050.6	896.8	843.7	806.3	782.2
LSD = 62.6										
Assessment 6										
O	1150.9	1098.5	1132.2	1096.0	1082.0	1054.7	1080.7	1021.8	947.2	988.6
E	1123.1	1034.1	1101.6	1107.3	1100.1	1030.1	1082.3	1014.6	921.5	907.3
L	1161.3	1068.7	1148.5	1109.3	1114.9	1003.7	1108.7	1047.5	984.4	980.8
D	1150.1	1109.8	1129.4	1146.6	1116.3	1014.8	1096.6	967.6	898.9	832.9
LSD was not significant										
Assessment 7										
O							1124.7	998.0	922.9	980.6
E							1116.5	976.8	918.2	900.7
L							1108.0	1054.0	970.0	1008.5
D							1112.5	982.5	906.5	859.0
LSD was not significant										

* Schooner

Appendix 11

Incidence of leaf infection on individual barley lines at each assessment, in yield assessment trials

Treatment	Barley line 765/51	943/51	215/51	669/39	87/39	116/39	207/39	S*	Galleon	Clipper
Assessment 1										
O	0.18	0.10	0.05	0.05	0.07	0.01	0.03	0.03	0.00	0.01
E	0.11	0.04	0.10	0.05	0.08	0.05	0.03	0.13	0.07	0.01
L	0.06	0.09	0.08	0.04	0.00	0.00	0.00	0.03	0.03	0.01
D	0.04	0.06	0.00	0.15	0.04	0.03	0.04	0.10	0.03	0.11
Assessment 2										
O	0.07	0.03	0.04	0.01	0.04	0.06	0.04	0.09	0.00	0.01
E	0.08	0.08	0.12	0.06	0.06	0.20	0.08	0.36	0.24	0.49
L	0.13	0.20	0.13	0.04	0.03	0.09	0.01	0.03	0.01	0.04
D	0.04	0.12	0.21	0.12	0.11	0.21	0.09	0.41	0.33	0.38
Assessment 3										
O	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
E	0.01	0.05	0.27	0.00	0.06	0.03	0.25	0.44	0.58	0.82
L	0.01	0.00	0.07	0.00	0.00	0.00	0.15	0.00	0.01	0.00
D	0.01	0.05	0.21	0.00	0.20	0.07	0.17	0.54	0.55	0.68
Assessment 4										
O	0.00	0.00	0.03	0.00	0.00	0.00	0.04	0.01	0.03	0.16
E	0.01	0.20	0.32	0.00	0.41	0.08	0.25	0.59	0.61	0.72
L	0.00	0.00	0.07	0.00	0.00	0.01	0.01	0.04	0.03	0.18
D	0.00	0.11	0.19	0.00	0.35	0.09	0.18	0.64	0.60	0.75
Assessment 5										
O	0.00	0.00	0.08	0.00	0.01	0.00	0.09	0.03	0.00	0.30
E	0.00	0.44	0.44	0.00	0.65	0.01	0.22	0.59	0.50	0.61
L	0.00	0.08	0.34	0.00	0.03	0.01	0.06	0.13	0.11	0.28
D	0.01	0.62	0.29	0.00	0.94	0.05	0.33	0.82	0.41	0.86
Assessment 6										
O	0.00	0.00	0.02	0.02	0.03	0.00	0.15	0.72	0.24	0.28
E	0.03	0.51	0.30	0.02	0.80	0.13	0.18	0.84	0.77	0.68
L	0.00	0.25	0.18	0.00	0.48	0.02	0.05	0.72	0.70	0.48
D	0.00	0.90	0.43	0.00	0.78	0.10	0.49	1.00	0.99	1.00
Assessment 7										
O							0.09	0.13	0.29	0.70
E							0.15	0.81	0.77	0.86
L							0.13	0.64	0.82	0.87
D							0.38	0.96	0.90	0.97

* = Schooner

Appendix 12

Zadoks' decimal code for the growth stages of cereals

Code*		Code	
0	Germination	5	Inflorescence emergence
00	Dry seed	50	}First spikelet of inflorescence
01	Start of imbibition	51	} just visible
02	-	52	]1/4 of inflorescence emerged
03	Imbibition complete	53	]
04	-	54	}1/2 of inflorescence emerged
05	Radicle emerged from caryopsis	55	}
06	-	56	]3/4 of inflorescence emerged
07	Coleoptile emerged from caryopsis	57	]
08	-	58	}Emergence of inflorescence completed
09	Leaf just at coleoptile tip	59	}
1.	Seedling growth	6	Anthesis
10	First leaf through coleoptile	60	}Beginning of anthesis(not easily
11	First leaf unfolded*	61	} detected in barley)
12	2 leaves unfolded	62	-
13	3 leaves unfolded	63	-
14	4 leaves unfolded	64	}Anthesis half-way
15	5 leaves unfolded	65	}
16	6 leaves unfolded	66	-
17	7 leaves unfolded	67	-
18	8 leaves unfolded	68	}Anthesis complete
19	9 or more leaves unfolded	69	}
2	Tillering	7	Milk development
20	Main shoot only	70	-
21	Main shoot and 1 tiller	71	Caryopsis water ripe
22	Main shoot and 2 tillers	72	-
23	Main shoot and 3 tillers	73	Early milk
24	Main shoot and 4 tillers	74	-

Appendix 12 (continued)

25	Main shoot and 5 tillers	75	Medium milk(Increase in solids of liquid endosperm notably when crushing the caryopsis)
26	Main shoot and 6 tillers	76	-
27	main shoot and 7 tillers	77	Late milk
28	Main shoot and 8 tillers	78	-
29	Main shoot and 9 or more tillers	79	-
3	Stem elongation	8	Dough development
30	Pseudo stem erection	80	-
31	1st node detectable	81	-
32	2nd node detectable	82	-
33	3rd node detectable	83	Early dough
34	4th node detectable	84	-
35	5th node detectable	85	Soft dough (Fingernail impression not held)
36	6th node detectable	86	-
37	Flag leaf just visible	87	Hard dough(Fingernail impression held)
38	-	88	-
39	Flag leaf ligule/collar just visible	89	-
4	Booting	9	Ripening
40	-	90	-
41	Flag leaf sheath extending	91	Caryopsis hard(Difficult to divide by thumbnail)
42	-	92	Caryopsis hard (Can no longer be dented by thumbnail)
43	Boots just visibly swollen	93	Caryopsis loosening in daytime
44	-	94	Over-ripe,straw dead and collapsing
45	Boots swollen	95	Seed dormant
46	-	96	Viable seed giving 50% germination
47	Flag leaf sheath opening	97	Seed not dormant
48	-	98	Secondary dormancy induced
49	First awns visible	99	Secondary dormancy lost

* Even code numbers refer to crops in which this stage is reached by all shoots simultaneously and odd numbers to unevenly developing crops when 50% of the shoots are at the stage given.

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