VIRULENCE SPECTRUM, MOLECULAR CHARACTERISATION AND FUNGICIDE SENSITIVITY OF THE SOUTH AFRICAN *RHYNCHOSPORIUM SECALIS* POPULATION

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DECLARATION

I, the undersigned, hereby declare that the work contained in this dissertation is my own original work and that I have not previously in its entirety or in part submitted it at any university for a degree.

SUMMARY

Barley leaf scald, caused by *Rhynchosporium secalis,* is the most important disease of barley (*Hordeum vulgare*) in the Western Cape province of South Africa. The disease was first reported from South Africa in 1937. The present study is the first attempt to characterise the South African *R. secalis* population. Topics such as pathogenesis-related proteins, virulence spectra, variability of pathotypes, sources of variation, host resistance, breeding strategies, molecular characterisation and fungicide sensitivity are summarised in Part 1 of this dissertation. In succeeding Parts the focus is on the characteristics of the local *R. secalis* population regarding virulence spectrum, DNA polymorphisms, *in vitro* as well as *in vivo* fungicide sensitivity. These aspects are treated as separate entities, leading to some duplication which is unavoidable.

In Part 2 the virulence spectra of 50 *R. secalis* isolates from a population in the Western Cape province were determined. Twenty-one races were detected using 17 differential barley cultivars. The two most prevalent races, namely races 4 and 7 had three and four virulence genes respectively. Both race 4 and 7 were virulent on the most susceptible cultivars, namely West China, Steudelli, C.I.8618 and C.I.2226. Considering the resistance genes reported for cultivars Atlas 46, Turk, and C.I.3515 which showed no susceptible cultivar-pathogen interaction, it would appear that the *Rh-Rh3-Rh4* complex is primarily involved in conferring resistance to the local *R. secalis* isolates.

A total of 20 races (47 isolates) characterised in Part 2 were selected for further characterisation by means of DNA fingerprinting. In Part 3 an anonymous multilocus DNA probe was used to characterise the genotypic structure of these isolates by means of RFLP analysis. No correlation between any particular fingerprint pattern, race, district, field or lesion was found. The two most prevalent races, 4 and 7, did not share the same genotypes, even when isolated from the same field or lesion. The genotypic diversity of the isolates studied was 46.5% of the theoretical maximum diversity. The high level of genotypic variation observed in the South African *R. secalis* population resembled the genotypic diversity observed in other cereal pathogens with known sexual structures. Although no teleomorph has yet been observed, these data suggest that sexual recombination may operate within the local population of *R. secalis.*

In South Africa barley scald is primarily controlled by means of fungicides. The continued use of fungicides on cereal crops results in the build-up of fungicide resistance in the population, which could lower the efficacy of these compounds. These aspects were investigated in Part 4, where isolates (collected during 1993 to 1995) were evaluated *in vitro* for sensitivity to triadimenol, tebuconazole, flusilazole and propiconazole. The sensitivity fluctuated but in 1995 isolates were significantly less sensitive towards triadimenol than in the previous two years. In a second experiment, isolates collected from two fields with a 5-6 year-history of triadimenol seed treatments and tebuconazole applications, were evaluated for their fungicide sensitivity. A significant positive correlation was observed between tebuconazole and triadimenol sensitivity among *R. secalis* populations from these fields. However, such a correlation was not found within the *R. secalis* population collected during 1993-1995 where shorter crop rotation patterns and a range of fungicides was applied. In a third experiment, the fungicide sensitivity of local *R. secalis* isolates was evaluated towards two new triazole fungicides, namely bromuconazole and triticonazole. Correlation coefficients observed between these new triazoles and those previously applied in South Africa were not significantly positive. The lack of significant cross-resistance has important practical implications regarding the management of fungicide resistance.

In Part 5, isolates with different minimum inhibitory concentration (MIC) towards tebuconazole *in vitro* (1, 3 and 10 µg/ml) were compared *in vivo*. The aim of this study was to determine how MIC values would influence virulence (leaf area affected) and sporulation. Results indicated that all isolates were equally fit to induce lesions and sporulate in the absence of tebuconazole. Thus no fitness cost was associated with the degree of tebuconazole sensitivity in the present study. All *R. secalis* isolates were able to induce lesions on tebuconazole treated leaves, but differed significantly with respect to the percentage leaf area affected. Isolates, least sensitive (MIC = 10 µg/ml) towards tebuconazole were more adapted on tebuconazole treated leaves, being able to repeatedly cause larger lesions than sensitive *R. secalis* isolates (MIC = 1 µg/ml). Sporulation was not significantly different between isolates on lesions of untreated or tebuconazole treated leaves. Larger leaf areas affected and adequate sporulation suggest that a less sensitive population would result in more disease in tebuconazole treated fields.

In conclusion, this study revealed the variability associated with the South African *R. secalis* population regarding virulence spectrum and genotypic structure. The data in this study suggest that it is likely that the local population will easily adapt to newly introduced, single gene resistance. For more durable resistance, higher levels of quantitative resistance should be introduced. This type of resistance is, however, more difficult to identify and incorporate than single gene resistance. Consequently, barley scald control will remain dependent on the efficacy of fungicide applications. Furthermore, the lack of cross-resistance and low frequency of resistant isolates indicates a low risk for the development of fungicide resistance in the local *R. secalis* population. Other factors such as current crop rotation practices and the range of fungicides being applied also contribute to this low risk level. However, the status of these factors can change over time. The *in vivo* tebuconazole sensitivity study has indicated that a resistant field population of *R. secalis* may be able to build-up. It is, therefore, necessary to monitor the fungicide sensitivity of *R. secalis* isolates at timely intervals with view to successful barley cultivation in the future.

OPSOMMING

Blaarvlek op gars (*Hordeum vulgare*), veroorsaak deur *Rhynchosporium secalis*, is die belangrikste siekte van gars in die Wes-Kaap provinsie van Suid-Afrika. Die voorkoms van *R. secalis* op gars is in Suid-Afrika vir die eertse keer in 1937 gerapporteer. Hierdie studie is die eerste poging tot karakterisering van die plaaslike *R. secalis*-populasie. Aspekte soos proteïene betrokke by patogenese, virulensiespektra, variabiliteit van patotipes, bronne van variasie, gasheerweerstand, teeltprogramme, molekulêre karakterisering en swamdodersensitiwiteit word in Deel 1 van die tesis opgesom. In die daaropvolgende gedeelte is die fokus op die karakterisering van die *R. secalis*-populasie en behels DNA karakterisering, virulensiespektrum, en swamdodersensitiwiteit *in vitro* asook *in vivo*.

In Deel 2 is die virulensiespektra van 50 *R. secalis* isolate van 'n populasie in die Wes-Kaap geëvalueer teenoor 17 differensiëel weerstandbiedende gars kultivars en hieruit is 21 rasse geïdentifiseer. Die twee mees algemene rasse (rasse 4 en 7), met onderskeidelik drie en vier virulensie gene, het virulent vertoon teenoor die mees vatbare kultivars soos West China, Steudelli, C.I.8618 en C.I.2226. Geen vatbare kultivar-patogeen interaksies is met kultivars Atlas 46, Turk en C.I.3515, wat al drie die *Rh-Rh3-Rh4* kompleks dra, gevind nie. Dit wil dus voorkom asof hierdie genekompleks effektiewe gasheerweerstand teen die plaaslike *R. secalis* isolate kan bied.

'n Totaal van 20 rasse (47 isolate), gekarakteriseer in Deel 2, is geselekteer vir verdere karakterisering met behulp van DNA bandpatrone. In Deel 3 is 'n anonieme multilokus DNA peiler gebruik om deur middel van RFLP analise die genotipiese struktuur van hierdie *R. secalis*-isolate te bepaal. Geen assosiasie is gevind tussen DNA bandpatroon en ras, distrik, garsland of letsel nie. Die twee rasse (4 en 7) wat mees algemeen voorkom, het nie dieselfde bandpatroon vertoon nie, ook nie dié afkomstig vanuit dieselfde garsland of letsel nie. Die genotipiese diversiteit van isolate was 46.5% van die teoretiese maksimum diversiteit. Die hoë vlak van variasie waargeneem in die *R. secalis* populasie is soortgelyk aan variasie waargeneem in ander graanpatogene wat oor 'n geslagtelike stadium in die lewenssiklus beskik. Alhoewel geen geslagtelike stadium tot dusver geidentifiseer is nie, dui die vlak van variasie daarop dat geslagtelike rekombinasie moontlik wel plaasvind binne die plaaslike *R. secalis* populasie.

In Suid-Afrika word blaarvlek op gars primêr deur swamdoders beheer. Die toenemende gebruik van swamdoders op graangewasse veroorsaak moontlik 'n opbou van swamdoderweerstand in die populasie. Dit kan die effektiwiteit van swamdoders verlaag. Hierdie veronderstelling is in Deel 4 ondersoek, waar die sensitiwiteit van isolate *in vitro* teenoor triadimenol, tebukonasool, flusilasool en propikonasool geëvalueer is. Die triasool sensitiwiteit van *R. secalis* isolate wat gedurende die 1993-1995 seisoen versamel is het gewissel en slegs vir triadimenol was daar 'n tendens na meer weerstandbiedenheid. 'n Swamdoder-evaluasie is in 'n aparte eksperiment op isolate gedoen wat versamel is vanaf twee garslande met 'n 5-6 jaar geskiedenis van triadimenol saadbehandelings en tebukonasool bespuitings. 'n Betekenisvolle positiewe korrelasie is waargeneem tussen tebukonasool en triadimenol sensitiwiteit in *R. secalis* isolate afkomstig vanaf hierdie twee garslande. 'n Soortgelyke korrelasie is egter nie gevind in die populasie wat gedurende die 1993-1995 seisoene versamel is nie. Laasgenoemde kan moontlik toegeskryf word aan korter wisselboupatrone en die toediening van 'n verskeidenheid van swamdoders. In 'n derde eksperiment is die sensitiwiteit van plaaslike *R. secalis* isolate teenoor twee nuwe triasole, naamlik bromukonasool en tritikonasool getoets. Die korrelasie waargeneem tussen die twee nuwe triasole en triasool swamdoders reeds voorheen in gebruik in die Wes-Kaap was nie betekenisvol positief nie. Die gebrek aan betekenisvolle kruis-weerstandbiedendheid het belangrike praktiese implikasies vir die bestuur van swamdoder-weerstandbiedendheid.

In Deel 5 is isolate met wisselende minimum inhiberende konsentrasies (MIKs) teenoor tebukonasool *in vitro* (1, 3 en 10 µg/ml) en *in vivo* vergelyk. Die doel van hierdie studie was om te bepaal hoe wisselende MIK-waardes virulensie (blaaroppervlakte geïnfekteer) en sporulasie sal beïnvloed. Resultate dui daarop dat alle *R. secalis* isolate in hierdie studie ewe fiks was om, in die afwesigheid van tebukonasool, letsels te induseer en te sporuleer. Die bevinding is dat die verlies in fiksheid nie geassosieer is met die mate van tebukonasool weerstand nie. Alle *R. secalis* isolate het die vermoë gehad om letsels op tebukonasool-behandelde blare te veroorsaak maar het betekenisvol verskil ten opsigte van die blaaroppervlakte geaffekteer. Isolate wat minder sensitief (MIK = 10 µg/ml) teenoor tebukonasool *in vitro* is, het meer aangepastheid op tebukonasool-behandelde blare getoon. Gevolglik het hierdie isolate herhaaldelik meer letsels veroorsaak as sensitiewe isolate (MIK = 1 µg/ml). Sporulasie

het nie betekenisvol verskil tussen isolate vanaf letsels op ondehandelde of tebukonsool-behandelde blare nie. Hierdie resultate dui egter daarop dat 'n minder sensitiewe populasie tot meer siektevoorkoms in tebukonasool-bespuite lande kan lei.

Die studie het die veranderlike karakter van die Suid-Afrikaanse *R. secalis*-populasie aangaande virulensiespektrum en genotipiese struktuur blootgelê. Dit is dus baie moontlik dat die *R. secalis*-populasie maklik sal aanpas by teelmateriaal met nuwe enkelgeen-weerstand. Vir volgehoue gasheerweerstand is dit egter nodig dat hoër vlakke van kwantitatiewe weerstand ingeteel moet word. In die praktyk is hierdie tipe weerstand egter baie moeiliker om te identifiseer en by nuwe teelmateriaal in te sluit as in die geval van enkelgeen-weerstand. Dit bring mee dat blaarvlekbeheer afhanklik bly van swamdodertoedienings as beheermaatreël. Die resultate van hierdie studie dui daarop dat daar tans 'n lae risiko vir die ontwikkeling van swamdoderweerstand in die plaaslike populasie is, as gevolg van die afwesigheid van kruisweerstandbiedendheid en die lae voorkoms van weerstandbiediende isolate. Ander faktore soos die wisselboustelsels wat toegepas word en die verskeidenheid van swamdoders toegedien dra ook daartoe by. Ten spyte hiervan kan die status van hierdie faktore egter oor tyd verander. Die *in vivo* tebukonasool studie het daarop gedui dat 'n weerstandbiedende veldpopulasie van *R. secalis* die potensiaal het om te vermeerder. Gevolglik is die tydige monitering van swamdodersenisitiwiteit van *R. secalis* isolate noodsaaklik om ‘n volhoubare garsproduksie te verseker.

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Fungi - a mutable and treacherous tribe – Albrecht von Haller, 1745

CONTENTS

1. *Rhynchosporium secalis,* the cause of barley scald – a review

ABSTRACT

Various aspects of leaf scald disease, caused by *Rhynchosporium secalis,* are reviewed. Topics dealt with include the biology, symptomatology, epidemiology, host resistance, breeding strategies, fungicide sensitivity and molecular characterisation. Specific attention was given to the variable nature of *R. secalis,* with specific reference to pathogenesis-related proteins, virulence spectrum, variability of pathotypes and sources of variation.

INTRODUCTION

The scald fungus was first described in 1897 by Oudemans under the name *Marssonina secalis.* However, in 1919 Davis established a new combination as *Rhynchosporium secalis* (Oudem.) Davis, the Latin binomial by which the fungus is known today (Caldwell, 1937).

On barley (*Hordeum vulgare*) *R. secalis* causes a disease commonly referred to as barley scald, *Rhynchosporium* leaf blotch, barley leaf blotch, *Rhynchosporium* scald or simply scald. Scald is regarded as a serious disease of barley, with *R. secalis* being pathogenic on a broad range of grasses in the Poaceae family. *R. secalis* is primarily found in cool, moist regions of the world and has been recorded from barley on all five continents. Barley scald is of economic importance in many countries such as Britain (James *et al.*, 1968), Australia (Khan, 1986), Germany (Beer, 1988) and South Africa (Trench *et al.*, 1992). A loss in 1000-kernel weight due to barley scald is primarily the cause of yield loss (James *et al.*, 1968; Beer, 1988). However, if a high infection level is present at an early growth stage, then a reduction in grains per ear may play a more important role (Schaller, 1951).

BIOLOGY

Rhynchosporium secalis is classified as a mitosporic fungus since no meiotic state has been correlated with the genus *Rhynchosporium* (Hawksworth *et al.*, 1995). Conidia are elongate, cylindrical, hyaline and two-celled with the upper cell typically beak-shaped. *R. secalis* conidia are usually 15-20 µm long and 3-5 µm wide (Fig. 1). However, some collections have been found to be more variable, as summarised by Beer (1991).

Rhynchosporium secalis is a hyphomycete, and conidia are produced on a superficial, fertile stromata present on infected barley stubble at the start of the growing season. Conidia are splash dispersed onto barley seedlings and one or more germ tubes can develop within 24 h from either of the two cells of a conidium (Caldwell, 1937; Hoseman & Branchard, 1985). Germ tubes usually remain short and form appresoria which are circular or elongate and sometimes not easily distinguished from germ tubes. *R. secalis* penetrates the host directly through the cuticle (Caldwell, 1937; Ayesu-Offei & Clare, 1970; Jones & Ayres, 1974). Occasionally penetration will take place without the formation of an appresorium (Ayesu-Offei & Clare, 1970). According to Ryan & Clare (1975), light reduces conidial germination and suppresses germ tube elongation.

After penetration, the pathogen grows for 10-14 days beneath the cuticle (Jones & Ayres, 1972). *R. secalis* utilises soluble nutrients present in the intercellular spaces of the leaf and is able to form a subcuticular mycelium which develops into a stroma. A week before the first symptoms appear, *R. secalis* induces an increase in the permeability of the underlying host cells which results in an increase of nutrients (Jones & Ayres, 1972). In culture *R. secalis* is capable of producing a complex of cellulose degrading enzymes (Olutiola & Ayres, 1973), and similar enzymes may be involved in the *in vivo* degradation of cell walls. Mycelial growth is intercellular at first but as epidermal cells lose their turgor and collapse, growth becomes intracellular (Ayesu-Offei & Clare, 1970; Jones & Ayres, 1974). Collapsed cells are externally visible as a blue-green water-soaked area (Caldwell, 1937; Ayesu-Offei & Clare, 1970; Hoseman & Branchard, 1985). Ayesu-Offei & Clare (1971) noted that more than one toxic metabolite was associated with these symptoms. Short lengths of the plasmalemma of epidermal cells may become detached from the cell wall near

subcuticular hyphae but ultimately the cell wall is degraded and replaced by hyphae (Ryan & Grivel, 1974). The pectic and cuticular layers remain largely intact in the leaf lesions until conidia are produced (Ryan & Grivel, 1974). Conidia are produced on small extensions of the subcuticular mycelium, which protrude through the leaf cuticle above the underlying stroma (Howlett & Cooke, 1987). Only in the late stages of lesion development is the cuticle replaced by masses of conidia and mycelium (Howlett & Cooke, 1987). The intact cuticle protects the stroma from adverse conditions during the greater part of the spore production period (Howlett & Cooke, 1987).

Under optimum conditions symptoms may appear within 8 - 14 days after inoculation (Owen, 1963; Hoseman & Branchard, 1985; Cromey, 1987). Compared to other important leaf diseases of barley such as powdery mildew (*Erysiphe graminis*) and leaf rust (*Puccinia hordei*), the latent period for *R. secalis* is relatively long.

SYMPTOMATOLOGY AND EPIDEMIOLOGY

Barley scald is primarily a foliage disease but may also attack older leaf sheaths to a lesser degree. In the early stages of disease development, the lesions are blue-grey in colour and have a water-soaked appearance. Individual lesions vary in length from 4-25 mm and 1-7 mm in width (Caldwell, 1937), but as the number of lesions increase and coalesce, the total area diseased sometimes occupies the whole lamina (James, 1967). The edges of the lesions are sharply bordered by a broad dark-brown margin (Amelung & Beer, 1984), which may be surrounded by a chlorotic region (Shipton, 1974) (Fig. 2). The centre of lesions soon die and assume a light grey (Caldwell, 1937; James, 1967) or beige colour (Amelung & Beer, 1984). As a result of dew or rain drops running down to the junction between the lamina and leaf sheath, infections often occur at the base of the leaf blade. Growth of the fungus across the whole lamina then cuts the supply of nutrients and the whole leaf wilts and dies back (Bartels, 1928; Kiewnick, 1977).

Rhynchosporium secalis was also associated with brown necrosis on nodal portions of barley (Arai, 1996). Plants with such symptoms were observed in the Hokuriku district of Japan and exclusively in fields with severe leaf scald symptoms

caused by *R. secalis*. Infected nodal portions became fragile and were easily broken. This happened at the latter half of the ripening stage until harvest (Arai, 1996).

Barley scald development is greatly influenced by weather conditions. The optimum temperature for conidial germination is between 15°C and 25°C (Ryan & Clare, 1975). At least 95% relative air humidity is needed for successful conidial germination and adequate sporulation. However, after penetrating the cuticle, the fungus is less influenced by temperature and humidity changes (Caldwell, 1937; Fowler & Owen, 1971). Conidia are dispersed by rain splash and it seems that short intense showers are more effective in spreading spores than longer periods of continuous rain (Stedman, 1980; Fitt *et al.*, 1986). Dispersal from infected leaves or straw usually does not exceed 1 m in height (Stedman, 1980). Early in the season the disease progress spreads horizontally (until EC30), and during stem elongation (EC31-EC37) the vertical dispersal of conidia to the new upper leaves becomes important (Ayesu-Offei & Carter, 1971; Polley, 1971; Fitt *et al.*, 1988).

The period of shooting was identified as the critical phase for barley scald development during an investigation on the disease progress in the winter barley/scald system in Germany (Beer, 1988). Without sufficient rainfall during this period further disease development is limited. Therefore, it is not possible until shooting is completed to make a reliable forecast on barley scald development after flowering (EC61). Before the milky ripe stage *R. secalis* is able to destroy most of the assimilatory (photosynthesis) area of the three upper leaves (which include the flag leaf), resulting in considerable loss in grain yield. *R. secalis* infection results in the decrease of chlorophyll and ascorbic acid content (Drapatyi, 1978), as well as affecting the carbon dioxide assimilation in visibly affected parts of the leaves (Martin, 1982). Therefore, net photosynthesis in flag leaves is reduced in proportion to the amount of diseased leaf tissue (Martin, 1982).

Residue (stubble and straw) from the preceding crop is a major source of primary inoculum (Caldwell, 1931; Evans, 1969; Stedman, 1982; Mayfield & Clare, 1984). Viability of conidia on infected barley on the soil surface under field conditions varied from 6 - 12 months (Bartels, 1928; Skoropad, 1966). It seemed that the deeper infected material was worked into the soil, the shorter the survival period (Drapatyi, 1973). A correlation between the amount of barley stubble debris from the previous crop and the severity of *R. secalis* infection was demonstrated by Evans

(1969) and Polley (1971). Infection may also originate from infected seed or small pieces of debris introduced with it (Kay & Owen, 1973b; Webster & Jackson, 1976). Volunteer barley plants and other grass species in the family Poaceae provide an additional source of initial inoculum (Caldwell, 1937; Ali & Boyd, 1973; Kay & Owen, 1973a; Stedman, 1977; Ali, 1981; Cromey & Mulholland, 1987). Beer (1991) drew-up an extensive list of potential grass hosts reported for *R. secalis*. While primary inoculum is a source of disease when plants emerge, secondary inoculum on basal leaves is the main source of disease at stem extension, especially on early sown crops (Davis & Fitt, 1992).

Several additional practices have been observed which induce higher incidence of barley scald. These include expansion of barley growing areas (Doling, 1964), larger areas under susceptible varieties (Brown, 1979), shortened or eliminated rotations (Skoropad, 1960), retention of stubble and plant debris (Skoropad, 1960), use of combine harvesters which scatter straw over the field (Doling, 1964), increased use of nitrogen fertilisers (Ozoe, 1956; Doling, 1964; Couture & Isfan, 1986), earlier sown barley (Jenkins & Jemmett, 1967) and supplementary irrigation (Seidel *et al.*, 1982).

TOXIC METABOLITES

After penetration of the cuticle, fungal growth is limited to intercellular spaces of the leaf during the early stages of interaction between *R. secalis* and its host (Ayesu-Offei & Claire, 1971). The availability of nutrients is critical for the pathogen at this stage. Therefore, to release nutrients from host cells, it is necessary to produce toxic compounds that are capable of moving across cell walls (Wevelsiep *et al.*, 1993).

Ayesu-Offei & Clare (1971) demonstrated that sterilised culture filtrates of *R. secalis* were able to induce visible symptoms of the barley scald disease. They concluded that toxic metabolites of *R. secalis* were, therefore, responsible for disease symptoms in infected barley plants. The toxins also seemed to be non-host specific (Ayesu-Offei & Claire, 1971). In their investigation the toxic effect was not removed by dialysis or autoclaving, and it was suspected that these metabolites were polypeptides or glycopeptides.

Auriol *et al.* (1978) and Mazars *et al.* (1983) confirmed Ayesu-Offei and Clare's (1971) speculation that the toxic metabolites produced by *R. secalis* were indeed glycopeptides. Auriol *et al.* (1978) collectively called these toxins rhynchosporides (popular name). These metabolites are a toxic family of β1-4 glucosides linked 1-0 α to 1,2 propanediol, including the glucoside, the cellobioside, the cellotrioside and cellotetraoside (Strobel, 1982).

Beltran & Strobel (1978) demonstrated the involvement of membrane proteins in the binding of glucose, cellobiose and cellotrioside. Distinct differences in the affinity of purified proteins from susceptible and resistant barley lines towards the rhynchosporides were found. The specific binding activity of rhynchosporosides was higher to proteins of susceptible barley lines (Beltran & Strobel, 1978; Mazars *et al.*, 1983). Cellotrioside induced symptoms corresponding to the resistance or susceptibility of barley lines tested with natural infection (Auriol *et al.*, 1984), whereas glucoside, cellobioside and cellotetraoside have generalised effects that did not vary between resistant and susceptible barley plants. It seems that the mechanism involved with toxin binding regarding rhynchosporosides is analogous to the model of helminthosporoside (Strobel, 1975). In this model lectin-like proteins are involved in toxin binding and the greater the binding the more susceptible the plant is to the toxin.

In 1991 Wevelsiep *et al.* (1991) identified a small family of necrosis-inducing peptides (NIP1, 2 and 3) in culture filtrates of *R. secalis* as well as in infected leaves of a susceptible barley cultivar. NIP2 (a non-glycosylated peptide) was present in culture filtrates of all seven *R. secalis* races analysed. NIP3 (a glycopeptide) was found in culture filtrates of six races in widely varying amounts (Wevelsiep *et al.*, 1991). NIP1 (a non-glycosylated peptide), however, was detected only in culture filtrates of race US238.1 (Wevelsiep *et al.*, 1991). The toxin molecules are relatively small (<10kDa) and their movement across the cell wall are most probably unobstructed. These necrosis-inducing peptides (NIPs) induce necrosis not only in barley but in other cereal hosts as well, and are thus classified as non-host selective toxins. NIP3 and NIP1 stimulated ATPase activity in barley leaf and bean leaf tissue (Wevelsiep *et al.*, 1991). This mode of action is similar to fusicoccin produced by *Fusicoccum amygdali* and typical for non-host selective toxins by affecting a fundamental process common in all plants (Marrè, 1979). NIP2 seems to have a

different mode of action since it had no influence on ATPase activity but was as toxic to barley as NIP3.

ELICITORS AND PATHOGENESIS-RELATED PROTEINS ASSOCIATED WITH BARLEY SCALD

Apart from being able to influence ATPase activity NIP1 had elicitor activity which was not observed for NIP3. NIP1 had race specific elicitor activity only to barley cultivars carrying resistance gene *Rrs1* (Hahn *et al.*, 1993). NIP1, which was detected only in culture filtrates of *R. secalis* race US238.1 was found to elicit m-RNA accumulation of a thaumatin-like (TL) protein and peroxidase in Rrs1 cultivars. This is the earliest induced defence response of barley to *R. secalis* infection detected thus far.

TL proteins, like peroxidase, are pathogenisis-related proteins believed to be part of the defence response of mono- and dicotyledonous plants (Kerby & Somerville, 1989; Rebmann *et al.*, 1991). The TL protein PRHv-1 was found to be encoded by a gene family located on barley chromosome 1 (Hahn *et al.*, 1993).

The accumulation of PRHv-1 mRNA also seemed to be associated with cultivars (e.g. cultivar Atlas 46) that carry the *Rrs1* gene derived from Turk but peculiarly not in other cultivars reported to carry the *Rrs1* gene. Resistance gene *Rrs1*, which was mapped to chromosome 3 (Dyck & Schaller, 1961a; Bockelmann *et al.*, 1977), is regarded to be part of a complex of closely linked genes (*Rrs1-Rrs3-Rrs4*) (Dyck & Schaller, 1961b; Dyck & Schaller, 1961a; Starling *et al.*, 1971). Alternatively, a multiple allelic series has also been suggested for the *Rrs1* locus (Habgood & Hayes, 1971). Many barley cultivars are reported to carry the resistance factor *Rrs1* (=*Rh*) (Habgood & Hayes, 1971; Shipton, 1974; Beer, 1991). However, it has not been proven that the same gene is present in all of these cultivars. This may explain why cultivars Hudson and Brier which supposedly have the resistant gene *Rrs1* responded weakly to NIP1 as elicitor. Unexpectedly PRHv-1 mRNA also accumulated in the cultivar Kitchen, though to a lesser extent. It may be that this cultivar also has the co-dominant gene *Rrs1*, or that the co-dominant *Rrs9* gene present in Kitchen (Baker & Larter, 1963) may be a *Rrs1* allele (Hahn *et al.*, 1993). It is thought that the plant's basic defence response in susceptible cultivars, not carrying

the resistant *Rrs1* gene (found in Turk), is either delayed or not triggered (Hahn *et al.*, 1993). Consequently, the function of *Rrs1* may be to link cultivar-specific recognition to the basic defence response of the host-susceptible species, barley, upon attack by *R. secalis* (Heath, 1981; Knogge, 1991).

Rohe *et al.* (1995) found that all fungal races avirulent on barley cultivars *Rrs1* resistance genotype carry and express the *nip1* gene and secrete an elicitor-active NIP1 polypeptide. It was demonstrated that NIP1 produced a product of the fungal avirulence gene avrsRrs1 which is complementary to resistance gene *Rrs1* (Rohe *et al.*, 1995). This interaction between *R. secalis* and barley conform to the gene-for-gene hypothesis in which a plant with a particular resistance gene recognises a pathogen by a virulence factor (Flor, 1955; 1971). In the study by Rohe *et al.* (1995) twelve races were analysed for the presence of the *nip1* gene to enable a correlation with their virulence phenotype on Rrs1 plants. Two races had the *nip1* gene and rendered NIP1 proteins inactive as elicitors of defence responses on plants carrying resistance gene *Rrs1* (Rohe *et al.*, 1995). Recognition by the host plant was eluded by either deletion of the encoding gene or alteration of the primary structure of the gene product in these two races.

VIRULENCE SPECTRUM

Rhynchosporium secalis populations from different barley producing countries differ notably in virulence spectra (Table 1). Furthermore, considerable pathogenic variation also occurs among single conidium isolates from the same lesion as between isolates from different lesions collected from the same or different locations (Brown, 1985).

Races are strains defined by host specificity and are also referred to as pathotypes (Yoder *et al.*, 1986). The *R. secalis* population from each barley producing area in the world is comprised of many unique races which differ in their ability to attack different barley cultivars. Race specialisation in *R. secalis* was first demonstrated in Argentina by Sarsola & Campi (1947), and pathogenic variability in *R. secalis* populations has been studied in several countries since then (Table 1). Disease reaction data obtained by inoculating host differentials with fungal isolates often reveal important properties of pathogen populations. Highly variable isolates of

this pathogen have been reported from California, Western Canada, Southern Ontario in Canada, Denmark, and Italy, whereas considerably less variability has been reported from Finland, New Zealand and South-Eastern Australia (Table 1). The data in Table 1 suggests that the UK also has a population with less variation, but recent research has provided evidence for the presence of several more races (A.C. Newton, personal communication). The *R. secalis* population from Finland was the only population with no significant differences in specific pathogenicity (Robinson *et al.*, 1996). However, comparisons between virulence spectra of specific populations should be done with caution, especially as numbers of isolates tested, as well as numbers of differentials often vary considerably (Table 1). The effect of inoculum concentration on host reaction (Jackson & Webster, 1976b), as well as rating schemes employed may also markedly influence the results of such studies. Jackson & Webster (1976b) found that conidium concentrations of 1×10^5 and 2×10^5 conidia/ml, resulted in the clearest differentiation between resistance and susceptibility on all cultivars in the differential host range. Lower concentrations ($1x10^2$, $1x10^3$, $1x10^4$, $5x10^4$ conidia/ml) allowed disease escape of some cultivars that were susceptible at the higher concentration.

Crandall (1987) found that *R. secalis* contains a greater number of virulence genes than resistance genes present in the host. He speculated that selection pressure towards greater virulence might occur from unidentified field resistance in diverse barley genotypes in breeder plots and wild grasses. In a study conducted by Ali & Boyd (1973) the most virulent *R. secalis* isolate proved to be one from *Hordeum leporinum*. The possibility was considered that selection pressures stemming from the extensive hectares of native *Hordeum* species may influence the variability of *R. secalis* in Western Australia (Ali & Boyd, 1973). Brown (1990) was the first to consider the pathogenic variability of isolates from barley grass (*H. leporinum* and *H. murinum*) in South Eastern Australia, where barley grass represents an extensive source of inoculum. Cultivars Hudson and Osiris were resistant to isolates from cultivated barley, but not to barley grass isolates in South Eastern Australia (Brown, 1990). Cultivar C.I. 3515 was the only cultivar resistant to isolates from both barley grass and cultivated barley in South Eastern Australia.

There is much dispute whether the stabilising selection theory (*i.e.* races with unnecessary virulence are less fit) proposed by Vanderplank (1968), apply to the

barley scald complex. Jørgensen & Smedegaard-Petersen (1995) claimed that their data and that of others (Hansen & Magnus, 1973; Ali *et al.*, 1976; Jackson & Webster, 1976b) suggest that the stabilising selection theory does not apply to this pathogen. Other results, however, suggest that the theory does apply to the barley leaf-scald complex (Williams & Owen, 1973; Jackson & Webster, 1976a). Jackson & Webster (1976a) found that the population shifted towards simpler pathogenic races after two disease cycles. These results are in contrast with observed frequencies in a previous study by Jackson & Webster (1976b), which indicated that fitness properties are considered to be characteristics of isolates rather than of races.

The studies of Jørgensen & Smedegaard-Petersen (1995) and others (Caldwell, 1937; Müller, 1954; Dodov, 1964; Kajiwara, 1969) indicate that *R. secalis* has a preference for a particular host species. However, it is also possible that isolates of *R. secalis* from other hosts may adapt to barley and *vice versa* when optimum conditions for the fungus prevails (Kay & Owen, 1973a). Experiments showed that isolates of *R. secalis* from rye (*Secale cereale*), couch grass (*Elymus repens*) and wall barley (*H. murinum*) increased their aggressiveness to barley after serial passages through barley leaves (Kay & Owen, 1973a). Ali & Boyd (1973) examined the pathogenic variability of *R. secalis* isolates on different host genera of the Poaceae and on selected barley cultivars. They found that *R. secalis* did not exhibit strict host specialisation and that much inter- and intra-isolate variability existed in pathogenicity characteristics.

VARIABILITY OF PATHOTYPES

The variable nature of *R. secalis* was first recognised by researchers who studied the pathogenicity and virulence of the barley scald pathogen (Jackson & Webster, 1976a; Ceoloni, 1980; Xue & Hall, 1991; Jørgensen & Smedegaard-Petersen, 1995). Variability in virulence was reported in single spore isolates, isolates from the same lesion and populations. A number of explanations for this variability were given, some of which were proven and others remain speculation.

Investigations on the pathogenic variation among isolates of *R. secalis* populations conflict with each other (Brown, 1985). There are numerous factors that could contribute to these apparent discrepancies. Firstly, the same set of differential cultivars has not been used for each study and resistance genes in each differential

have not been completely determined (Ceoloni, 1980). Thus, as a common seed source is not used, differences could be due to the presence of different resistance genes, even in varieties with the same name. Secondly, environmental conditions could also provide a possible explanation for the differences observed (Owen, 1963; Williams & Owen, 1973). More pathogenicity groups were differentiated under fluctuating temperature conditions compared to race evaluation under standardised conditions (Owen, 1963; Williams & Owen, 1973). There is a possibility that different temperature fluctuations within a season influence the race composition of a population from year to year, especially since race composition of a population has been reported to vary from year to year (Zhang *et al.*, 1992). It has been demonstrated that *R. secalis* isolates reacted differently to temperature regarding lesion development (Salamati & Magnus, 1997). The most aggressive isolate caused severe disease irrespective of temperature (56-70% of the leaf area infected). However, disease severity caused by the least aggressive isolate was significantly higher at optimum temperature compared with a sub-optimum temperature (13°C) (Salamati & Magnus, 1997).

Owen (1963) found that variation in symptom expression occurred between plants of a cultivar in a single pot, and that six of the seven cultivars tested did not give a consistent reaction to 90% of the isolates tested. He also concluded that more consistent results would be obtained if environmental conditions during incubation were better controlled. In Owen's experiment, plants were moved to a corridor where the temperature varied from 15°C to 25°C after being inoculated and kept for 48 h within a Perspex chamber at approximately 17°C. Seedlings in the corridor were subjected to daily maximum temperatures of 25-37°C, and minima of 14-21°C. This post-inoculation environment (in the corridor) caused chlorosis leading to necrosis of all variety seedlings tested and especially effected Modoc and Osiris. As a result of unclear and irregular varietal reactions, distinct physiologic races could not be designated (Owen, 1963).

Williams & Owen (1973) aimed in a subsequent study to eliminate uncontrolled variables and to develop techniques for clear, reproducible varietal reactions. An improved experiment was therefore conducted in a controlled environment (17°C). Despite these improvements many seedlings of Atlas, Modoc, Perfect, Trebi and West China developed symptoms which varied from small water-

soaked areas at leaf edges to extensive chlorosis (>20% leaf area). These manifestations may have been caused by a high level of background resistance, or it may have been resistant reactions characteristic for a particular gene (Williams & Owen, 1973). The authors furthermore suggested that resistance in Atlas, Modoc and West China was unstable or that the seed used in the study was not uniform.

Zhang *et al.* (1987), however, pointed out that many studies (Jackson *et al.*, 1978; Muona, 1980; Jackson *et al.*, 1982; Webster *et al.*, 1986) obtained highly repeatable results by using the inoculation and incubation method described by Jackson & Webster (1976b). In the latter method, barley plants were grown in a greenhouse maintained at 15-25°C and inoculated at the one and a half to three leaf stage. A day prior to inoculation, plants were placed in a controlled environment chamber maintained at 15°C. A conidial suspension adjusted to 2 x 10^5 conidia/ml was sprayed onto 70 plants with a DeVilbiss adjustable tip atomiser attached to an air hose. The inoculum was allowed to dry on plants for a period of 3 h. Test plants were transferred to a controlled environment chamber with 100% relative humidity. After 48 h plants were returned to the greenhouse. Symptom expression on inoculated barley plants was not influenced by the variation in temperature (15-25°C) in the greenhouse (Jackson & Webster, 1976b).

However, Jackson & Webster (1976a) observed high levels of intra-isolate variation. After being passed through a susceptible cultivar, four out of 12 single-spore isolates differed in virulence from their parent isolate on two or three of the 14 barley differentials. Jackson & Webster (1976a) speculated that there must be some unknown mechanism of recombination and segregation. Hansen & Magnus (1973) also found high levels of intra-isolate variation in virulence of single-spore *R. secalis* isolates. Ali & Boyd (1973) found that intra-isolate variability depended on the host-isolate combination and the conditions of the experiment. Hansen & Magnus (1973) thought it highly unlikely that environmental conditions played an important role in the display of such great variation in virulence. They speculated that the variation must be explained in terms of segregation of virulence genes. Hence, they proposed that an isolate must be considered as a population carrying virulence genes in which mutation, selection and recombination are operating. Great variability in pathogenicity to certain rice cultivars were also noted in subcultures of uninucleate

conidia arising from single-spore cultures of *Magnaporthe grisea*, the rice blast fungus (Giatong & Frederiksen, 1969).

SOURCES OF VARIATION - MECHANISMS INVOLVED IN ASEXUAL REPRODUCTION

The versatility found in asexual filamentous fungi has been mainly attributed to mutation, heterokaryosis, chromosomal polymorphisms and transposable genetic elements (Caten & Jinks, 1966; Hansen & Magnus, 1973; Kinsey & Helber, 1989; McDonald & Martinez, 1991; Kistler & Miao, 1992; Goodwin *et al.*, 1994).

Rhynchosporium secalis is able to recombine asexually with considerable frequency, despite the apparent absence of sexual reproduction. This may be explained in terms of segregation of virulence genes due to mutation, migration, selection or possibly parasexual recombination (Hansen & Magnus, 1973; Goodwin *et al.*, 1994). Newman & Owen (1985) inoculated barley plants with a mixture of two *R. secalis* isolates, each characterised by an isozyme profile, and found novel combinations of isozyme patterns in their progeny. This indicated that some form of asexual recombination had occurred which he speculated may be a result of either the parasexual cycle or cytoplasmic modifier genes. However, results obtained by Goodwin *et al.* (1994) indicated that the parasexual cycle, probably at most plays a minor role in generating genetic variability in *R. secalis* populations in nature. Mutation and migration seem to be important sources of variation but the mutation rate for various traits can differ, e.g. the mutation rate for pathogenicity appeared to be much higher than that for isozymes (Goodwin *et al.*, 1994).

Heterokaryosis followed by fusion of two dissimilar nuclei to produce a heterozygous diploid is implicit in the parasexual cycle. Genetic recombination in which there is no fine co-ordination between recombination, segregation and reduction, as there is in meiosis, has been termed parasexual recombination (Tinline & MacNeill, 1969). Heterokaryosis takes place after anastomosis and the condition wherein genetically unlike nuclei occupy a common cytoplasm has been termed heterokaryosis. Anastomosis of hyphae will be dependent on the vegetative compatibility of different strains. It has been found that where incompatibility occurs in homothallic imperfect fungi, it is restricted to gene exchange through

heterokaryosis (Caten & Jinks, 1966). During the isolation and regeneration of *R. secalis* protoplasts, 90-100% were uninucleate when obtained from conidia, and 70-80% uninucleate when obtained from mycelium (Martinez-Espinoza & Sands, 1995). The remaining protoplasts had two nuclei. This could be an indication of the chances (20-30%) of heterokaryon formation in mycelium. However, it was not clear whether genetically different nuclei occupied a common cytoplasm (Martinez-Espinoza & Sands, 1995).

Studies of electrophoretic karyotypes of plant pathogenic fungi indicated that chromosomal polymorphisms is another potential source of genetic variation, and may take the form of variation in chromosome size and/or number (McDonald & Martinez, 1991; Kistler & Miao, 1992). Kistler & Miao (1992) suggested that the occurrence and extent of chromosomal polymorphism is inversely correlated to the frequency of meiosis. Imperfect fungi are more likely to have extensive chromosomal polymorphisms than fungi that carry out meiosis, because meiosis is the process that selects against many aberrations that leads to detectable chromosome polymorphism (e.g. deletion, reciprocal translocation, aneuploidy, etc.) (Kistler & Miao, 1992).

Filamentous fungi also have transposable genetic elements which may be a source of variation (Kinsey & Helber, 1989). Transposons can be defined as specialised DNA sequences which can jump or transpose on to any DNA molecule in the same cell. Fungal transposons may be transmitted between nuclei of a heterokaryon by vegetative transmission through a population of nuclei.

McDermott *et al.* (1989) analysed 163 *R. secalis* isolates, collected from two experimental barley populations in California, for variations in four isozymes. High gametic disequilibrium values were obtained based on gene frequencies of isozyme loci, providing no evidence of genetic recombination. Therefore, instead of recombination, it was suggested that some form of balancing selection, which causes fitness to vary with genotypic frequencies, was responsible for the maintenance of variation in *R. secalis* (McDermott *et al.*, 1989). However, comparison of frequencies of alleles of another isozyme study showed little association with the allelic state at other loci which pointed to the presence of a sexual cycle in the Australian population of *R. secalis* (Burdon *et al.*, 1994). No sexual stage for *R. secalis* has been reported but effects of a very elusive teleomorph were evident.

HOST RESISTANCE

Many barley varieties and lines with scald resistance have been reported but little is known about their genetic basis. Most of the known resistance genes are jointly carried in 24 differential barley cultivars and were summarised by Goodwin *et al.* (1990). Riddle & Briggs (1950) were the first to identify a single dominant gene in La Mesita and its derivatives, Trebi and Modoc. In addition, the derivatives possessed a recessive gene for resistance. Turk carries two genes, of which one gene is allelic to a common gene in La Mesita and its derivatives. Bryner (1957) reported the resistance in Brier which was conditioned by a single dominant gene. Four genes, designated as *Rh2, Rh3, Rh4* and *Rh5* were identified, of which *Rh2* and *Rh5* were independent of each other while a very close linkage existed between *Rh3* and *Rh4* (Dyck & Schaller, 1961b; Dyck & Schaller, 1961a). $Rh4^2$ appeared to be allelic to the *Rh4* locus and *Rh* appeared to be allelic to both *Rh3* and *Rh4*. Together these loci are referred to as the *Rh-Rh3-Rh4* locus complex on chromosome 3. However, Habgood & Hayes (1971) suggested the possibility of a multiple allelic series instead of a tightly linked locus complex.

Considerable variation in pathogenicity was found among Western Australian isolates of *R. secalis* when tested in winter and summer against a differential set of barley cultivars (Ali & Boyd, 1973). Only 10 out of 27 barley genotypes evaluated gave the same disease reaction in summer as well as in winter, and eight of these ten genotypes were resistant to all the isolates tested. The eight resistant genotypes were cultivars Atlas 46, Atlas 57, Hudson, Psaknon, Osiris, Sultan, Turk and Trebi (Ali & Boyd, 1973). It is also known that some resistance genes have certain temperature thresholds, for example, the effectiveness of the recessive genes *rh6* and *rh7* identified by Baker & Larter (1963) was impaired above 25°C.

Races from various *R. secalis* populations in the world revealed differences in the resistance of cultivars previously thought to carry identical resistance genes. In California 18 races of *R. secalis* differed in their ability to attack La Mesita and Osiris which have the *Rh4* gene in common (Jackson & Webster, 1976b). Similary, Brier and Hudson also differed in susceptibility towards 11 races, though they had the *Rh* gene in common (Jackson & Webster, 1976b). Italian isolates of *R. secalis* were also able to differentiate between the latter two cultivars (Brier and Hudson) previously

assumed to have identical resistance factors (Ceoloni, 1980). Brown (1990) reported differences regarding resistance genes previously described for Hudson, Brier, Atlas 46, C.I. 3515 and Osiris, when inoculated with *R. secalis* isolates of South Eastern Australia. Therefore, apparent genetic variability among differentials, even within individual cultivars, influence the outcome of studies on virulence spectra. The authors explain these differences as either unrecognised differences in the genes described or the presence of previously undetected genes for resistance.

Nevertheless, on comparing host resistance studies summarised in Table 1, the cultivar Atlas 46 could be singled out as the cultivar resistant to *R. secalis* populations in Norway, Western Australia, Italy, Victoria Australia, New Zealand, South Eastern Australia & Southern Ontario. The cultivar Atlas 46 was introduced in California during 1947, at which time the cultivar was considered resistant. Atlas 46 was infected at several localities in California in 1953 and was found to be extremely susceptible in all parts of the state by 1956 (Houston & Ashtworth, 1957). Virulence tests carried out in California against Atlas 46 with 1973 isolates of *R. secalis* showed susceptible reactions against 26 races representing 39% of the sample (Jackson & Webster, 1976b), which indicated that *R. secalis* can overcome race-specific resistance.

Hansen & Magnus (1973) did not detect any virulence associations between different host differentials among isolates of *R. secalis* collected throughout Norway. However, Zhang *et al.* (1992), using almost the same set of host differentials, found a close association of virulence genes in isolates collected in California, even in isolates collected eleven years apart. Zhang *et al.* (1992) favoured the hypothesis that the highly conserved virulence associations reflect certain virulence gene combinations maintained by natural selection. Furthermore, asexual recombinants can be readily recovered in glasshouse studies (Jackson & Webster, 1976a; Newman & Owen, 1985) and asexual reproduction in the absence of selection would not be sufficient to hold the genotype together (Zhang *et al.*, 1992).

Promising sources of resistance to *R. secalis* in *H. vulgare* accessions from Turkey have been identified and characterised by identification of isolate specific resistance (Penner *et al.*, 1998).

BREEDING STRATEGIES

Race specific resistance may be short-lived in view of genetic variation and the capacity for virulence variability in the pathogen population over a small area (Xue & Hall, 1991; McDonald *et al.*, 1999). Accordingly, a highly variable *R. secalis* population as found in Canada, may require breeding strategies that rely on the incorporation of more than one single major gene (Tekauz, 1991). None of the Canadian *R. secalis* isolates tested was virulent on both Atlas 46 and C.I. 3515. Therefore, a combination of their resistance genes *Rh2*, *Rh3* and *Rh10* might provide effective protection against the *R. secalis* population in Canada. As a result of the considerable variation in virulence, a strategy which involves general or non-specific resistance was considered more valuable for lasting resistance against the Western Australian population of *R. secalis* (Ali & Boyd, 1973).

Change of seedling to adult-plant resistance is another factor to take into account when breeding for resistance. Eight barley cultivars were susceptible as seedlings but resistant or moderately resistant as adult-plants when inoculated with a highly virulent *R. secalis* isolate collected in Alberta, Canada (Xue *et al.*, 1995). These cultivars were Leduc, Tukwa, Ac Lacombe, Bridge, Brier, HBB323, Noble and Phoenix. Bonanza and Klages were susceptible as seedlings and remained susceptible as adult-plants. Furthermore, no genotypes resistant as seedlings and susceptible as adult-plants were found (Xue *et al.*, 1995). According to Xue *et al.* (1995), the seedling test should be used in breeding programmes to develop qualitative resistance (where a specific gene, control resistance). Adult-plant reactions and quantitative resistance present in genotypes must be evaluated under field conditions since environmental conditions may influence resistance.

MOLECULAR CHARACTERISATION

Two hundred and eighty-eight isolates of *R. secalis* from the UK were characterised by isozymes, providing a convenient natural marker system with which to study asexual recombination (Newman, 1985). Much variation was found amongst α-esterase patterns of *R. secalis* isolates, but no correlation with geographical origins within the UK were reported. α-Esterase bands 8 and 9 formed the only allozyme

polymorphism that could be identified in the *R. secalis* population which also appeared to be a stable polymorphism and seemed to follow natural selection.

Classification of 163 isolates for four putative isozyme systems, a colony colour dimorphism and 20 ribosomal DNA restriction fragment length variants revealed 49 different multilocus haplotypes (McDermott *et al.*, 1989). rDNA RFLP analysis produced 29 different patterns among the 163 isolates. In contrast only two electromorphs were detected for each of the four enzymes (phosphoglucoisomerase, phosphoglucomutase, leucine aminopeptidase, β-glucosidase) tested, and only two colony-colour morphs (black and cream) were found on PDA.

Rhynchosporium secalis populations were also compared on a global format (Goodwin *et al.*, 1993). Electrophoretic phenotypes of 150 *R. secalis* isolates originating from Australia, Europe, USA and the United Kingdom were determined for each of the 8 enzymes (Goodwin *et al.*, 1993). In a test of 54 enzyme systems the enzymes aconitate hydratase, catalase, leucine aminopeptidase, glucose-6-phosphate dehydrogenase, β-glucosidase, hexokinase and phosphoglucomutase gave the clearest and most consistent bands. No overall geographical pattern could be correlated to the variability in allele frequencies found in the eight electrophoretic loci. However, after cluster analysis on allele frequencies, which was then converted to genetic distance, some smaller groupings were detected based on geographical location. Populations of *R. secalis* in New South Wales and Victoria grouped together, and populations from Idaho and Montana grouped closely with that from Oregon. The percentage of polymorphic loci ranged from 0% in Pennsylvania to 75% in Norway. The lowest multilocus diversity and lowest allelic diversity were found in Australia but no genetic differentiation between isolates collected from *H. leporinum* and *H. vulgare* were observed. Distinct differences among geographically separated *R. secalis* populations were observed and high levels of genetic variation were maintained in many locations (Goodwin *et al.*, 1993). Goodwin *et al.* (1993) concluded that *R. secalis* populations in Australia, Europe and the USA had allelic and multilocus diversities which were generally high compared to most fungi.

A survey of electrophoretic variation in 89 isolates of *R. secalis* collected from cultivated and wild barley grass in Victoria, New South Wales and Tasmania detected 37 distinct multi-locus isozyme phenotypes (Burdon *et al.*, 1994). An average of 2.5 alleles at each of 11 loci were detected in 5 enzyme systems (Burdon *et al.*, 1994). At

five loci, two alleles each occurred at frequencies exceeding 0.2. Comparisons of the frequencies of alleles at these loci showed little association with host or pathogenicity of the isolates.

McDonald *et al.* (1999) was the first to use anonymous RFLP markers to examine the genetic structure of *R. secalis* populations. The study showed that Australian populations of *R. secalis* were highly variable and that the great majority of genetic variation was distributed within fields on a fine spatial scale (McDonald *et al.*, 1999). Among the 265 fungal isolates analysed, 214 distinct genotypes were identified. Average genotype diversity (genotype diversity calculated according to Stoddart & Taylor (1988) within a population was 65% of its theoretical maximum and the genotype diversity within five populations studied varied from 45-97%.

FUNGICIDE SENSITIVITY

Imidazole and triazole fungicides inhibit the C14 demethylation step in fungal ergosterol biosynthesis and are called demethylation inhibitors (DMIs) (Copping *et al.*, 1984). Terms such as "sterol inhibitors" (SIs), "ergosterol biosynthesis inhibitors" (EBIs), or "sterol biosynthesis inhibitors" (SBIs) describe the broad group of inhibitors of sterol demethylation with DMIs being the most important subgroup of SBIs. DMIs belong to the group of site-specific fungicides, which are in general more prone to resistance than multi-site inhibitors (Dekker, 1985). In spite of a single-site mode of action, the development of pathogen populations resistant to DMIs has been relatively slow. Development of resistance to DMIs was also different from the experience with some earlier site-specific fungicides such as the benzimidazoles or phenylamides. There is increasing evidence that the difference in resistance development can be explained by different types of selection leading to the build-up of resistant subpopulations, which might proceed through a disruptive selection (qualitative response) or a directional selection (quantitative response). Words such as "directional", "quantitative", "multi-step" or "continuous" are used to describe fungicide resistance that develops gradually. In contrast, a sudden loss of fungicide effectiveness as well as clear-cut sensitive and resistant populations with widely differing response, is referred to as "disruptive", "qualitative", "single-step" or "discontinuous" resistance. Directional selection is most likely responsible for

resistance development to DMIs (Köller & Scheinpflug, 1987; Skylakakis, 1987). Field isolates resistant to triadimenol have been described for *Erysiphe graminis* (Butters *et al.*, 1984; De Waard *et al.*, 1986), *Pyrenophora teres* (Sheridan *et al.*, 1985) and *R. secalis* (Kendall *et al.*, 1993). Triadimenol sensitivity ranged from sensitive to resistant for *E. graminis* f.sp. *hordei*, and *P. teres* with ED_{50} values 0.002-1.4 mg/l and 2-25 mg/l respectively. *R. secalis* collected from a field where triadimenol sprays were no longer effective had a mean MIC value of 42.3 µg/ml.

Cross-resistance studies are a useful tool to assess the risk of resistance development against fungicides. Cross-resistance to fungicides has been defined as resistance to two or more fungicides as a result of the same genetic factor (Georgopoulos, 1977). Cross-resistance can be determined by correlation analysis, and a significant positive correlation between two fungicide sensitivities indicates cross-resistance. Fungal genes may control resistance to one or two fungicides and depending on the frequency of genes conveying resistance, the fungicide management strategy will be influenced. Correlation coefficients may therefore change and it has been reported that correlation coefficients regarding resistance to DMIs differed between *P. teres* populations (Peever & Milgroom, 1993).

At a time when DMI fungicides were being introduced in the UK, an *in vitro* assay of 30 isolates of *R. secalis* isolates collected during 1975 to 1981 was conducted to determine the variation in sensitivity to triadimenol (Hollomon, 1984). Minimum inhibitory concentrations (MICs) of triadimenol varied from 0.2-3.2 µg/ml (Hollomon, 1984). Eleven of the isolates collected in 1981 had previously been subjected to fungicides (triadimenol, fuberidazole, triadimefon or prochloraz). However, the latter isolates did not differ in triadimenol sensitivity from isolates collected at untreated sites and those collected before the widespread use of these triazoles. Although somewhat limited in scope, these results provided the first indication of the variation in fungicide sensitivity of *R. secalis* to triadimenol in the UK. Other DMI sensitivity surveys in the UK were conducted by Jones (1990) with *R. secalis* isolates collected in 1987 and 1989. In 1987, the *in vitro* sensitivity of 464 *R. secalis* isolates (collected from 95 random selected farms), ranged from 0.8-12.8 mg/l for triadimenol, and 0.008-1.0 mg/l for propiconazole. In 1989, the sensitivity of 151 *R. secalis* isolates (collected from 13 of 95 farms), ranged from 0.08-51.2 mg/l for triadimenol and 0.05-1.6 mg/l for propiconazole. According to the proportion of

isolates with a 0.2 mg/l MIC, it was concluded that a reduction in sensitivity to propiconazole occurred between 1987 and 1989. These results were compared with the MIC values determined by Hollomon (1984), where one out of 30 *R. secalis* isolates tested in that particular study had a MIC of 3.2 mg/l. The MIC values of triadimenol in 1987 were 6.4 mg/l or higher for 46% of the 464 isolates in the sample. Jones (1990) concluded that the population had become less sensitive to triadimenol since the 1987 and 1981 surveys.

During 1987-1990, Kendall *et al.* (1993) did *in vitro* fungicide sensitivity tests on 2000 *R. secalis* isolates from the UK. The average MIC value for triadimenol was 5.23 µg/ml in 1987 and 40.16 µg/ml in 1990. A distinctly bimodal population distribution regarding triadimenol sensitivity was observed in 1987, which pointed to a disruptive selection unlike the directional selection usually described for DMIs (Kendall *et al.*, 1993). Bimodal and unimodal distributions (of the degree of sensitivity) are respectively characteristic of the disruptive and directional patterns of resistance development (Brent, 1995). By 1990 the wild-type population had largely been replaced by a less sensitive population. From 1986 onwards nearly 2000 isolates were also assayed for propiconazole sensitivity. Little change occurred before 1988, but by 1990 the mean sensitivity had declined nearly eightfold ($P = 0.001$). Unlike triadimenol, changes in propiconazole sensitivity involved a gradual shift of a unimodal population (Kendall *et al.*, 1993).

Benzimidazole fungicides (methyl-benzimidazole-carbamate-generating [MBC] fungicides) such as carbendazim, act by affecting tubulin synthesis (Davidse, 1973). MBC resistance has been reported in cereal pathogens such as *Tapesia yallundae* and *Septoria tritici* (Griffin & Fisher, 1985; King & Griffin, 1985). MBC-resistant field strains of *R. secalis* were first detected in 1990, 15 years after benzimidazole fungicides were first used in UK barley crops. The MBC-resistant field strains were not less fit than wild-type ones, and must have played an important role in the build-up of MBC-resistance (Kendall *et al.*, 1994). Strains of *R. secalis* resistant to carbendazim have also been found in crops of winter barley in England, Wales and Northern Ireland during 1992 and 1993 (Phillips & Locke, 1994; Taggart *et al.*, 1994). Carbendazim resistance was particularly prevalent in wetter regions, which are more conducive to proliferation of *R. secalis* (Taggart *et al.*, 1999). As applications of carbendazim were increased, it was found that a higher correlation

between frequency of carbendazim resistance and disease control emerged (Taggart *et al.*, 1999). The effects of fungicides used to control *R. secalis* in winter barley were investigated at sites in Northern Ireland where benzimidazole resistance was present in *R. secalis* populations. Carbendazim contributed little to disease control whereas propiconazole-containing treatments performed well (Taggart *et al.*, 1998). Treatments containing carbendazim, either alone or in mixture, caused an increase in the proportion of carbendazim-resistant isolates within *R. secalis* populations (Taggart *et al.*, 1998).

Effective disease control in the field largely depends on the extent of the population shift towards resistance and may or may not be affected (Köller & Scheinpflug, 1987; Skylakakis, 1987). It is generally accepted today that a small population of resistant genotypes already exists in the field before the first application of a fungicide. This resistant subpopulation increases under the pressure of the fungicide rather than being generated by mutagenic action of the compound itself (Köller & Scheinpflug, 1987). *R. secalis* isolates collected from fungicide treated field plots were subjected to four transfers through fungicide sprayed barley plants in the greenhouse (Hunter *et al.*, 1986). Lesions of *R. secalis* developed on plants sprayed with 1000 mg/l carbendazim or triadimenol and 10 mg/l prochloraz or propiconazole. The decreased sensitivity to fungicides were maintained after five transfers through unsprayed plants. The latter isolates also retained normal pathogenicity as well as an ability to grow and sporulate *in vitro* and *in vivo*. However, conclusions derived from such successive transfers should be treated with great care since these experiments only represent a small segment of genotypes present in the field (Köller, 1988).

Recently, a new class of fungicide has been introduced, namely the strobilurins, of which kresoxim-methyl is a member. This fungicide was found active against the barley scald fungus (Jensen *et al.*, 1995). Kresoxim-methyl has both protective and curative qualities and inhibits mitochondrial electron transport at the site of the bc_1 complex. Kresoxim-methyl can be described as a quasi-systemic fungicide since the active ingredient mainly spreads out over the leaf surface by the diffusion of gasses. Leaf-penetration of kresoxim-methyl takes place through the stomata as well as through the wax layer and cuticle. The fungicide is especially effective against fungi that primarily colonise the epidermal cell layers. A slow

uptake of the fungicide and low vapour pressure are the main reasons for it having a prolonged effectiveness. Kresoxim-methyl has not yet been registered for use against *R. secalis* in South Africa but it would be advisable to determine the baseline sensitivity of the local *R. secalis* population before its introduction and application. This would be very useful to determine shifts in fungicide sensitivity in future.

REFERENCES

Ali, S. M. (1981). Barley grass as a source of pathogenic variation in *Rhynchosporium secalis*. *Australian Journal of Agricultural Research* **32**, 21-25.

Ali, S. M. & Boyd, W. J. R. (1973). Host range and physiologic specialisation in *Rhynchosporium secalis*. *Australian Journal of Agricultural Research* **25**, 21-31.

Ali, S. M., Mayfield, A. H. & Clare, B. G. (1976). Pathogenicity of 203 isolates of *Rhynchosporium secalis* on 21 barley cultivars. *Physiological Plant Pathology* **9**, 135-143.

Amelung, D. & Beer, W. W. (1984). Symptomatik der Rhynchosporium-Blattfleckenkrankheit an Gerste und ihre Differenzierung von anderen, ähnlichen Befallsbildern. *Nachrichtenblatt fuer den Deutschen Pflanzenschutzdienst in der DDR* **38**, 180.

Arai, M. (1996). Brown necrosis symptoms on nodal portions of barley infected with *Rhynchosporium secalis*. *Annals of the Phytopathological Society of Japan* **63**, 254-257.

Auriol, P., Mazars, C. & Rafenomamanjara, D. (1984). Approches biochimiques du role des rhynchosporosides dans la rhynchosporiose de l'orge (*Rhynchosporium secalis*). *Agronomie* **4**, 689-690.

Auriol, P., Strobel, G., Beltran, P. J. & Gray, G. (1978). Rhynchosporoside, a host selective toxin produced by *Rhynchosporium secalis*, the causal agent of scald disease of barley. *Proceedings of the National Academy of Sciences U.S.A.* **75**, 4339-4343.

Ayesu-Offei, E. N. & Carter, M. V. (1971). Epidemiology of leaf scald of barley. *Australian Journal of Agricultural Research* **22**, 383-390.

Ayesu-Offei, E. N. & Claire, B. G. (1971). Symptoms of scald disease induced by toxic metabolites of *Rhynchosporium secalis*. *Australian Journal of Biological Sciences* **24**, 169-174.

Ayesu-Offei, E. N. & Clare, B. G. (1970). Processes in the infection of barley leaves by *Rhynchosporium secalis*. *Australian Journal of Biological Sciences* **23**, 299-307.

Baker, R. J. & Larter, E. N. (1963). The inheritance of scald resistance in barley. *Canadian Journal of Genetics and Cytology* **5**, 445-449.

Bartels, F. (1928). Studien über *Marssonina graminicola* und der Immunität im Pflanzenbereich. *Phytopathologische Zeitschrift* **1**, 73-114.

Beer, W. W. (1988). Investigations on the disease progress in the winter barley/leaf blotch (*Rhynchosporium secalis*) system. *Zeitschrift fuer Pflanzenkrankheiten und Pflansenschutz* **95**, 16-24.

Beer, W. W. (1991). Leaf blotch of barley (*Rhynchosporium secalis*). *Zentralblatt fuer Mikrobiologie* **146**, 339-358.

Beltran, J. P. & Strobel, G. (1978). Rhynchosporoside-binding proteins of barley. *FEBS Letters* **96**, 34-36.

Bockelmann, H. E., Sharp, E. L. & Eslick, R. F. (1977). Trisomic analysis of genes for resistance to scald and net blotch in several barley cultivars. *Canadian Journal of Botany* **55**, 2142-2148.

Brent, K. (1995). *Fungicide resistance in crop pathogens: How can it be managed?* Groupement International des Associations Nationales de Fabricants de Produits Agrochimiques, Brussels.

Brown, A. G. P. (1979). Barley diseases in Western Australia. 1. Field experiments and surveys, 1971-1975. *Barley Diseases in Australasia*, 1-10.

Brown, J. S. (1985). Pathogenic variation among isolates of *Rhynchosporium secalis* from cultivated barley growing in Victoria Australia. *Euphytica* **34**, 129-133.

Brown, J. S. (1990). Pathogenic variation among isolates of *Rhynchosporium secalis* from barley grass growing in South Eastern Australia. *Euphytica* **50**, 81-89.

Bryner, C. S. (1957). Inheritance of scald resistance in barley. *Dissertation Abstracts* **27**, 2752.

Burdon, J. J., Abbott, D. C., Brown, A. H. D. & Brown, J. S. (1994). Genetic structure of the scald pathogen (*Rhynchosporium secalis*) in South East Australia: Implications for control strategies. *Australian Journal of Agricultural Research* **45**, 1445-1454.

Butters, J., Clark, J. & Hollomon, D. W. (1984). Resistance to inhibitors of sterol biosynthesis in barley powdery mildew. *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* **49**, 143-51.

Caldwell, R. M. (1931). Host specialisation and parasitism of the genus *Rhynchosporium*. *Phytopathology* **21**, 109-110.

Caldwell, R. M. (1937). *Rhynchosporium* scald of barley, rye and other grasses. *Journal of Agricultural Research* **55**, 175-198.

Caten, C. E. & Jinks, J. L. (1966). Heterokaryosis: Its significance in wild homothallic ascomycetes and fungi imperfecti. *Transactions of the British Mycological Society* **49**, 81-93.

Ceoloni, L. (1980). Race differentiation and search for sources of resistance to *Rhynchosporium secalis* in barley in Italy. *Euphytica* **29**, 547-553.

Copping, L. G., Birchmore, R. J., Wright, K. & Godson, D. H. (1984). Structure-activity relationships in a group of imidazole-1-carboxamides. *Pesticide Science* **15**, 280-284.

Couture, L. & Isfan, D. (1986). Effet de la fertilisation azotee sur le development de la rhynchosporiose de l'orge. *Canadian Journal of Plant Science* **66**, 795-799.

Crandall, B. A. (1987). *Studies on the interaction of Rhynchosporium secalis on barley composite cross populations and pure line cultivars from California and Montana*. Ph.D., University of California Davis.

Cromey, M. G. (1987). Pathogenic variation in *Rhynchosporium secalis* on barley in New Zealand. *Journal of Agricultural Research* **30**, 95-99.

Cromey, M. G. & Mulholland, R. I. (1987). Host specialisation of *Rhynchosporium secalis* in New Zealand. *Journal of Agricultural Research* **30**, 345-348.

Davidse, L. C. (1973). Anti-mitotic activity of methyl benzimidazole-2-yl-carbamate (MBC) in *Aspergillus nidulans*. *Pesticide Biochemistry and Physiology* **3**, 317.

Davis, H. & Fitt, B. D. L. (1992). Seasonal changes in primary and secondary inoculum during epidemics of leaf blotch (*Rhynchosporium secalis*) on winter barley. *Annals of Applied Biology* **121**, 39-49.

De Waard, M. A., Kipp, E. M. C., Hom, N. M. & Van Nistelrooy, J. G. M. (1986). Variation in sensitivity to fungicides which inhibit ergosterol biosynthesis in wheat powdery mildew. *Netherlands Journal of Plant Pathology* **92**, 21-32.

Dekker, J. (1985). The development of resistance to fungicides. In *Progress in Pesticide Biochemistry* (eds. D. H. Hutson & T. R. Roberts). Vol. 4, pp. 165-218. John Wiley & Sons, New York.

Dodov, D. N. (1964). Immunitctni prouchvaniya pri bolestta listen prigor po Echcminka - *Rhynchosporium secalis* (Oudem) Davis. *RAM* **43**, 248.

Doling, D. A. (1964). Leaf blotch of barley. *Agriculture* **71**, 412-414.

Drapatyi, N. A. (1973). Rinkhosporioz jacmenja. *Zašcita Rastenii (Soviet Union)* **12**, 18-19.

Drapatyi, N. A. (1978). *Rinkhosporioz (okajmlennaja pjatnistost') jacmenja v. uslovijakh severno-zapadnykh rajonov ukrainy*. Ph.D., Ministry of Agriculture of the USSR, Agricultural Institute "V.V. Dokucaev".

Dyck, P. L. & Schaller, C. W. (1961a). Association of two genes for scald resistance with specific barley chromosomes. *Canadian Journal of Genetic Cytology* **3**, 165-169.

Dyck, P. L. & Schaller, C. W. (1961b). Inheritance of resistance in barley to several physiologic races of the scald fungus. *Canadian Journal of Genetic Cytology* **3**, 153-164.

Evans, S. G. (1969). Observations of the development of leaf blotch and net blotch of barley from barley debris, 1968. *Plant Pathology* **18**, 116-118.

Fitt, B. D. L., Creighton, N. F., Lacey, M. E. & McCartney, H. A. (1986). Effects of rainfall intensity and duration on dispersal of *Rhynchosporium secalis* conidia from infected barley leaves. *Transactions of the British Mycological Society* **86**, 611-618.

Fitt, B. D. L., McCartney, H. A., Creighton, N. F., Lacey, M. E. & Walklate, P. J. (1988). Dispersal of *Rhynchosporium secalis* conidia from infected barley leaves or straw by simulated rain. *Annual Applied Biology* **112**, 49-59.

Flor, H. H. (1955). Host-parasite interaction in flax rust - Its genetics and other implications. *Phytopathology* **45**, 680-685.

Flor, H. H. (1971). Current status of the gene-for-gene concept. *Annual Review of Phytopathology* **9**, 275-296.

Fowler, A. M. & Owen, H. (1971). Studies on leaf blotch of barley (*Rhynchosporium secalis*). *Transactions of the British Mycological Society* **56**, 137-152.

Georgopoulos, S. G. (1977). Development of fungal resistance to fungicides. In *Antifungal Compounds* (eds. M. R. Siegel & H. D. Sisler). Vol. 2, pp. 439-495. Marcel Dekker, New York.

Giatong, P. & Frederiksen, R. A. (1969). Pathogenic variability and cytology of monoconidial subcultures of *Pyricularia oryzae*. *Phytopathology* **59**, 1152-1157.

Goodwin, S. B., Allard, R. W. & Webster, R. K. (1990). A nomenclature for *Rhynchosporium secalis* pathotypes. *Phytopathology* **80**, 1330-1337.

Goodwin, S. B., Saghai-Maroof, M. A., Allard, R. W. & Webster, R. K. (1993). Isozyme variation within and among populations of *Rhynchosporium secalis* in Europe, Australia and the United States. *Mycological Research* **97**, 49-58.

Goodwin, S. B., Webster, R. K. & Allard, R. W. (1994). Evidence for mutation and migration as sources of genetic variation in populations of *Rhynchosporium secalis*. *Genetics* **84**, 1047-1053.

Griffin, M. J. & Fisher, N. (1985). Laboratory studies on benzimidazole resistance in *Septoria tritici*. *EPPO Bulletin* **15**, 505-511.

Habgood, R. M. & Hayes, J. D. (1971). The inheritance of resistance to *Rhynchosporium secalis* in barley. *Heredity* **27**, 25-37.

Hahn, M., Jungling, S. & Knogge, W. (1993). Cultivar specific elicitation of barley defence reactions by the phytotoxic peptide NIP1 from *Rhynchosporium secalis*. *Molecular Plant- Microbe Interactions* **6**, 745-754.

Hansen, L. R. & Magnus, H. A. (1973). Virulence spectrum of *Rhynchosporium secalis* in Norway and sources of resistance in barley. *Phytopathologische Zeitschrift* **76**, 303-313.

Hawksworth, D. L., Kirk, B. C., Sutton, B. C. & Pegler, D. N. (1995). *Ainsworth & Bisby's Dictionary of the Fungi.* 8th ed. CAB International, Cambridge.

Heath, M. C. (1981). A generalised concept of host-parasite specificity. *Phytopathology* **71**, 1121-1123.

Hollomon, D. W. (1984). A laboratory assay to determine the sensitivity of *Rhynchosporium secalis* to the fungicide triadimenol. *Plant Pathology* **33**, 65-70.

Hoseman, D. & Branchard, M. (1985). Étude *in vitro* de la Rhynchosporiose de l'orge: Cycle de Reproductio du Parasite - Histopathologie de l'hôte. *Phytopathologische Zeitschrift* **112**, 127-142.

Houston, B. R. & Ashtworth, L. J. J. (1957). Newly determined races of the barley scald fungus in California. *Abstract in Phytopathology* **47**, 525.

Howlett, S. G. & Cooke, B. M. (1987). Scanning electron microscopy of sporulation in *Rhynchosporium secalis*. *Transactions of the British Mycological Society* **88**, 547-577.

Hunter, T., Jordan, V. W. L. & Kendall, S. S. (1986). Fungicide sensitivity changes in *Rhynchosporium secalis* in glasshouse experiments. *British Crop Protection Conference - Pests and Diseases*, 523-530.

Jackson, L. F., Kahler, A. L., Webster, R. K. & Allard, R. W. (1978). Conservation of scald resistance in barley composite cross populations. *Phytopathology* **68**, 645-650.

Jackson, L. F. & Webster, R. K. (1976a). The dynamics of a controlled population of *Rhynchosporium secalis*, changes in race composition and frequencies. *Phytopathology* **66**, 726-728.

Jackson, L. F. & Webster, R. K. (1976b). Race differentiation, distribution and frequency of *Rhynchosporium secalis* in California. *Phytopathology* **66**, 719-725.

Jackson, L. F., Webster, R. K., Allard, R. W. & Kahler, A. L. (1982). Genetic analysis of changes in scald resistance in barley composite cross. *Phytopathology* **72**, 1069-1072.

James, W. C. (1967). Assessment of barley leaf blotch and its effect on spring barley yield. *Proceedings of the 4th British Insecticide and Fungicide Conference*, 111-114.

James, W. C., Jenkins, J. E. E. & Jemmett, J. L. (1968). The relationship between leaf blotch caused by *Rhynchosporium secalis* and losses in grain yield of spring barley. *Annals of Applied Biology* **62**, 273-288.

Jenkins, J. E. E. & Jemmett, J. L. (1967). Barley leaf blotch. *National Agricultural Advisory Service Quarterly Review* **75**, 127-132.

Jensen, E. K., Clausen, M. & Jørgensen, L. M. (1995). Strobilurins a new group of fungicides. *12th Danish Plant Protection Conference - Pests and Diseases*, 101-114.

Jones, D. R. D. (1990). Sensitivity of *Rhynchosporium secalis* to DMI fungicides. *Brighton Crop Protection Conference - Pests and Diseases*, 1135-1140.

Jones, P. & Ayres, P. G. (1972). The nutrition of the subcuticular mycelium of *Rhynchosporium secalis* (barley leaf blotch): permeability changes induced in the host. *Physiological Plant Pathology* **2**, 383-392.

Jones, P. & Ayres, P. G. (1974). *Rhynchosporium* leaf blotch of barley studied during the subcuticular phase by electron microscopy. *Physiological Plant Pathology* **4**, 229-233.

Jørgensen, H. J. L. & Smedegaard-Petersen, V. (1995). Pathogenic variation of *Rhynchosporium secalis* in Denmark and sources of resistance in barley. *Plant Disease* **79**, 297-301.

Kajiwara, T. (1969). Comparative studies on the pathogenicity of barley and rye scald fungus, *Rhynchosporium secalis* (Oudem) Davies. *Review of Applied Mycology* **48**, 316.

Kay, J. G. & Owen, H. (1973a). Host range of *Rhynchosporium secalis*. *Transactions of the British Mycological Society* **60**, 413-422.

Kay, J. G. & Owen, H. (1973b). Transmission of *Rhynchosporium secalis* on barley grain. *Transactions of the British Mycological Society* **60**, 405-411.

Kendall, S., Hollomon, D. W., Ishii, H. & Heany, S. P. (1994). Characterisation of benzimidazole-resistant strains of *Rhynchosporium secalis*. *Pesticide Science* **40**, 175-181.

Kendall, S. J., Hollomon, D. W., Cooke, L. R. & Jones, D. R. (1993). Changes in sensitivity to DMI fungicides in *Rhynchosporium secalis*. *Crop Protection* **12**, 357-362.

Kerby, K. & Somerville, S. (1989). Enhancement of specific intercellular peroxidase following inoculation of barley with *Erysiphe graminis* f.sp. *hordei*. *Physiological and Molecular Plant Pathology* **35**, 323-337.

Khan, T. N. (1986). Effects of fungicide treatments on scald (*Rhynchosporium secalis* (Oud)J Davis) infection and yield of barley in Western Australia. *Australian Journal of Experimental Agriculture* **26**, 231-235.

Kiewnick, L. (1977). Zum Auftreten von *Rhynchosporium secalis* an Wintergerste. *Gesonde Pflanzen* **29**, 174-175.

King, J. E. & Griffin, M. J. (1985). Survey of benomyl resistance in *Pseudocercosporella herpotrichoides* on winter wheat and barley in England and Wales in 1983. *Plant Pathology* **34**, 272-283.

Kinsey, J. A. & Helber, J. (1989). Isolation of a transposable element from *Neurospora crassa*. *Proceedings of the National Academy of Science* **86**, 1929-1933.

Kistler, H. C. & Miao, V. P. W. (1992). New modes of genetic change in filamentous fungi. *Annual Review of Phytopathology* **30**, 131-152.

Knogge, W. (1991). Plant resistance genes for fungal pathogens - Physiological models and identification in cereal crops. *Zeitschrift fuer Naturforschung Section C Biosciences* **46**, 969-981.

Köller, W. (1988). Sterol demethylation inhibitors: Mechanism of action and resistance. In *Pesticide Resistance in North America* (ed. C. J. Delp), pp. 79-88. American Phytopathological Society, St Paul, Minnesota.

Köller, W. & Scheinpflug, H. (1987). Fungal resistance to sterol biosynthesis inhibitors: A new challenge. *Plant Disease* **71**, 1066-1074.

Marrè, E. (1979). Fusicoccin: a tool in plant physiology. *Annual Review of Plant Physiology* **30**, 273-288.

Martin, P. J. (1982). *Effects of Rhynchosporium on net photosynthesis*. Rep. No. 84. Long Ashton Research Station, Bristol.

Martinez-Espinoza, A. D. & Sands, D. C. (1995). Isolation and regeneration of *Rhynchosporium secalis* protoplasts, the causal agent of barley scald. *Revista-Mexicana-de-Micologia* **11**, 155-164.

Mayfield, A. H. & Clare, B. G. (1984). Effects of common stubble treatments and sowing sequences on scald disease (*Rhynchosporium secalis*) in barley crops. *Australian Journal of Agricultural Research* **35**, 799-805.

Mazars, C., Auriol, P. & Rafenomananjara, D. (1983). Rhynchosporosides binding by barley proteins. *Phytopathology* **73**, 1071-1078.

McDermott, J. M., McDonald, B. A., Allard, R. W. & Webster, R. K. (1989). Genetic variability for pathogenicity, isozyme, ribosomal DNA and colony color variants in populations of *Rhynchosporium secalis*. *Genetics* **122**, 561-565.

McDonald, B. A. & Martinez, J. P. (1991). DNA fingerprinting of the plant pathogenic fungus *Mycosphaerella graminicola* (anamorph, *Septoria tritici*). *Experimental Mycology* **15**, 146-158.

McDonald, B. A., Zhan, J. & Burdon, J. J. (1999). Genetic structure of *Rhynchosporium secalis* in Australia. *Phytopathology* **89**, 639-645.

Müller, E. (1954). Versuche mit dem Rhynchosporium-Schorf von Gerste und Roggen. *Review of Applied Mycology* **33**, 149.

Muona, O. A. (1980). *Evolution of correlated systems in barley: morphological and enzyme polymorphism, quantitative characters and disease resistance*. Ph.D., California Davis.

Newman, P. L. (1985). Variation amongst isozymes of *Rhynchosporium secalis*. *Plant Pathology* **34**, 329-337.

Newman, P. L. & Owen, H. (1985). Evidence of asexual recombination in *Rhynchosporium secalis*. *Plant Pathology* **34**, 338-340.

Olutiola, P. A. & Ayres, P. G. (1973). A cellulase complex in culture filtrates of *Rhynchosporium secalis* (barley leaf blotch). *Transactions of the British Mycological Society* **60**, 273-282.

Owen, H. (1963). Physiological races of *Rhynchosporium secalis* on cultivated barley. *Transaction of the British Mycological Society* **46**, 604-608.

Ozoe, S. (1956). Studies on the *Rhynchosporium* scald of barley and its control. *Shimane Prefectural Agricultural Institute Bulletin* **1**, 1-122.

Peever, T. L. & Milgroom, M. G. (1993). Genetic correlations in resistance to sterol biosynthesis-inhibiting fungicides in *Pyrenophora teres*. *Phytopathology* **83**, 1076-82.

Penner, G. A., Legge, W. G. & Tekauz, A. (1998). Identification of isolate specific sources of scald resistance in Turkish barley (*Hordeum vulgare*) accessions. *Euphytica* **99**, 111-114.

Phillips, A. N. & Locke, T. (1994). Carbendazim resistance to *Rhynchosporium secalis* in England and Wales. *BCPC Monograph* **60**, 251-254.

Polley, R. W. (1971). Barley leaf blotch epidemics in relation to weather conditions with observations on the overwintering of the disease on barley debris. *Plant Pathology* **20**, 184-190.

Rebmann, G., Hertig, C., Bull, J., Mauch, F. & Dudler, R. (1991). Cloning and sequencing of cDNA encoding a pathogen induced putative peroxidase of wheat (*Triticum aestivum* L.). *Plant Molecular Biology* **16**, 329-331.

Riddle, O. C. & Briggs, F. N. (1950). Inheritance of resistance to scald in barley. *Hilgardia* **20**, 19-27.

Robinson, J., Lindqvist, H. & Jalli, M. (1996). Genes for resistance in barley to Finnish isolates of *Rhynchosporium secalis*. *Euphytica* **92**, 295-300.

Rohe, M., Gierlich, A., Hermann, H., Hahn, M., Schmidt, B., Rosahl, S. & Knogge, W. (1995). The race-specific elicitor, NIP1, from the barley pathogen, *Rhynchosporium secalis*, determines avirulence on host plants of the Rrs1 resistance genotype. *EMBO Journal* **14**, 4168-4177.

Ryan, C. C. & Clare, B. G. (1975). Effects of light, temperature and period of leaf surface wetness on infection of barley by *Rhynchosporium secalis*. *Physiological Plant Pathology* **6**, 93-103.

Ryan, C. C. & Grivel, C. J. (1974). An electron microscope study of the outer layers of barley leaves infected with *Rhynchosporium secalis*. *Australian Journal of Plant Physiology* **1**, 313-317.

Salamati, S. & Magnus, H. A. (1997). Leaf blotch severity on spring barley infected by isolates of *Rhynchosporium secalis* under different temperature and humidity regimes. *Plant Pathology* **46**, 939-945.

Sarsola, J. A. & Campi, M. D. (1947). Reacción de algunas cebadas con respecto a *Rhynchosporium secalis* en Argentina. *Revista de Investigaciones Agropecuarias* **1**, 243-260.

Schaller, C. W. (1951). The effect of mildew and scald infection on yield and quality of barley. *Agronomy Journal* **43**, 183-188.

Seidel, D., Sierigk, U. & Edner (1982). Zur (kologie und Schadwirkung von *Rhynchosporium secalis* (Oudem.) Davis. *Schaderreger in der industriemä(igen Getreideproduktiion*, S33.

Sheridan, J. E., Grbavac, N. & Sheridan, M. H. (1985). Triadimenol sensitivity in *Pyrenophora teres*. *Transactions of the British Mycological Society* **85**, 338-341.

Shipton, W. A. (1974). Scald of barley. *Review of Plant Pathology* **53**, 839-861.

Skoropad, W. P. (1960). Barley scald in the Prairie provinces of Canada. *Commonwealth Phytopathological News* **6**, 25-27.

Skoropad, W. P. (1966). Sporulating potential of *Rhynchosporium secalis* on naturally infected leaves of barley. *Canadian Journal of Plant Science* **46**, 243-247.

Skylakakis, G. (1987). Changes in the composition of pathogen populations caused by resistance to fungicides. In *Populations of Plant Pathogens: Their dynamics and Genetics* (eds. M. S. Wolfe & C. E. Caten), pp. 227-237. Blackwell Scientific Publications, Oxford.

Starling, T. M., Roane, C. W. & Chi, K. R. (1971). Inheritance of reaction to *Rhynchosporium secalis* in winter barley cultivars. *Proceedings of the 2nd International Barley Genetics Symposium*, 513-519.

Stedman, O. J. (1980). Dispersal of spores of *Rhynchosporium secalis* from a dry surface by water drops. *Transactions of British Mycological Society* **75**, 339-340.

Stedman, O. J. (1982). The effect of three herbicides on the number of spores of *Rhynchosporium secalis* on barley stubble and volunteer plants. *Annual Applied Biology* **100**, 271-279.

Stedman, S. G. (1977). Effect of paraquat on the number of spores of *Rhynchosporium secalis* on barley and volunteers. *Plant Pathology* **26**, 112-120.

Stoddart, J. A. & Taylor, J. F. (1988). Genotypic diversity: estimation and prediction in samples. *Genetics* **118**, 705-711.

Strobel, G. A. (1975). A mechanism of disease resistance in plants. *Scientific American* **232**, 80-88.

Strobel, G. A. (1982). Phytotoxins. *Annual Review of Biochemistry* **51**, 309-333.

Taggart, P. J., Cooke, L. R. & Mercer, P. C. (1994). Benzimidazole resistance in *Rhynchosporium secalis* in Northern Ireland and its implications for the disease control. *BCPC Monograph* **60**, 243-246.

Taggart, P. J., Cooke, L. R., Mercer, P. C. & Shaw, M. W. (1998). Effects of fungicides used to control *Rhynchosporium secalis* where benzimidazole resistance is present. *Crop Protection* **17**, 727-734.

Taggart, P. J., Locke, T., Philips, A. N., Pask, N., Hollomon, D. W., Kendall, S. J., Cooke, L. R. & Mercer, P. C. (1999). Benzimidazole resistance in *Rhynchosporium secalis* and its effect on barley leaf blotch control in the UK. *Crop Protection* **18**, 239-243.

Tekauz, A. (1991). Pathogenic variation in *Rhynchosporium secalis* on barley in Canada. *Canadian Journal of Plant Pathology* **13**, 298-304.

Tinline, R. D. & MacNeill, B. H. (1969). Parasexuality in plant pathogenic fungi. *Annual Review of Phytopathology* **7**, 147-170.

Trench, T. N., Wilkinson, D. J. & Esterhuysen, S. P. (1992). *South African Plant Disease Control Handbook.* Farmer Support Group, University of Natal, Pietermaritzburg.

Vanderplank, J. E. (1968). *Disease resistance in plants.* Academic Press, New York.

Webster, R. K. & Jackson, L. F. (1976). Seed and grasses as possible sources of *Rhynchosporium secalis* for barley in California. *Plant Disease Reporter, Washington* **60**, 233-236.

Webster, R. R., Saghai-Maroof, M. A. & Allard, R. W. (1986). Evolutionary responses of barley composite cross II to *Rhynchosporium secalis* analyzed by pathogenic complexity and by gene-by-race relationships. *Phytopathology* **76**, 661-668.

Wevelsiep, L., Koggel, K. H. & Knogge, W. (1991). Purification and characterisation of peptides from *Rhynchosporium secalis* inducing necrosis in barley. *Physiology and Molecular Plant Pathology* **39**, 417-482.

Wevelsiep, L., Rupping, E. & Knogge, W. (1993). Stimulation of barley plasmalemma H+-ATPase by phytotoxic peptides from the fungal pathogen *Rhynchosporium secalis*. *Plant Physiology* **101**, 297-301.

Williams, R. J. & Owen, H. (1973). Physiologic races of *Rhynchosporium secalis* on barley in Britain. *Transaction of the British Mycological Society* **60**, 223-234.

Xue, A. G., Burnett, P. A., Helm, J. & Rossnagel, B. G. (1995). Variation in seedling and adult-plant resistance to *Rhynchosporium secalis* in barley. *Canadian Journal of Plant Pathology* **17**, 46-48.

Xue, G. & Hall, R. (1991). Components of parasitic fitness in *Rhynchosporium secalis* and quantitative resistance to scald in barley as determined with a dome inoculation chamber. *Canadian Journal of Plant Pathology* **13**, 19-25.

Yoder, O. C., Valent, B. & Chumley, F. (1986). Genetic nomenclature and practice for plant pathogenic fungi. *Phytopathology* **76**, 383-385.

Zhang, Q., Webster, R. K. & Allard, R. W. (1987). Geographical distribution and associations between resistance to four races of *Rhynchosporium secalis*. *Phytopathology* **77**, 352-357.

Zhang, Q., Webster, R. K., Grandall, B. A., Jackson, L. F. & Saghai-Maroof, M. A. (1992). Race composition and pathogenicity associations of *Rhynchosporium secalis* in California. *Phytopathology* **82**, 798-803.

Table 1. A chronological list of investigations regarding the virulence spectra of some *Rhynchosporium secalis* populations in the world

No. of pathotypes/ races	No. of isolates	No. of cultivars	Cultivars resistant	Cultivars susceptible	Country	Reference
11 different virulence genes	72	11	Hudson	Prefect & Wong	Norway	Hansen & Magnus (1973)
Nt	27	27	Atlas 46, Atlas 57, Hudson, Osiris, Psaknon, Sultan, Turk & Trebi	Dampier	Western Australia	Ali & Boyd (1973)
2	122	15	La Mesita, Osiris & Trebi	Cambrinus	UK	Williams & Owen (1973)
75	175	14	-	-	California, USA	Jackson & Webster (1976a)
17	100	13	Atlas, Atlas 46 & Osiris	Nt	Italy	Ceoloni (1980)
5	319	15	C.I. 3515, C.I. 4364, Abbyssinian, Atlas 46, Hudson, La Mesita, Modoc, Nigrinudum, Osiris, Turk & Wisconsin Winter X Glabron	Atlas, Brier, Clipper & Steudelli	Victoria, Australia	Brown (1985)
4	149	18	Abyssinian , Atlas 46, Hudson, Ilia, Kitchen, Modoc, Priver, Tipper & Trebi	Atlas, Brier, Koru, La Mesita, Magnum, Turk, Triumph & Wisconsin Winter X Glabron	New Zealand	Cromey (1987)
20	276	15	C.I. 3515	-	South Eastern Australia	Brown (1990)

Table 1. (continued) A chronological list of investigations regarding the virulence spectra of some *Rhynchosporium secalis* populations in the world

No. of pathotypes/ races	No. of isolates	No. of cultivars	Cultivars resistant	Cultivars susceptible	Country	Reference
45	111	10	-	-	Western Canada	Tekauz (1991)
20	352	5	Atlas & Atlas 46	OAC Acton & OAC Halton	South-Ontario, Canada	Xue & Hall (1991)
180 (1983)	275	14	nt	nt	California, USA	Zhang *et al.* (1992)
183 (1984)	273					
28	38	23	Atlas & Osiris	nt	Denmark	Jørgensen & Smedgaard-Petersen (1995)
0	20	18	C.I. 2376, C.I. 5581, Armelle, Atlas, Atlas 46, Atrada x Atlas, Brier, Hudson, Jet, Kitchen, La Mesita, Magnum, Modoc, Nigrinudum, Osiris & Wisconsin Winter x Glabron	Algerian	Finland	Robinson *et al.* (1996)
24	42	32	Osiris	Gospeck, Sakigake & Sultan	Norway	Salamati & Tronsmo (1997)

nt = not clearly specified.

- = no cultivar uniformly susceptible or resistant towards all isolates tested.

0 = no significant differences in specific pathogenicity.

Fig. 1. Scanning electron micrograph of conidia of *Rhynchosporium secalis* with the upper cell typically beak-shaped. Bar = 5 µm

Fig. 2. Fully-developed lesions caused by *Rhynchosporium secalis* showing typical dark brown margins surrounded by chlorotic regions.

2. Virulence spectrum of the *Rhynchosporium secalis* population in the Western Cape province

ABSTRACT

The virulence spectra of 50 *Rhynchosporium secalis* isolates from a population in the Western Cape province of South Africa were determined. Twenty-one races were detected using 17 differential barley cultivars. The two most prevalent races, namely 4 and 7, had three and four virulence genes respectively. Both race 4 and 7 were virulent on the most susceptible cultivars West China, Steudelli, C.I.8618 and C.I.2226. Considering the resistance genes reported for the cultivars Atlas 46, Turk, and C.I.3515 which showed no susceptible cultivar-pathogen interaction, it would appear that the *Rh-Rh3-Rh4* complex is primarily involved in conferring resistance to the local *R. secalis* isolates.

INTRODUCTION

Rhynchosporium secalis (Oudem.) Davis, the cause of barley leaf scald, is reported to be most severe on barley (*Hordeum vulgare*) in the cool, moist areas of the temperate zones (Taggart *et al.*, 1999). The *R. secalis* population from each of the barley producing areas in the world is comprised of many unique races that differ in their ability to attack different barley cultivars (Jackson & Webster, 1976a; Ceoloni, 1980; Xue & Hall, 1991; Jørgensen & Smedegaard-Petersen, 1995). Race specialisation in *R. secalis* was first demonstrated in Argentina by Sarsola & Campi (1947) and genetic variability in *R. secalis* populations has been studied in several countries since then (Jackson & Webster, 1976a; Ceoloni, 1980; Xue & Hall, 1991; Jørgensen & Smedegaard-Petersen, 1995). Disease reaction data obtained by inoculating host differentials with fungal isolates often reveal important properties of pathogen populations. From the literature it is evident that *R. secalis* is pathogenically highly variable, consisting of populations which differ in virulence spectra (Part 1, Table 1). High variability in the pathogen has been reported from the USA (California), Canada (Western Canada, Southern Ontario), Denmark and Italy, whereas considerably less variation has been reported from Finland, South-Eastern Australia and New Zealand

(Williams & Owen, 1973; Jackson & Webster, 1976b; Ceoloni, 1980; Cromey, 1987; Brown, 1990; Tekauz, 1991; Xue & Hall, 1991; Robinson *et al.*, 1996). Although the study of Williams & Owen (1973) suggests that the UK has a population with less variation (2 races identified among 122 isolates), recent research has provided evidence for the presence of several more races (A.C. Newton, personal communication).

Barley scald is an economically important disease in South Africa. Clipper dominates the malting barley industry in the Western Cape of South Africa, and thus not much variation in virulence would be expected within the *R. secalis* population of the Western Cape. Despite this, barley breeders have not yet been able to develop a good malting quality, scald resistant cultivar. To date the race composition of local *R. secalis* populations has not been determined, neither have possible resistance sources been identified. The objectives of this study were therefore to characterise the virulence spectrum of *R. secalis* populations in the Western Cape province and to evaluate the local *R. secalis* isolates against most of the existing resistance sources in barley using standard differential cultivars. These data will lay the foundation for future breeding strategies towards resistance against *R. secalis* in South Africa.

MATERIALS AND METHODS

Isolates

Barley leaves with leaf scald symptoms were collected from 29 different locations (24 different farms) within the Western Cape province during the 1993-1995 growing seasons. To isolate the fungus a leaf segment with a scald lesion was cut and surface sterilised (30 sec in alcohol, 120 sec in 1% aqueous NaOCl, 30 sec in alcohol), rinsed in sterile distilled water and placed on moist filter paper in a Petri dish sealed with Parafilm. After two days lesions were scraped with a scalpel, rinsed in a drop of water on a 1.2% water agar (WA) plate, spores distributed over the surface of the WA plate, the plate inverted and incubated overnight. Germinated spores were located under a stereo microscope and transferred to lima bean agar (LBA, 124 g lima beans, 12 g agar, 1 l water) plates amended with 0.05 g/l streptomycin. Resulting single-spore colonies were subsequently stored on lima bean agar slants under sterile mineral oil at 3°C.

Differential cultivars

Fifty isolates were characterised by inoculating 17 differential barley cultivars (Table 1) which provided a spectrum of interaction phenotypes. Line B87/14 (Small Grain Institute, South Africa) was also included in the study. Differential cultivars were kindly provided by the Victorian Institute for Dryland Agriculture (Australia) and the National Small Grain Centre (USA). Genes for resistance against *R. secalis* have been reported for all the cultivars except C.I.2226. Each cultivar was grown as groups of five plants in 15 cm diameter plastic pots in a glasshouse where the temperature ranged from 17-20°C. The susceptible cultivar Clipper was planted in the middle of each pot to check the viability of the inoculum. The experimental design was a randomised block with two replications of each cultivar-isolate combination. The experiment was repeated.

Inoculation and disease rating

Rhynchosporium secalis cultures were incubated on LBA plates at 17 ±1°C in the dark for 2 weeks. Conidial suspensions were obtained from these cultures by adding sterile distilled water to the plates and scraping the conidia from the surface. The resulting conidial suspensions were adjusted to approximately 3 x 10^5 spores/ml by adding sterile distilled water.

Barley seedlings at 2 to 3 leaf stage (approximately two-wk-old), were first sprayed with a 0.1% aqueous solution of Tween 20 in order to wet the leaves, after which a conidial inoculum was sprayed until run-off by applying approximately 125 ml of inoculum to a set of test plants. Inoculated seedlings were transferred to a dew chamber where the plants were kept for 48h in the dark at approximately 17°C and a relative humidity of 95-98%. Seedlings were then transferred to a glasshouse.

After 14 days seedlings were rated for disease symptoms according to a scale described by Jackson & Webster (1976b) where 0 = no visible symptoms; 1 = very small lesions confined to leaf margins; 2 = small lesions not confined to leaf margins; 3 = large coalescing lesions, involving the majority of the leaf area; 4 = total collapse of the leaf with no distinct lesions. Average disease ratings were used to classify the cultivars according to its reaction, where an index of 0-2 was indicative of a resistant reaction and 3-4 indicated a susceptible reaction. Reactions between some cultivar-isolate combinations could not be uniformly categorised in either the susceptible or

resistant group, in which case the reaction type was classified according to the most dominant rating observed.

Cluster analysis

Statistical analysis was performed using SAS (Statistical Analysis System, Cary, NC). Cluster analysis was performed using PROC CLUSTER with Density Linkage and the k=. (kth-nearest neighbour method) option. The number of clusters were assessed graphically with a dendrogram. The dendrogram was bisected when the level of similarity (R-squared value) reached 75%.

RESULTS

Twenty-one races were identified from 50 isolates when evaluated against 17 differential cultivars and line B87/14 (Table 2). Races varied from having a simple (one virulence gene) to a complex (12 virulence genes) virulence spectrum.

More than half of the *R. secalis* isolates tested had four or less virulence genes, which were also the highest association of virulence genes (>59%) among the isolates tested. Races 4 and 7 were the two most prevalent races, being found over 5 districts within the barley producing area of the Western Cape (Fig. 1). These two races were found at 18 of the 29 fields (15 of the 24 farms) where samples were taken, but were virulent to only four cultivars, namely Steudelli, West China, C.I.8618 and C.I.2226. The frequency in association of virulence genes found in races 4 and 7 were 88% and 60% respectively. Races with 6 and more virulence genes were characterised by the occurrence of one isolate per race, and a low association (<13%) of virulence genes among the isolates tested.

Twelve clusters were resolved among 21 races (Fig. 2). The first cluster contained 10 races. Races included in this cluster were virulent on a mean of four differentials. The remaining eleven clusters each consisted of a single race.

All isolates were virulent to the susceptible cultivar Clipper, and 98%, 96%, 90% and 64% were virulent towards barley cultivars Steudelli, West China, C.I.8618 and C.I.2226. The remainder of the cultivars (Abyssinian, La Mesita, Modoc, Wisconsin Winter x Glabron, Jet, Nigrinudum and C.I.4364) were susceptible to 6-34% of the isolates tested, and 14-57% of the races were virulent towards these cultivars. La Mesita was susceptible to 6% of the isolates and 14% of the races while

C.I.4364 was susceptible to 34% of the isolates and 57% of the races. No susceptible cultivar-pathogen interactions were observed for Atlas 46, Turk, CI3515 and line B87/14. One isolate was found to be virulent on Osiris and Psaknon and two isolates were virulent on Brier.

DISCUSSION

The virulence spectrum of the *R. secalis* population from the Western Cape province showed considerable variation and carries unnecessary virulence genes which is quite unexpected, since susceptible barley cultivars are grown throughout the province. Most of the isolates from the same field and even same lesion differed in virulence spectrum, which confirmed a similar observation made by Brown (1990). Unnecessary virulence in *R. secalis* populations has previously been recorded in Norway and Western Australia (Ali, 1981; Salamati & Tronsmo, 1997). Variation in Norway has been explained by a foreign seed source, which may have been contaminated, whereas in Australia it has been explained by the extensive hectares of *Hordeum* species. However, in South Africa neither of these explanations is applicable, since barley seed is multiplied locally for commercial use, and fungicide seed treatments are standard practice. Furthermore, barley grass is not the predominant grass weed and does not occupy vast areas in the barley producing area of the Western Cape.

With the number of cultivars (17) used in this study it should be possible to detect 131 072 ($= 2^{17}$) races if they were present in the population. Twenty-one races were revealed when 50 isolates were tested and >50% of these races were respectively represented by only one isolate each. It is however, more than likely that a greater sample size would reveal more races. Despite this variation, less virulent races dominated, with races 4 and 7 being the most prominent. These two races showed a high (99%) homology in regard to their virulence spectra, with only cultivar C.I.2226 distinguishing the two races from each other. The three most common races (56% of isolates tested) were fixed for virulence on three differentials, fixed for avirulence on 13, and varied for only two differentials. Furthermore, races in cluster 1 represented 76% of isolates tested in the study and were virulent to a mean of only four differentials (Fig. 2). Although *R. secalis* populations are potentially variable, it seems that under conditions which do not demand a variety of virulence genes, the

population tends to be dominated by simple races. There is also an indication that the local *R. secalis* population is dominated by an association of virulence genes characteristic of races with less virulence, compared to the more complex races which were less prevalent. These observations support the stabilising theory (races with unnecessary virulence are less fit) proposed by Vanderplank (1968). Due to evidence supporting the stabilising theory (Williams & Owen, 1973; Jackson & Webster, 1976a) and evidence against the theory (Hansen & Magnus, 1973; Ali *et al.*, 1976; Jørgensen & Smedegaard-Petersen, 1995), conflicting statements were made regarding this for *R. secalis*. Selection pressure differs from country to country, and factors such as commercially grown cultivars, seed sources, environment and alternative hosts influence the level of variability in *R. secalis* populations. However, it is evident from the conflicting statements that stabilising selection is not the only element shaping the virulence spectra of *R. secalis* populations worldwide. Hansen & Magnus (1973) were the first to suggest that the variation may be explained in terms of segregation of virulence genes, which may implicate parasexual or sexual recombination.

Cultivars Atlas 46, Turk, C.I.3515 and line B87/14 proved to be useful sources of resistance. Although Osiris, Psaknon and Brier were also promising, they were marginally susceptible. Steudelli, which was highly susceptible in this study was also not found to be promising in other studies (Jackson & Webster, 1976b; Brown, 1985; Brown, 1990; Zhang *et al.*, 1992). Considering the resistance genes reported for the cultivars Atlas 46, Turk, and C.I.3515, which showed no susceptible cultivar-pathogen interaction, it seems that the *Rh-Rh3-Rh4* complex is primarily involved in conferring resistance to the local *R. secalis* isolates (Table 1). Cultivars that proved to be resistant in this study, also showed resistance against *R. secalis* populations of other countries in the world such as Australia (West Australia, South Australia; Victoria); Italy; New Zealand; Canada (South-Ontario) and Finland (Ali & Boyd, 1973; Ceoloni, 1980; Brown, 1985; Cromey, 1987; Brown, 1990; Xue & Hall, 1991). Stacking of race specific genes will be a useful strategy, but the capacity of the *R. secalis* population for variation in virulence within a small area (lesion, plant, field) may still reduce the effective period of such a combination of race specific resistance. The importance of quantitative resistance for scald control has been frequently stated (Brown, 1985; Cromey, 1987; Tekauz, 1991) and although practically more difficult

to identify and incorporate, levels of quantitative resistance should ultimately be introduced in breeding programmes for durable resistance.

REFERENCES

Ali, S. M. (1981). Barley grass as a source of pathogenic variation in *Rhynchosporium secalis*. *Australian Journal of Agricultural Research* **32**, 21-25.

Ali, S. M. & Boyd, W. J. R. (1973). Host range and physiologic specialisation in *Rhynchosporium secalis*. *Australian Journal of Agricultural Research* **25**, 21-31.

Ali, S. M., Mayfield, A. H. & Clare, B. G. (1976). Pathogenicity of 203 isolates of *Rhynchosporium secalis* on 21 barley cultivars. *Physiological Plant Pathology* **9**, 135-143.

Brown, J. S. (1985). Pathogenic variation among isolates of *Rhynchosporium secalis* from cultivated barley growing in Victoria Australia. *Euphytica* **34**, 129-133.

Brown, J. S. (1990). Pathogenic variation among isolates of *Rhynchosporium secalis* from barley grass growing in South Eastern Australia. *Euphytica* **50**, 81-89.

Ceoloni, L. (1980). Race differentiation and search for sources of resistance to *Rhynchosporium secalis* in barley in Italy. *Euphytica* **29**, 547-553.

Cromey, M. G. (1987). Pathogenic variation in *Rhynchosporium secalis* on barley in New Zealand. *Journal of Agricultural Research* **30**, 95-99.

Hansen, L. R. & Magnus, H. A. (1973). Virulence spectrum of *Rhynchosporium secalis* in Norway and sources of resistance in barley. *Phytopathologische Zeitschrift* **76**, 303-313.

Jackson, L. F. & Webster, R. K. (1976a). The dynamics of a controlled population of *Rhynchosporium secalis*, changes in race composition and frequencies. *Phytopathology* **66**, 726-728.

Jackson, L. F. & Webster, R. K. (1976b). Race differentiation, distribution and frequency of *Rhynchosporium secalis* in California. *Phytopathology* **66**, 719-725.

Jørgensen, H. J. L. & Smedegaard-Petersen, V. (1995). Pathogenic variation of *Rhynchosporium secalis* in Denmark and sources of resistance in barley. *Plant Disease* **79**, 297-301.

Robinson, J., Lindqvist, H. & Jalli, M. (1996). Genes for resistance in barley to Finnish isolates of *Rhynchosporium secalis*. *Euphytica* **92**, 295-300.

Salamati, S. & Tronsmo, A. M. (1997). Pathogenicity of *Rhynchosporium secalis* isolates from Norway on 30 cultivars of barley. *Plant Pathology* **46**, 416-424.

Sarsola, J. A. & Campi, M. D. (1947). Reacción de algunas cebadas con respecto a *Rhynchosporium secalis* en Argentina. *Revista de Investigaciones Agropecuarias* **1**, 243-260.

Taggart, P. J., Locke, T., Philips, A. N., Pask, N., Hollomon, D. W., Kendall, S. J., Cooke, L. R. & Mercer, P. C. (1999). Benzimidazole resistance in *Rhynchosporium secalis* and its effect on barley leaf blotch control in the UK. *Crop Protection* **18**, 239-243.

Tekauz, A. (1991). Pathogenic variation in *Rhynchosporium secalis* on barley in Canada. *Canadian Journal of Plant Pathology* **13**, 298-304.

Vanderplank, J. E. (1968). *Disease resistance in plants.* Academic Press, New York.

Williams, R. J. & Owen, H. (1973). Physiologic races of *Rhynchosporium secalis* on barley in Britain. *Transaction of the British Mycological Society* **60**, 223-234.

Xue, G. & Hall, R. (1991). Components of parasitic fitness in *Rhynchosporium secalis* and quantitative resistance to scald in barley as determined with a dome inoculation chamber. *Canadian Journal of Plant Pathology* **13**, 19-25.

Zhang, Q., Webster, R. K., Grandall, B. A., Jackson, L. F. & Saghai-Maroof, M. A. (1992). Race composition and pathogenicity associations of *Rhynchosporium secalis* in California. *Phytopathology* **82**, 798-803.

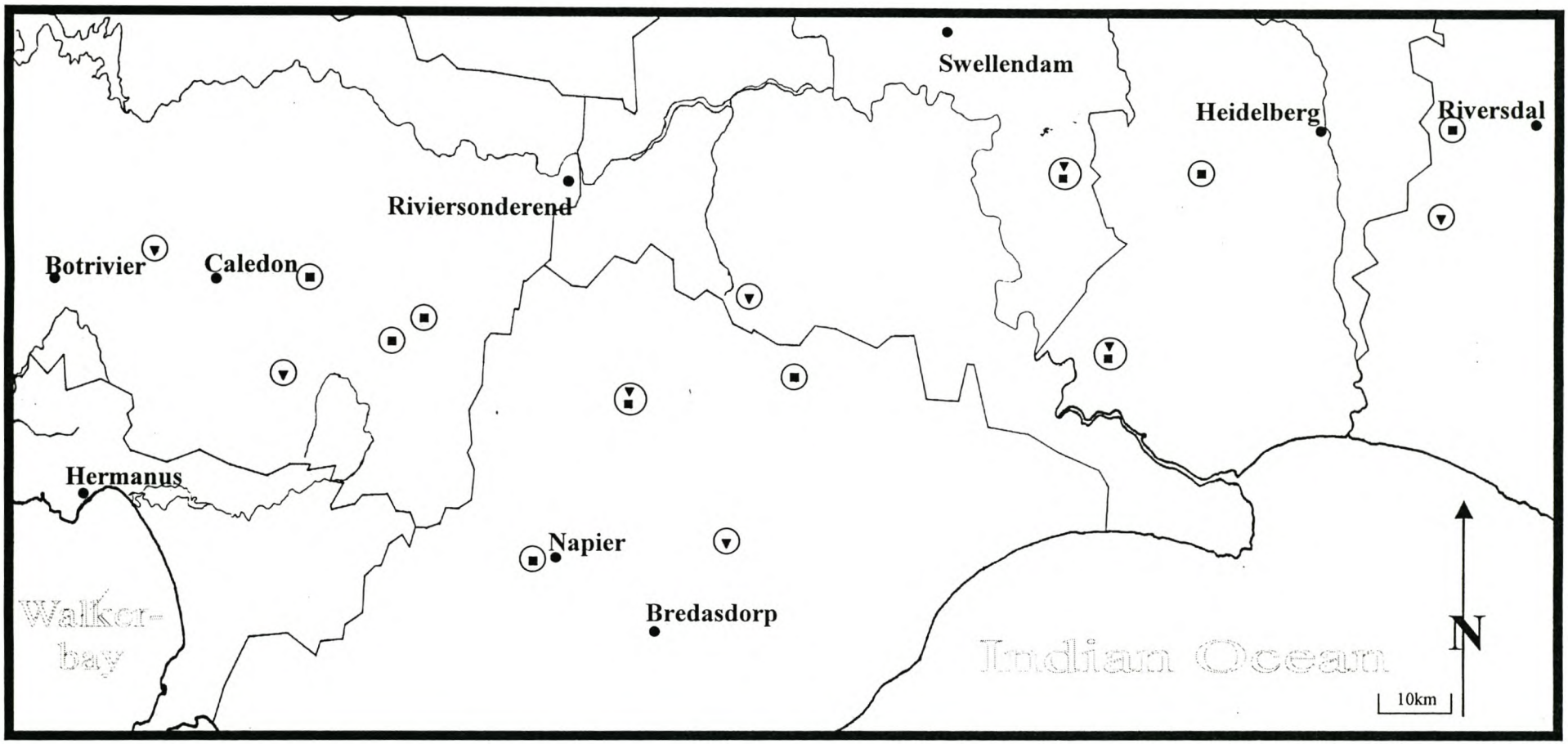

Fig. 1. Localities in the Western Cape province where symptomatic barley leaves were collected for the characterisation of *Rhynchosporium secalis* isolates. (○) = Farms where the two most prominent races (race 4 ■ and race 7▼) of the local *Rhynchosporium secalis* population were found. ● = Towns.

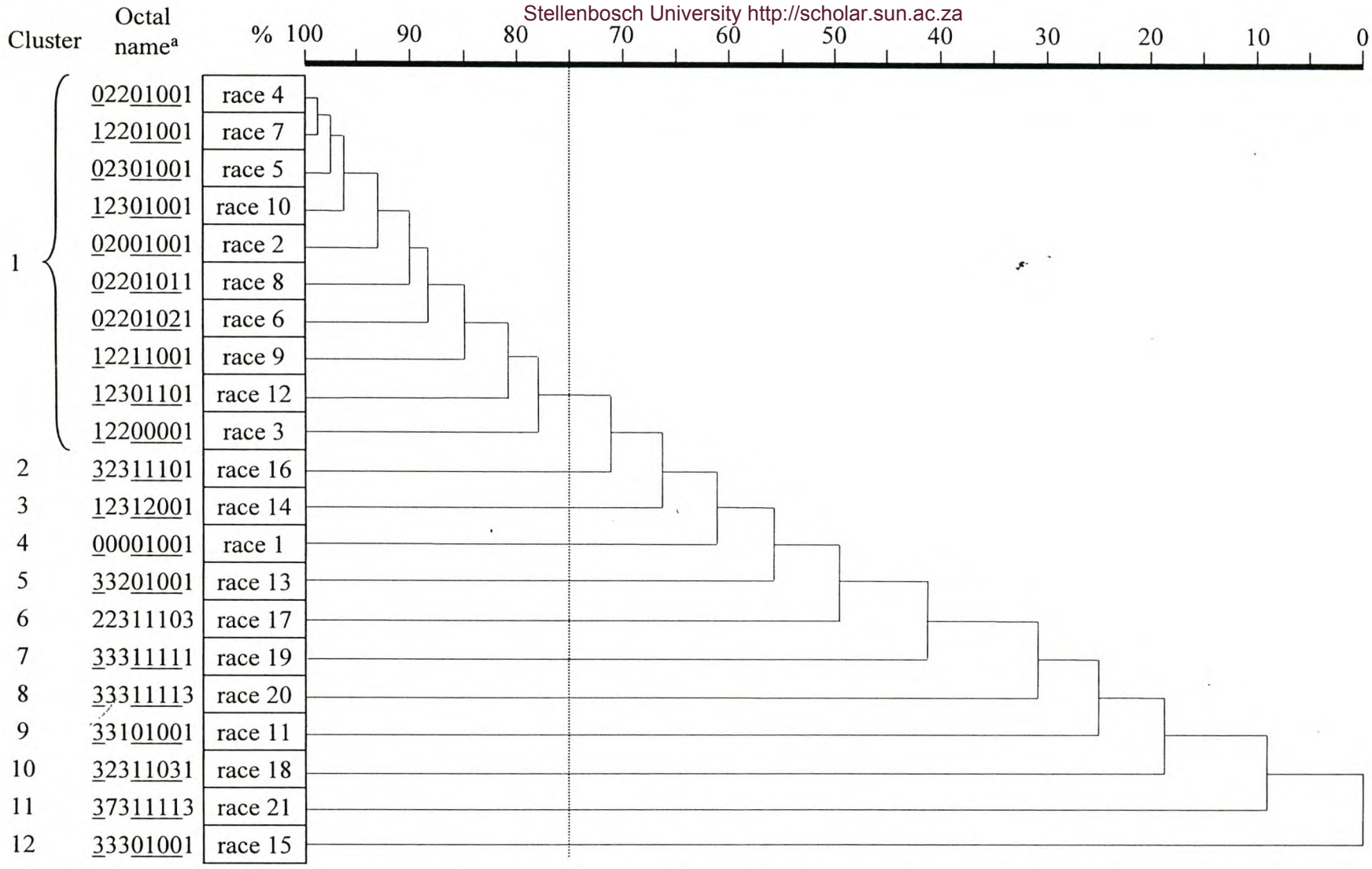

Fig. 2. A dendrogram of homology (%) between *Rhynchosporium secalis* isolates according to their virulence to a set of differential cultivars using the kth-nearest neighbour method of cluster analysis. (dendrogram bisected at 75% similarity as indicated by the dotted line) [a] According to the octal nomenclature proposed by Goodwin et al. (1990) (Abysinian was used as the source of the *Rh9* resistance instead of Kithchin and cultivars proposed for digit 8 were not evaluated in this study, instead Jet and C.I.2226 were incorporated).

Table 1. Genetic designation of 17 differential barley cultivars used to differentiate between 50 *Rhynchosporium secalis* isolates from South Africa[a]

Cultivars	Genes	References
B87/14	not characterised	
Atlas 46	*Rh2* and *Rh3*	Dyck & Schaller (1961)
	Rh	Habgood & Hayes (1971)
	One dominant genes at the *Rh-Rh3-Rh4* complex	Starling *et al.* (1971)
Turk	*Rh3* and *Rh5*	Dyck & Schaller (1961)
	Rh and *rh6*	Habgood & Hayes (1971)
	One dominant genes at the *Rh-Rh3-Rh4* complex	Starling *et al.* (1971)
Osiris	*Rh4*	Dyck & Schaller (1961)
	Rh^4 ,*Rh10* & *rh6*	Habgood & Hayes (1971)
Psaknon	1 to 3 dominant genes	Ali, 1975
C.I. 3515	Rh^4 & *Rh10*	Habgood & Hayes (1971)
	One dominant genes at the *Rh-Rh3-Rh4* complex	Starling *et al.* (1971)
Abyssinian	*Rh9*	Baker & Larter (1963)
La Mesita	*Rh4*	Dyck & Schaller (1961)
	Rh^4 & *Rh10*	Habgood & Hayes (1971)
	One dominant genes at the *Rh-Rh3-Rh4* complex	Starling *et al.* (1971)
Brier	*Rh* & *rh6*	Habgood & Hayes (1971)
	One dominant genes at the *Rh-Rh3-Rh4* complex	Starling *et al.* (1971)
Modoc	*Rh4*	Dyck & Schaller (1961)
	Rh^2 & *rh6*	Habgood & Hayes (1971)
	One dominant genes at the *Rh-Rh3-Rh4* complex	Starling *et al.* (1971)

Table 1. Continued

Cultivars	Genes	References
Wisconsin Winter x Glabron	*Rh*^{3}	Habgood & Hayes (1971)
Jet	*rh6* & *rh7*	Baker & Larter (1963)
	rh^5	Habgood & Hayes (1971)
Nigrinudum	*rh8*	Habgood & Hayes, (1971)
Steudelli	*rh6* & *rh7*	Baker & Larter (1963)
C.I. 4364	*rh11*	Habgood & Hayes (1971)
C.I. 2226	Not characterised	
West China	Two dominant genes	Ali (1975)
C.I. 8618	One dominant gene	Starling *et al.* (1971)

[a] Table adapted from Goodwin *et al.* (1990)

Table 2. Disease reaction[a] of 50 South African isolates of *Rhynchosporium secalis* on 17 barley cultivars and line B87/14

No. of isolates	Race	B87/14	At.46[b]	Turk	Osiris	Psak.[c]	C.I.3515[d]	Aby[e]	LaM.[f]	Brier	Modoc	W.W.G.[g]	Jet	Nig.[h]	Steud.[i]	C.I.4364	C.I.2226	W.C.[j]	C.I.8618
2	1	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
2	2	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-
1	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
11	4	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+
1	5	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+
1	6	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	+	+
11	7	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+
1	8	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	+
3	9	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	+	+	+
6	10	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
1	11	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-
1	12	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+
1	13	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	+	+	+
1	14	-	-	-	+	-	-	+	-	-	-	-	-	+	+	+	+	+	+
1	15	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+
1	16	-	-	-	-	-	-	+	-	-	+	-	+	-	+	+	+	+	+
1	17	-	-	-	-	-	-	+	+	-	+	-	-	-	+	+	+	+	+
1	18	-	-	-	-	-	-	+	-	+	-	+	+	-	+	+	+	+	+
1	19	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	+	+
1	20	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+
1	21	-	-	-	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+

[a] - + resistant, + = susceptible
[b] Atlas 46
[c] Psaknon
[d] Cereal Inventory Number, Agricultural Research Service, United States Department of Agriculture
[e] Abyssinian
[f] La Mesita
[g] Wisconsin Winter x Glabron
[h] Nigrinudum
[i] Steudelli
[j] West China

3. Genotypic variation in *Rhynchosporium secalis* pathotypes collected in the Western Cape province of South Africa

ABSTRACT

An anonymous multilocus DNA probe was used to characterise the genotypic structure of *Rhynchosporium secalis* isolates previously characterised according to their virulence spectra on a set of differential barley cultivars. The maximum percentage of genotypic diversity of 47 *R. secalis* isolates from the Western Cape province of South Africa was 46.5%. In comparison with diversity observed at DNA level, less variation was observed in pathogenicity for *R. secalis*. DNA polymorphisms in *R. secalis* seemed to be independent of variation in virulence. No correlation between any particular fingerprint pattern, race, district, field or lesion was observed. The two most frequently observed races, 4 and 7, did not share the same genotypes, even when isolated from the same field or lesion. The high level of genotypic variation observed in the South African *R. secalis* population resembled the genotypic diversity observed in other cereal pathotypes with known sexual states. Although no teleomorph has yet been observed, these data suggest that sexual recombination may operate within the South African population of *R. secalis*.

INTRODUCTION

The barley scald fungus *Rhynchosporium secalis* (Oud.) Davis is an important pathogen of barley and occurs in all barley producing countries including South Africa. Barley scald has been responsible for yield reductions of up to 37% in South Africa (Scott *et al.*, 1992). Considerable variation in pathogenicity has been reported to exist in populations of *R. secalis* (Owen, 1963; Ali & Boyd, 1973; Hansen & Magnus, 1973; Williams & Owen, 1973; Jackson & Webster, 1976a; Brown, 1985; Zhang *et al.*, 1992). Although new resistant barley cultivars are routinely introduced, this pathogen has been found to easily overcome race-specific resistance(Jackson & Webster, 1976b). High levels of virulence polymorphism have also been observed within fields, and in lesions on the same leaf (Brown, 1985). The mechanism of this

variation for pathogenicity is, however, still a topic of debate(Hansen & Magnus, 1973; Newman & Owen, 1985; Burdon *et al.*, 1994; Goodwin *et al.*, 1994) since no teleomorph has yet been reported for *R. secalis*. Furthermore, *R. secalis* pathotypes appear to be unstable and results could often not be repeated between laboratories(Owen, 1963; Hansen & Magnus, 1973; Williams & Owen, 1973; Ali *et al.*, 1976; Jørgensen & Smedegaard-Petersen, 1995). Hansen and Magnus(1973) found large intra-isolate variation, and were able to recover multiple pathotypes from plant lesions infected with a culture derived from a single conidium. However, those pathotypes were never confirmed by DNA fingerprinting techniques, and thus contamination cannot be ruled out. Zhang *et al.*(1987) pointed out that many studies(Jackson *et al.*, 1978; Muona, 1980; Jackson *et al.*, 1982; Webster *et al.*, 1986) had highly repeatable results and reported no intra-isolate variability when the inoculation and incubation method of Jackson and Webster(1976b) was used.

It is well-known that different environmental conditions during pathogenicity studies will affect pathotype analysis(Khan & Boyd, 1969; Shipton *et al.*, 1973; Rufty *et al.*, 1981; Bonman *et al.*, 1987). In previous studies dealing with *R. secalis*, different rating scales, differential cultivars, as well as different environmental conditions were used to determine pathotypes, making a comparison of results impossible(Brown, 1985; Jørgensen & Smedegaard-Petersen, 1995). However, neutral molecular markers such as RFLPs (which are highly repeatable) and isozymes, have proven to be useful techniques to compare the variability of *R. secalis* populations from different studies (Goodwin *et al.*, 1993; Burdon *et al.*, 1994; McDonald *et al.*, 1996).

Recently, McDonald *et al.*(1999) developed anonymous RFLP markers for use in *R. secalis*. Probes were selected from a random sample of total genomic DNA fragments of *R. secalis*. These probes were used to assay the genetic structure of *R. secalis* in Australia where genetic variation on a fine (within 3.24 m^2) and large scale (within 200 m^2) was investigated(McDonald *et al.*, 1999). The majority of the genetic diversity was observed on a fine spatial scale. Since the virulence spectra of the isolates tested were not known, McDonald *et al.*(1999) could only speculate about the possible relationships between virulence and genetic diversity. The objective of the present study was thus to investigate the genotypic variation of South African *R. secalis* isolates using RFLP fingerprinting and to determine if any relationship existed between genotypes and pathotypes.

MATERIALS AND METHODS

Isolates

The 47 *R. secalis* isolates investigated were selected in a previous study where they had been characterised according to their virulence spectra on a set of 17 differential barley cultivars(Robbertse *et al.*, 2000). The resistance genes reported for this set of differential barley cultivars were summarised by Goodwin *et al.*(1990). The 47 isolates represented 20 races, originating from 27 different fields in the Western Cape province (Table 1). Ten fields were represented by one isolate each, 14 fields were represented by two isolates (where isolates from 7 fields originated from the same lesion) and the remaining three fields were represented by three isolates from three different lesions in each field (Table 1).

Culture and harvesting of fungal strains

A spore suspension of each *R. secalis* isolate was inoculated into flasks with liquid lima bean broth (62g/l1 distilled water) (pH 5.9) and grown at 20°C in a shake incubator (120 rev/min). After 2 weeks the mycelium was harvested through a sieve and squeezed between filter paper to remove excess liquid. The dried mycelium was immersed into liquid nitrogen, ground with a mortar and pestle to a fine powder, and transferred aseptically into sterile Eppendorf tubes.

Solutions for DNA preparation

Extraction buffer: 200 mmol Tris HCl pH 8.5, 250 mmol NaCl, 25 mmol EDTA, 0.5% SDS; phenol; RNAse A: e.g. Boehringer Mannheim No. 109169, 10 mg/ml in ddH_20, boiled for 30 min, stored at -20°C.

DNA preparation

Ground mycelium in an Eppendorf tube was resuspended in 3 volumes SDS extraction buffer and mixed homogeneously. Samples were placed in a boiling bath for 2 min and afterwards snap frozen by floating tubes in liquid nitrogen for 2 min. Tubes were later placed in a water bath at 60°C for 10 min to thaw the samples. One volume phenol was added to one volume of the sample, inverted for 10 min and centrifuged for 20 min in an Eppendorf centrifuge (14 000 g). The upper aqueous

phase was immediately removed and transferred to a sterile Eppendorf tube. Phenol-chloroform-isoamylalchohol (2:1:1) was added to one volume of the sample, inverted for 10 min and centrifuged for 10 min at 14 000 g. The upper phase was transferred into a sterile Eppendorf tube and mixed with ca. 1 volume isopropanol and 20 µl sodium acetate. DNA precipitated visibly into a lump and was incubated overnight at –20°C for total precipitation. The lump of DNA was spooled out and transferred to a sterile 2 ml tube containing 1 ml TE (pH 8.0). Twenty-five microlitres RNAse A solution was added and the sample incubated overnight at 37°C. Proteinase K (15 µl, 2%) and SDS (5 µl, 20%) were added to the solution and incubated at 60°C for 2 hours. Phenol and chloroform-isoamylalchohol were added to the sample (1:1:2) and inverted a few times before being centrifuged for 10 min at 14 000 g. The upper phase was transferred into a sterile Eppendorf tube and mixed with ca. 1 volume isopropanol and 20 µl sodium acetate (3 M, pH 6.0), and incubated at –20°C for 2 hours. The tube was centrifuged for 1 min to concentrate the precipitate into a DNA pellet. As much liquid as possible was decanted and the pellet rinsed with 70% ethanol, dried in an oven at 50°C for 30 min and resuspended in 50-100 µl TE (pH 8.0) according to the amount of DNA.

Enzyme digestion and DNA fingerprint

Total DNA (3 µg) was digested overnight with the restriction enzyme *Eco*R1. Digested DNA (2.5 µg per lane) was separated in an 0.8% agarose TAE (0.5 X) gel for 21 hours at 1.75 V/cm using a gel electrophoresis apparatus. Upward capillary transfer of DNA to a nylon^{+} membrane (Magnagraph-N+, MSI) was conducted overnight. DNAs were fixed to the membrane by UV cross-linking on a 312 nm transilluminator for 3 min. A PCR DIG Probe synthesis kit (Roche) was used to label the pRs26-DNA probe(McDonald *et al.*, 1999). Prehybridization and hybridization reactions were performed according to the manufacturer's instructions in a hybridization oven (Personalhyb, Stratagene) at 40°C. Hybridization reactions were allowed to proceed for 16-18 hours. After hybridisation the membrane was washed twice at room temperature in 2XSSC; 0.1% w/v SDS for 5 min each time, followed by two 15-min washes in 0.5XSSC; 0.1% w/v SDS at 65°C. Detection of the hybridised probe and bound antibody conjugate was performed using a chemiluminescent alkaline phosphatase substrate (CDP-Star, Roche) according to the manufacturers instructions. Film (X-Omat AR, Kodak) was placed on the membrane and exposed

for 10-20 min depending on the strength of the light signal. A multicopy DNA probe (pRs26) was kindly provided by Dr. B. McDonald (Federal Institute of Technology, ETH-Zentrum, Switzerland) who identified it as being useful for fingerprinting isolates of *R. secalis*(McDonald *et al.*, 1999).

Data analysis

Different multilocus haplotypes were assumed to represent different genotypes. The maximum percentage of genotype diversity ($\hat{G}$) and its variance were quantified according to Stoddart and Taylor[32].

Pathogenic diversity within and among clonal genotypes was quantified with the Shannon information statistic according to the description in Goodwin *et al.*(1995). This statistic can be partitioned into within- and among-genotype components(Goodwin *et al.*, 1992), analogous to Nei's gene diversity analysis(Nei, 1973), but is more suitable for phenotypic data.

RESULTS

The maximum percentage of genotypic diversity of 47 *Rhynchosporium secalis* isolates from the Western Cape was 46.5% of its theoretical maximum diversity according to the measure of Stoddart and Taylor(1988) (Table 2).

Rhynchosporium secalis isolates were genotypically more diverse than pathogenically and the spatial distribution of geno- and pathotypes differed (Fig. 1; Table 3). In four out of seven cases where isolates originated from the same lesion, they represented different fingerprints, whereas isolates within each lesion always differed in pathotype (Table 3). Genotypes 1 and 4 were scattered over two districts and genotype 5 was scattered over three districts (Table 3). Genotypes 1 and 5 were present in the 1993 as well as in the 1995 *R. secalis* population. No association between genotype characteristic and pathotype could be found, as isolates with the same genotype frequently represented different pathotypes (Table 3). The highest pathogenic variation was found in the most frequently observed genotype, namely genotype 1 (Table 4).

DISCUSSION

In this study it was shown that 47 South African isolates of *R. secalis* exhibited a high degree of genotypic diversity (determined with DNA fingerprinting), as well as high degrees of variation in pathogenicity (determined on a set of differential barley cultivars). High degrees of genotypic variation has also been reported for *R. secalis* in other studies(McDermott *et al.*, 1989; Burdon *et al.*, 1994; McDonald *et al.*, 1999), which is quite unexpected for a fungus with no known teleomorph.

No correlation between any particular multilocus haplotype and race at any district, field or lesion was observed. This supports the findings of isozyme studies by Newman(1985) and Burdon *et al.*(1994), who could not find any correlation between any particular isozyme band or pattern with either host cultivar or site. In the present study, isolates that were identical according to DNA fingerprinting (and presumed to be clonal), represented different pathotypes. In contrast, Goodwin *et al.*(1992) found association of particular electrophoretic genotypes (potentially clonal) with particular pathotypes within populations. However, in general no association between electrophoretic genotype and pathotype was evident(Goodwin *et al.*, 1992). It is possible, therefore, that the fingerprinting probe used in the present study may not be sensitive enough to distinguish between closely related clones. Little is known about the fingerprints produced by the pRS26 probe, as previous studies where it was employed did not include pathotype information. McDonald *et al.*(1999), using the method of Jeffreys *et al.*(1985), estimated that the average probability that two isolates chosen at random would have the same hybridization profile was 4×10^{-6}. They suggested, therefore, that pRS26 could be useful for DNA fingerprinting in other populations(McDonald *et al.*, 1999).

The maximum percentage of genotypic diversity was lower in the present study ($\hat{G}/N = 46.5$) than compared to the study of McDonald *et al.*(1999), where the average genotypic diversity within a field population was 65% of the theoretical maximum. Results of the present study were consistent with previous observations, where the majority of genetic variation was distributed within fields on a fine spatial scale and not between fields(McDonald *et al.*, 1999). This distribution of genotypic diversity has also been found in other cereal pathogens such as *Septoria tritici* and *Stagonospora nodorum*(Chen *et al.*, 1994; McDonald *et al.*, 1994). Both these species have known teleomorphs and it was demonstrated that the majority of genetic

variation within populations of these pathogens was due to sexual reproduction, and to a lesser extent due to migration(Chen *et al.*, 1994; McDonald *et al.*, 1994).

On inspecting the genotypic diversity of the two most frequently observed races in this study (4 and 7), it was found that they were genetically isolated from each other, sharing none of the same genotypes, even when isolated from the same field or lesion (Fig. 1). This is not totally unexpected, as the fingerprinting probe used was selected at random from the *R. secalis* genome, and therefore most probably not tightly linked to genes under selection such as virulence genes. Isolates within a pathotype were therefore not expected to all belong to a single multilocus haplotype. Eight different genotypes were identified among 11 isolates of each race. This could indicate that races 4 and 7 originated from genetically diverse source populations, and that polymorphism at the DNA level in *R. secalis* is independent of variation in virulence. Furthermore, the independent variation in virulence observed could probably be due to the lack of resistance genes in local barley cultivars. The presence of particular resistance genes would have selected for corresponding races, which in turn would result in their associated genotypes being carried along by hitch-hiking.

Because highly susceptible barley cultivars are commercially grown in the Western Cape (Trench *et al.*, 1992), a low level of selection pressure on the virulence genes in the *R. secalis* population would have been expected. However, some measure of selection did function, since more frequently observed races (races 1, 2, 4, 7, 9, 10) were readily found in the 1993 and 1995 seasons. Only two genotypes, genotype 1 (represented by 4 isolates in 1993 and 1 in 1995) and genotype 5 (represented by 2 isolates in 1993 and 2 in 1995) persisted over a period of two years. The same geno- and pathotype combinations (genotype 1 and race 7; genotype 5 and race 4) were observed in two of the three instances where genotypes persisted over two years. Races 4 and 7 were the two most prominent races, and these genotypes where probably carried over as a result of hitch-hiking.

Multiple pathotypes recovered from single plant lesions in the present study and previously reported by Brown (1985) may in part be a result of different genotypes being present within the same lesion (Table 3). It is evident that host selection in concert with a source of variation plays an important role in the race-structure of the *R. secalis* population. Regular recombination not only generates diversity by continually reshuffling genes, but also prevents the dominance of a few clones. Infrequent sexual reproduction together with highly adapted and widespread clones

would facilitate deterioration of host resistance or fungicide efficacy. Future research on the South African *R. secalis* population should focus on the genetic structure of a population within a field, as this may reveal the role of the hypothesised sexual cycle.

REFERENCES

Ali, S. M. & Boyd, W. J. R. (1973). Host range and physiologic specialisation in *Rhynchosporium secalis*. *Australian Journal of Agricultural Research* **25**, 21-31.

Ali, S. M., Mayfield, A. H. & Clare, B. G. (1976). Pathogenicity of 203 isolates of *Rhynchosporium secalis* on 21 barley cultivars. *Physiological Plant Pathology* **9**, 135-143.

Bonman, J. M., Vergel de Dios, T. I., Bandong, J. M. & Lee, E. J. (1987). Pathogenic variability of monoconidial isolates of *Pyricularia oryzae* in Korea and in the Philippines. *Plant Disease* **71**, 127-130.

Brown, J. S. (1985). Pathogenic variation among isolates of *Rhynchosporium secalis* from cultivated barley growing in Victoria Australia. *Euphytica* **34**, 129-133.

Burdon, J. J., Abbott, D. C., Brown, A. H. D. & Brown, J. S. (1994). Genetic structure of the scald pathogen (*Rhynchosporium secalis*) in South East Australia: Implications for control strategies. *Australian Journal of Agricultural Research* **45**, 1445-1454.

Chen, R. S., Boeger, J. M. & McDonald, B. A. (1994). Genetic stability in a population of a plant pathogenic fungus over time. *Molecular Ecology* **3**, 209-218.

Goodwin, S. B., Allard, R. W., Hardy, S. A. & Webster, R. K. (1992). Hierarchial structure of pathogenic variation among *Rhynchosporium secalis* populations in Idaho and Oregon. *Canadian Journal of Botany* **70**, 810-817.

Goodwin, S. B., Allard, R. W. & Webster, R. K. (1990). A nomenclature for *Rhynchosporium secalis* pathotypes. *Phytopathology* **80**, 1330-1337.

Goodwin, S. B., Saghai-Maroof, M. A., Allard, R. W. & Webster, R. K. (1993). Isozyme variation within and among populations of *Rhynchosporium secalis* in Europe, Australia and the United States. *Mycological Research* **97**, 49-58.

Goodwin, S. B., Sujkowski, L. S. & Fry, W. E. (1995). Rapid evolution of pathogenicity within clonal lineages of the potato late blight disease fungus. *Phytopathology* **85**, 669-676.

Goodwin, S. B., Webster, R. K. & Allard, R. W. (1994). Evidence for mutation and migration as sources of genetic variation in populations of *Rhynchosporium secalis*. *Genetics* **84**, 1047-1053.

Hansen, L. R. & Magnus, H. A. (1973). Virulence spectrum of *Rhynchosporium secalis* in Norway and sources of resistance in barley. *Phytopathologische Zeitschrift* **76**, 303-313.

Jackson, L. F., Kahler, A. L., Webster, R. K. & Allard, R. W. (1978). Conservation of scald resistance in barley composite cross populations. *Phytopathology* **68**, 645-650.

Jackson, L. F. & Webster, R. K. (1976a). The dynamics of a controlled population of *Rhynchosporium secalis*, changes in race composition and frequencies. *Phytopathology* **66**, 726-728.

Jackson, L. F. & Webster, R. K. (1976b). Race differentiation, distribution and frequency of *Rhynchosporium secalis* in California. *Phytopathology* **66**, 719-725.

Jackson, L. F., Webster, R. K., Allard, R. W. & Kahler, A. L. (1982). Genetic analysis of changes in scald resistance in barley composite cross. *Phytopathology* **72**, 1069-1072.

Jeffreys, A. J., Wilson, V. & Thein, S. L. (1985). Individual-specific 'fingerprints' of human DNA. *Nature* **316**, 76-79.

Jørgensen, H. J. L. & Smedegaard-Petersen, V. (1995). Pathogenic variation of *Rhynchosporium secalis* in Denmark and sources of resistance in barley. *Plant Disease* **79**, 297-301.

Khan, T. N. & Boyd, W. J. R. (1969). Environmentally induced variability in the host reaction of barley to net blotch. *Australian Journal of Biological Science* **22**, 1237-1244.

McDermott, J. M., McDonald, B. A., Allard, R. W. & Webster, R. K. (1989). Genetic variability for pathogenicity, isozyme, ribosomal DNA and colony color variants in populations of *Rhynchosporium secalis*. *Genetics* **122**, 561-565.

McDonald, B. A., Miles, J., Nelson, L. R. & Pettway, R. E. (1994). Genetic variability in nuclear DNA in field populations of *Stagonospora nodorum*. *Phytopathology* **84**, 250-255.

McDonald, B. A., Mundt, C. C. & Chen, R. S. (1996). The role of selection on the genetic structure of pathogen populations: Evidence from field experiments with *Mycosphaerella graminicola* on wheat. *Euphytica* **92**, 73-80.

McDonald, B. A., Zhan, J. & Burdon, J. J. (1999). Genetic structure of *Rhynchosporium secalis* in Australia. *Phytopathology* **89**, 639-645.

Muona, O. A. (1980). *Evolution of correlated systems in barley: morphological and enzyme polymorphism, quantitative characters and disease resistance*. Ph.D., California Davis.

Nei, M. (1973). Analysis of gene diversity in subdivided populations. *Proceedings of the National Acadamy of Science USA* **70**, 3321-3323.

Newman, P. L. (1985). Variation amongst isozymes of *Rhynchosporium secalis*. *Plant Pathology* **34**, 329-337.

Newman, P. L. & Owen, H. (1985). Evidence of asexual recombination in *Rhynchosporium secalis*. *Plant Pathology* **34**, 338-340.

Owen, H. (1963). Physiological races of *Rhynchosporium secalis* on cultivated barley. *Transaction of the British Mycological Society* **46**, 604-608.

Robbertse, B., Lennox, C. L., Van Jaarsveld, A. B., Crous, P. W. & Van der Rijst, M. (2000). Pathogenicity of the *Rhynchosporium secalis*population in the Western Cape province of South Africa. *Euphytica* **In press**.

Rufty, R. C., Hebert, T. T. & Murphy, C. F. (1981). Variation in virulence in isolates of *Septoria nodorum*. *Phytopathology* **71**, 593-596.

Scott, D. B., van Niekerk, H. A. & Paxton, T. G. (1992). Effect of propiconazole on necrotrophic fungi and yield of barley genotypes differing in susceptibility to *Rhynchosporium secalis*. *Crop Protection* **11**, 243-247.

Shipton, W. A., Khan, T. N. & Boyd, W. J. R. (1973). Net blotch of barley. *Review of Plant Pathology* **52**, 269-287.

Stoddart, J. A. & Taylor, J. F. (1988). Genotypic diversity: estimation and prediction in samples. *Genetics* **118**, 705-711.

Trench, T. N., Wilkinson, D. J. & Esterhuysen, S. P. (1992). *South African Plant Disease Control Handbook*. Farmer Support Group, University of Natal, Pietermaritzburg.

Webster, R. R., Saghai-Maroof, M. A. & Allard, R. W. (1986). Evolutionary responses of barley composite cross II to *Rhynchosporium secalis* analyzed by pathogenic complexity and by gene-by-race relationships. *Phytopathology* **76**, 661-668.

Williams, R. J. & Owen, H. (1973). Physiologic races of *Rhynchosporium secalis* on barley in Britain. *Transaction of the British Mycological Society* **60**, 223-234.

Zhang, Q., Webster, R. K. & Allard, R. W. (1987). Geographical distribution and associations between resistance to four races of *Rhynchosporium secalis*. *Phytopathology* **77**, 352-357.

Zhang, Q., Webster, R. K., Grandall, B. A., Jackson, L. F. & Saghai-Maroof, M. A. (1992). Race composition and pathogenicity associations of *Rhynchosporium secalis* in California. *Phytopathology* **82**, 798-803.

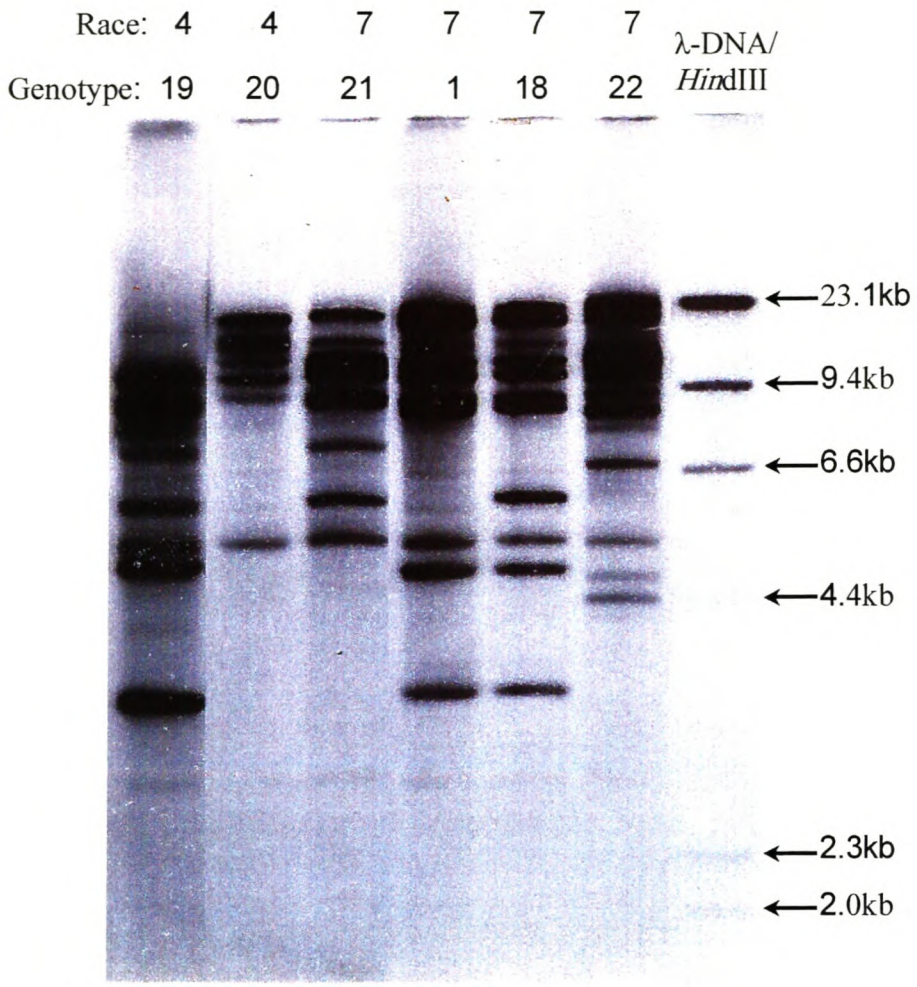

Fig. 1. DNA fingerprints of races 4 and 7 of *Rhynchosporium secalis*, produced by the multilocus probe pRS26 revealing six different genotypes. The last lane is a λ-*Hin*dIII standard and fragments are indicated in kilobases to the right.

Table 1. Collection data and race typification of *Rhynchosporium secalis* isolates studied.

Race	Isolates	Pathogenicity[a]	Octal name	District	Year isolated	Collector
1	11-1-1	00000000001000000l	00001001	Caledon	1993	CL Lennox
1	35-4-2	00000000001000000l	00001001	Philadelphia	1995	B Robbertse
2	3-1-3	00010000001000000l	02001001	Riversdal	1993	CL Lennox
2	30-5-1	00010000001000000l	02001001	Mooreesburg	1995	B Robbertse
3	37-4-2	01010010000000000l	12200001	Caledon	1995	B Robbertse
4	5-2-1	00010010001000000l	02201001	Swellendam	1993	CL Lennoc
4	6-5-2	00010010001000000l	02201001	Bredasdorp	1993	CL Lennox
4	9-3-2	00010010001000000l	02201001	Bredasdorp	1993	CL Lennox
4	9-4-1	00010010001000000l	02201001	Bredasdorp	1993	CL Lennox
4	10-3-3	00010010001000000l	02201001	Bredasdorp	1993	CL Lennox
4	12-1-3	00010010001000000l	02201001	Heidelberg	1993	CL Lennox
4	13-1-1	00010010001000000l	02201001	Heidelberg	1993	CL Lennox
4	15-1-1	00010010001000000l	02201001	Riversdal	1993	CL Lennox
4	31-6-3	00010010001000000l	02201001	Caledon	1995	B Robbertse
4	33-5-1	00010010001000000l	02201001	Caledon	1995	B Robbertse
4	37-1-1	00010010001000000l	02201001	Caledon	1995	B Robbertse
5	36-5-3	00010011001000000l	02301001	Caledon	1995	B Robbertse
6	8-3-1	00010010001001000l	02201021	Bredasdorp	1993	CL Lennox
7	3-2-1	01010010001000000l	12201001	Riversdal	1993	CL Lennox
7	4-5-1	01010010001000000l	12201001	Caledon	1993	CL Lennox
7	5-1-1	01010010001000000l	12201001	Swellendam	1993	CL Lennox
7	7-1-3	01010010001000000l	12201001	Caledon	1993	CL Lennox
7	7-4-1	01010010001000000l	12201001	Caledon	1993	CL Lennox
7	8-5-1	01010010001000000l	12201001	Bredasdorp	1993	CL Lennox
7	13-1-3	01010010001000000l	12201001	Heidelberg	1993	CL Lennox
7	14-2-2	01010010001000000l	12201001	Swellendam	1993	CL Lennox
7	38-4-1	01010010001000000l	12201001	Caledon	1995	B Robbertse
7	42-2-3	01010010001000000l	12201001	Caledon	1995	B Robbertse
7	45-8-6	01010010001000000l	12201001	Bredasdorp	1995	B Robbertse
8	33-5-2	00010010001000100l	02201011	Caledon	1995	B Robbertse
9	30-3-1	01010010101000000l	12211001	Mooreesburg	1995	B Robbertse
9	5-3-2	01010010101000000l	12211001	Swellendam	1993	CL Lennox
10	6-1-3	01010011001000000l	12301001	Bredasdorp	1993	CL Lennox
10	7-5-1	01010011001000000l	12301001	Caledon	1993	CL Lennox
10	12-1-2	01010011001000000l	12301001	Heidelberg	1993	CL Lennox
10	27-4-1	01010011001000000l	12301001	Caledon	1995	B Robbertse
10	28-3-3	01010011001000000l	12301001	Caledon	1995	B Robbertse
11	27-2-2	11011001001000000l	33101001	Caledon	1995	B Robbertse
12	39-1-1	01010011001010000l	12301101	Heidelberg	1995	B Robbertse
13	4-5-3	11011010001000000l	33201001	Caledon	1993	CL Lennox
14	35-2-3	01010011111000000l	12312001	Philadelphia	1995	B Robbertse
15	40-5-1	11011011001000000l	33301001	Swellendam	1995	B Robbertse
16	23-4-5	11010011101010000l	32311101	Bredasdorp	1994	CL Lennox
18	39-1-3	11010011101001100l	32311031	Heidelberg	1995	B Robbertse
19	11-1-3	11011011101010100l	33311111	Caledon	1993	CL Lennox
20	27-3-1	11011011101010101l	33311113	Caledon	1995	B Robbertse
21	28-3-1	11111011101010101l	37311113	Caledon	1995	B Robbertse

[a] According to the octal nomenclature proposed by Goodwin *et al.*[28]. Pathogenicity to the differential cultivars C.I.2226, Jet, Psaknon, West China, Nigrinudum, C.I. 3515, C.I. 8618, C.I. 4364, Abysinian, Osiris, Steudelli, Turk, Modoc, Brier, Wisconsin Winter x Glabron, Atlas 46, La Mesita and the susceptible cultivar Clipper are listed from left to right, where 1 = susceptible and 0 = not susceptible. (Abysinian was used as the source of the Rh9 resistance instead of Kithchin; cultivars proposed for digit 8 were not evaluated in this study, instead Jet and C.I.2226 were incorporated).

Table 2. Frequencies, diversity and clonal fraction of *Rhynchosporium secalis* genotypes.

Total sample	Total number of genotypes	No. of genotypes	Frequency	$\hat{G}$	$\hat{G}/N$	*Clonal fraction (%)*
47	32	24	1	21.87	46.5%	32%
		5	2			
		2	4			
		1	5			

Table 3. Pathogenicity and distribution of *Rhynchosporium secalis* genotypes.

Genotype	Race	Isolates	Year isolated	District
1	1	11-1-1*	1993	Caledon
1	6	8-3-1	1993	Bredasdorp
1	7	42-2-3	1993	Bredasdorp
1	7	8-5-1	1995	Caledon
1	19	11-1-3	1993	Caledon
2	7	4-5-1	1993	Caledon
2	7	7-1-3	1993	Caledon
2	7	7-4-1	1993	Caledon
2	10	7-5-1	1993	Caledon
3	13	4-5-3	1993	Caledon
4	3	37-4-2	1995	Caledon
4	7	45-8-6	1995	Bredasdorp
5	4	5-2-1	1993	Swellendam
5	4	15-1-1	1995	Caledon
5	4	31-6-3	1993	Riversdal
5	5	36-5-3	1995	Caledon
6	4	33-5-1	1995	Caledon
6	8	33-5-2	1995	Caledon
7	15	40-5-1	1995	Swellendam
8	16	23-4-5	1994	Bredasdorp
9	4	6-5-2	1993	Bredasdorp
9	4	9-4-1	1993	Bredasdorp
10	20	27-3-1	1995	Caledon
11	9	30-3-1	1995	Mooreesburg
12	10	6-1-3	1993	Bredasdorp
13	11	27-2-2	1995	Caledon
14	14	35-2-3	1995	Philadelphia
15	21	28-3-1	1995	Caledon
16	7	13-1-3	1993	Heidelberg
17	7	14-2-2	1993	Swellendam
18	2	3-1-3	1993	Riversdal
18	7	3-2-1	1993	Riversdal
19	4	12-1-3	1993	Heidelberg
19	10	12-1-2	1993	Heidelberg
20	4	37-1-1	1995	Caledon
21	7	5-1-1	1993	Swellendam
22	7	38-4-1	1995	Caledon
23	2	30-5-1	1995	Mooreesburg
24	12	39-1-1	1995	Heidelberg
25	1	35-4-2	1995	Philadelphia
26	4	9-3-2	1993	Bredasdorp
27	9	5-3-2	1993	Swellendam
28	10	28-3-3	1995	Caledon
29	18	39-1-3	1995	Heidelberg
30	4	13-1-1	1993	Heidelberg
31	4	10-3-3	1993	Bredasdorp
32	10	27-4-1	1995	Caledon

*Isolate number (location – lesion – spore).

Table 4. Mean pathogenic diversity within genotypes of the *Rhynchosporium secalis* population from the Western Cape province of South Africa.

Genotype	Mean pathogenic diversity	Normalized pathogenic diversity	Number of isolates
SA1	0.304508	0.439405	5
SA2	0.033079	0.047732	4
SA4	0.040773	0.058836	2
SA5	0.066157	0.095465	4
SA6	0.040773	0.058836	2
SA9	0	0	2
SA18	0.081547	0.117672	2
SA19	0.081547	0.117672	2

4. DMI sensitivity and cross-resistance patterns of *Rhynchosporium secalis* isolates from South Africa

ABSTRACT

Isolates of *R. secalis* were collected yearly from the Rûens area of the Western Cape during the 1993-1995 growing seasons. These isolates were evaluated *in vitro* to determine sensitivity to triazole fungicides (triadimenol, tebuconazole, flusilazole and propiconazole). The sensitivity fluctuated but in 1995 isolates were significantly less sensitive towards triadimenol than in the previous two years. In a second experiment, isolates collected from two fields with a 5-6 year history of triadimenol seed treatments and tebuconazole applications were evaluated for their fungicide sensitivity. A significant positive correlation was observed between tebuconazole and triadimenol sensitivity among *R. secalis* isolates from these fields. However, such a correlation was not found within the *R. secalis* population collected during 1993-1995 where shorter crop rotation patterns and a range of fungicides were applied. In a third experiment, the fungicide sensitivity of local *R. secalis* isolates was evaluated towards two new triazole fungicides, namely bromuconazole and triticonazole. Correlation coefficients observed between these new triazoles and those previously applied in South Africa were not significantly positive. The lack of significant cross-resistance has important practical implications for management of fungicide resistance.

INTRODUCTION

Barley scald, caused by *Rhynchosporium secalis* (Oud) J. Davis, is the most serious disease of barley in South Africa, with yield losses being mainly attributed to a reduction in 1000-kernel weight (Khan & Crosbie, 1988; Scott *et al.*, 1992). In South Africa, a yield increase of 37% was achieved after plants of the susceptible cultivar Clipper were sprayed with two applications of the triazole fungicide, propiconazole (Scott *et al.*, 1992). Triazole fungicides inhibit the C14 demethylation step in fungal ergosterol biosynthesis and are referred to as demethylation inhibitors (DMIs) (Copping *et al.*, 1984). It is generally accepted that a small population of resistant genotypes occur naturally in pathogen populations before the first fungicide applications (Brent, 1992). Under selection pressure from the triazole fungicides,

however, the fungal population can shift towards reduced sensitivity, and the proportion of resistant phenotypes may reach a level where satisfactory disease control is no longer achieved (Brent, 1992). This leads to the development of practical resistance.

Cross-resistance studies are useful in assessing the risk of resistance development. Cross-resistance to fungicides has been defined as resistance to two or more fungicides as a result of the same genetic factor (Georgopoulos, 1977). For *Pyrenophora teres* populations, it has been reported that correlation coefficients of resistance to DMIs may differ between populations within the species (Peever & Milgroom, 1993). A change in fungicide sensitivity involves changes in the frequencies of genes controlling fungicide resistance in the pathogen population. Fungal genes may control resistance to one or two fungicides and depending on their frequency will influence the fungicide management strategy to be followed.

Triadimenol has been used in South Africa since 1979 as a seed treatment for barley scald control. Foliar sprays such as propiconazole, flusilazole and tebuconazole were introduced during 1984, 1988 and 1989, respectively. Although the triazoles have been used extensively for almost two decades in South Africa, no information is available on the sensitivity and cross-resistance patterns of the *Rhynchosporium secalis* population to this fungicide class. The aim of this study was to determine the sensitivity and cross-resistance patterns of the *R. secalis* population against triadimenol, propiconazole, flusilazole, tebuconazole and two recently introduced triazoles, bromuconazole and triticonazole.

MATERIALS AND METHODS

Three experiments were conducted on *R. secalis* isolates from the Rûens area of the Western Cape, which is the main area where malting barley is grown in South Africa. In the first experiment, isolates were screened against four commonly used triazoles, namely triadimenol, propiconazole, flusilazole and tebuconazole. In the second experiment, two fields were selected with a longer history of fungicide usage (5-6 years). Isolates from these fields were screened against the commonly used fungicides, namely triadimenol and tebuconazole. In the third experiment, isolates were screened against two triazoles not previously used in the Western Cape, namely bromuconazole and triticonazole. The minimum inhibitory concentration (MIC) of

each fungicide was determined for each isolate. Isolates from the Rûens were also compared with the fungicide sensitivity of wild-type *R. secalis* isolates. The term wild-type is an arbitrary designation for one or more strains chosen deliberately as genetic standards (Yoder *et al.*, 1986). The *R. secalis* population in the Rûens has been continuously subjected to fungicide applications. It was, therefore, decided to collect isolates from fodder barley growing in the Swartland area, as this crop had not yet been subjected to fungicide applications, and wheat fields largely dominate the area. Subsequently these *R. secalis* isolates were considered as being unselected by fungicides and a comparison with wild-type isolates from Australia (kindly provided by H. Wallwork) and England (kindly provided by S. Kendall) showed that they had similar levels of fungicide sensitivity. These wild-type isolates were also considered as indicative of the base-line sensitivity.

Isolation and* in vitro *fungicide sensitivity assay

Leaf segments with scald lesions were surface sterilised, rinsed in sterile water, placed on moist filter paper in Petri dishes and incubated for two days at 17°C in the dark. Spores that developed on lesion surfaces were dislodged with a sterile scalpel. Spores were then transferred to water agar plates supplemented with streptomycin (0.05 g streptomycin sulphate/L) and incubated for 24 hours, after which time germinating single spores were transferred to lima bean agar (LBA) (62 g lima beans, 12 g Biolab agar/L). Single spore colonies were subsequently cultured on LBA and stored on malt extract agar (20 g malt extract, 12 g Biolab agar/L) slants under sterile mineral oil at 3°C in the dark.

For fungicide sensitivity testing, sterile LBA was amended with technical grade fungicide. The technical grade fungicides were dissolved in a 70% ethanol solution to create a stock solution of 4000 µg ai/mL. Fungicide concentrations of 0, 0.03, 0.1, 0.3, 1, 3, 10, 30 and 60 µg ai/mL were prepared by dilution of the stock solution into autoclaved LBA after being cooled to 50°C. Control plates consisted of LBA and ethanol (which never exceeded 1.1%). Medium was poured into Petri dishes (90 mm diam.), and a mycelial disk (3 mm diam.) cut from the edge of an actively growing culture with a cork borer, inverted and placed in the centre of each plate. Each isolate was tested on nine concentrations of each fungicide. Three isolates of known sensitivity were included with each set of tests to provide a check.

Fungal growth was assessed after 14 days of incubation at 17°C in the dark, and the minimum fungicide concentration required to inhibit growth (MIC) recorded.

Statistical analysis

All MIC values were transformed to log scale so as to normalise the observed variation as required by the analysis of variance and by the Pearson correlation coefficient. In the case of the wild-types hardly any variation was observed; F-values for comparisons involving the wild-types were therefore obtained by squaring Cochran's t-like statistic (Snedecor & Cochran, 1989, p. 97) involving two error mean squares: Error(a) = variance within wild-types; Error(b) = variance within other types.

Sensitivity towards triadimenol, propiconazole, flusilazole and tebuconazole

Rhynchosporium secalis isolates (50 isolates per year) collected during 1993 (12 fields), 1994 (12 fields) and 1995 (14 fields) were tested for their fungicide sensitivity to triadimenol, propiconazole, flusilazole and tebuconazole, which have been used frequently in the Western Cape (Table 1). All isolates were tested simultaneously towards each of the respective fungicides. Most samples (approximately 60%) were taken from fields where barley had been grown the previous year. These fields were not selected according to the amount of fungicides sprayed but randomly although with a bias towards barley production in previous years. Two to seven single spore isolates per field were evaluated to determine their fungicide sensitivity. Replicate samples from fields varied and the distance between samples varied from being in the same lesion to 25 m apart within each field. In total 150 isolates were randomly collected from 36 different barley fields scattered throughout the Rûens in the Western Cape. Fungicide sensitivity of isolates collected during 1993-1995 was compared with each other and with wild-type isolates.

Sensitivity of R. secalis isolates from fields with continuous triadimenol and tebuconazole applications

In order to evaluate the fungicide sensitivity of *R. secalis* populations subjected to continuous triazole applications, two commercial barley fields with known fungicide histories were chosen as collection sites in the Bredasdorp district. At locality A, tebuconazole was applied from 1991-1996, with the spray dosage varying from 150-175 g/ha. Additionally, seed was treated with triadimenol from 1991-1994 and with

triticonazole in 1995 and 1996. At locality B, the spray dosage of tebuconazole varied from 187.5-271 g/ha during 1992-1996, and seed was treated with triadimenol for 3 years (1992-1994), and with triticonazole the following two years (1995-1996). The dosage given for spray applications were represented by one tebuconazole spray per season. Within each field 7 locations were sampled, and ultimately 41 single spored isolates from locality A, and 45 single spored isolates from locality B were evaluated for their sensitivity towards tebuconazole and triadimenol. The distance between samples within location A and within location B varied from being in the same lesion to 50 m apart. Fungicide sensitivity of isolates from these two fields was compared with that of isolates from the first experiment and with wild-type isolates by means of an analysis of variance. Cross-resistance between tebuconazole and triadimenol was determined with Pearson's correlation analysis.

Sensitivity of isolates to bromuconazole and triticonazole and cross-resistance

Two triazoles, namely bromuconazole and triticonazole were registered for use against *R. secalis* in the Western Cape during 1995. Eighty *R. secalis* isolates collected prior to the widespread use of these fungicides in the area (prior to 1996) were subsequently tested for their sensitivity towards these fungicides and compared with wild-type *R. secalis* isolates. Cross-resistance patterns between bromuconazole and triticonazole and other triazoles previously used in the Western Cape were determined with Pearson's correlation analysis.

RESULTS

Obtaining great numbers of *R. secalis* isolates from fodder barley cultivars, to represent the wild-type population, proved difficult because of their resistance to *R. secalis*. Consequently only 11 wild-type isolates were collected from a fodder barley field for use in this study. This small number of isolates is probably not fully representative of the wild-type population.

Sensitivity towards triadimenol, propiconazole, flusilazole and tebuconazole

Average MIC values of *R. secalis* isolates collected in the Cape Rûens in 1993 were 9.8 µg/ml, 17.5 µg/ml, 2.6 µg/ml and 3.1 µg/ml when tested against triadimenol, propiconazole flusilazole and tebuconazole respectively. Average MIC values of *R.*

secalis isolates collected in 1994 were 12.7 µg/ml, 11 µg/ml, 3.7 µg/ml and 3.7 µg/ml when tested against triadimenol, propiconazole, flusilazole and tebuconazole respectively. Average MIC values of *R. secalis* isolates collected in 1995 were 18.8 µg/ml, 7.9 µg/ml, 1.1 µg/ml and 2.9 µg/ml when tested against triadimenol, propiconazole, flusilazole and tebuconazole respectively. In comparison, the MIC values of the wild-type isolates towards the same fungicides were 1.2 µg/ml, 0.8 µg/ml, 0.3 µg/ml and 0.4 µg/ml respectively. The fungicide sensitivity of *R. secalis* isolates collected during the 1993-1995 seasons was significantly different from that of wild-type isolates regarding the four triazoles evaluated (Tables 2-5). There were also significant differences in sensitivity towards flusilazole between isolates collected from different years (Table 3). In general the sensitivity fluctuated, but in 1995 isolates were significantly less sensitive towards triadimenol than in the previous two years (Tables 2,4,5).

Sensitivity of R. secalis isolates from fields with continuous triadimenol and tebuconazole applications

Average MIC values of isolates from locality A and B were respectively 13.95 µg/ml and 17.82 µg/ml when tested towards triadimenol. The triadimenol sensitivity of isolates from both fields were not significantly different from each other and not different to the triadimenol sensitivity found in the 1993-1995 isolates (Table 4). Isolates from locality A and B evaluated against tebuconazole had average MIC values of 3.72 µg/ml and 4.96 µg/ml respectively. The tebuconazole sensitivity of isolates from both fields were not significantly different from each other but differed significantly from the sensitivity observed in the 1993-1995 isolates (Table 5). Wild-type *R. secalis* isolates evaluated against triadimenol and tebuconazole had average MIC values of 1.2 µg/ml and 0.4 µg/ml respectively. *R. secalis* isolates from fields A and B were significantly different in their sensitivity towards triadimenol and tebuconazole compared to wild-type isolates (Table 4,5). The correlation coefficients between triadimenol and tebuconazole analysed from both localities were significantly positive (Table 6).

Sensitivity towards bromuconazole and triticonazole and cross-resistance patterns

Significant positive correlation coefficients were found between bromuconazole and three other fungicides namely triticonazole, tebuconazole and flusilazole (Table 7).

Significant positive correlation coefficients were observed between triticonazole and two other triazoles, tebuconazole and flusilazole. The correlation coefficients between both bromuconazole and triticonazole and the two remaining fungicides in the study, triadimenol and propiconazole were respectively not significant (Table 7).

Wild-type *R. secalis* isolates had average MIC values of 0.77 µg/ml and 1.36 µg/ml when tested against triticonazole and bromuconazole respectively. *R. secalis* isolates differed significantly in their triticonazole (Cochran's t' = 2.6058; P = 0.0179) and bromuconazole (Cochran's t' = 8.3859; P = 0.0000) sensitivity compared to the wild-type isolates.

DISCUSSION

The triazole sensitivity of *R. secalis* isolates collected during the 1993-1995 seasons fluctuated, showing no trend towards resistance build-up except towards triadimenol. In some cases, *R. secalis* isolates from 1995 were significantly more sensitive towards tebuconazole, propiconazole and flusilazole than in the previous years (Table 8). Isolates collected in 1995 were significantly less sensitive than isolates collected during the previous two years (Fig. 1, Table 8). The apparent fluctuation in sensitivity of isolates towards these triazoles could be explained by the several factors. Triadimenol is the active ingredient of Bavistin a seed treatment introduced in 1979 which dominated the market for years and was widely applied in the Western Cape. This exerted an enormous selection pressure on the *R. secalis* population. However, a wide range of triazole fungicides was available as foliar applications to control scald, which limited the selection pressure exerted by the foliar application of a specific triazole. A correlation analysis (Table 9) supported this theory and showed that no significant positive correlation ($P > 0.05$) between triadimenol and the other triazoles tested in this study occurred in the *R. secalis* population collected from 36 different fields during the 1993-1995 period. The lack of triadimenol cross-resistance in this population, use of different triazoles as foliar applications and crop rotation practices, possibly resulted in a fluctuating sensitivity of the population towards flusilazole, propiconazole and tebuconazole. However, as triadimenol was continuously applied throughout these fields, the shift detected towards this fungicide may be due to an evolutionary process.

In the second experiment investigating the effect of continuous fungicide use on fungicide sensitivity and cross-resistance patterns, however, it was evident that a significant positive correlation in sensitivity towards triadimenol and tebuconazole can develop (Table 6). These findings are in accordance with the observations made by Peever & Milgroom (1993) regarding the DMI sensitivity of *Pyrenophora teres,* namely that correlation relationships between DMIs differ among populations. It seems that correlation relationships can also differ among populations of *R. secalis*. The two fields from which isolates were collected for the second experiment are situated in an area where farmers have a rotation pattern with longer periods of barley production (5-6 years). Thus, longer periods of fungicide application occur than usually found in the Rûens area (2-3 years). Under this selection pressure one would expect selection of genes conveying a reduced sensitivity to both fungicides. This also suggests that the frequency of genes which control resistance to both triadimenol and tebuconazole is high, and seems similar to the population in the study by Kendall et al. (1993).

Cross-resistance has important practical implications for management of resistance. Uncorrelated coefficients suggest that different genetic factors control resistance to each DMI. No significant correlation coefficients indicate that no relation exists between these triazoles in a particular population and therefore no tendency of MIC values to increase together. These findings suggest, therefore, that combining or alternating these triazoles would be useful in controlling disease and managing resistance build-up in South African barley fields.

REFERENCES

Brent, K. (1992). Monitoring fungicide resistance: purposes, procedures and progress. In *Resistance 1991: Achievements and Developments in Combating Pesticide Resistance* (eds. I. Denholm, A. L. Devonshire & D. W. Hollomon), pp. 1-18. Elsevier, London.

Copping, L. G., Birchmore, R. J., Wright, K. & Godson, D. H. (1984). Structure-activity relationships in a group of imidazole-1-carboxamides. *Pesticide Science* **15**, 280-284.

Georgopoulos, S. G. (1977). Development of fungal resistance to fungicides. In *Antifungal Compounds* (eds. M. R. Siegel & H. D. Sisler). Vol. 2, pp. 439-495. Marcel Dekker, New York.

Kendall, S. J., Hollomon, D. W., Cooke, L. R. & Jones, D. R. (1993). Changes in sensitivity to DMI fungicides in *Rhynchosporium secalis*. *Crop Protection* **12**, 357-362.

Khan, T. N. & Crosbie, G. B. (1988). Effect of scald (*Rhynchosporium secalis* (Oud) J. Davis) infection on some quality characteristics of barley. *Australian Journal Experimental Agriculture* **28**, 783-785.

Peever, T. L. & Milgroom, M. G. (1993). Genetic correlations in resistance to sterol biosynthesis-inhibiting fungicides in *Pyrenophora teres*. *Phytopathology* **83**, 1076-82.

Scott, D. B., van Niekerk, H. A. & Paxton, T. G. (1992). Effect of propiconazole on necrotrophic fungi and yield of barley genotypes differing in susceptibility to *Rhynchosporium secalis*. *Crop Protection* **11**, 243-247.

Snedecor, G. W. & Cochran, W. G. (1989). *Statistical Methods.* 8th ed. Iowa State University Press, Iowa.

Yoder, O. C., Valent, B. & Chumley, F. (1986). Genetic nomenclature and practice for plant pathogenic fungi. *Phytopathology* **76**, 383-385.

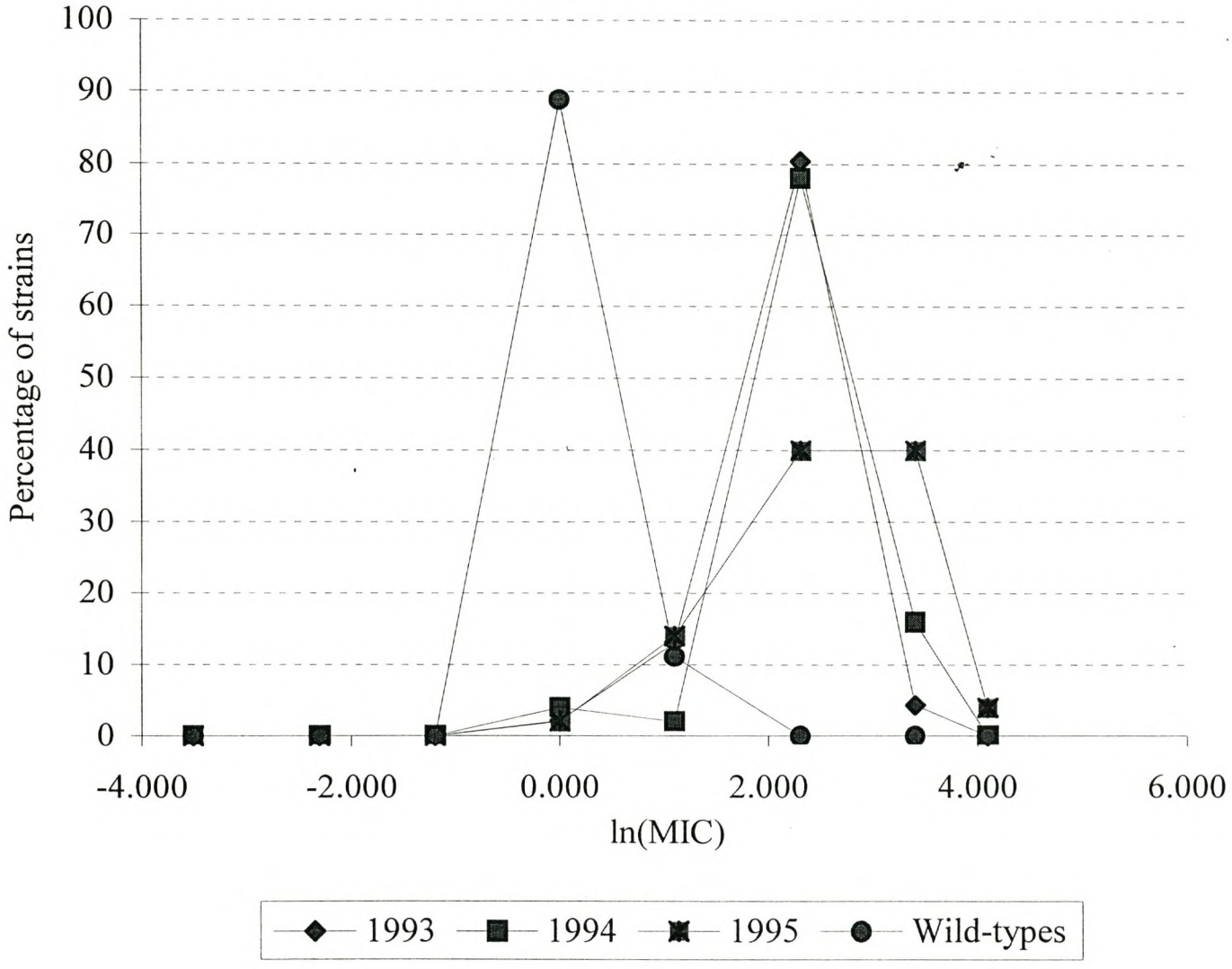

Fig. 1. Changes in sensitivity of *Rhynchosporium secalis* to triadimenol since 1993, and a comparison with wild-type isolates.

Table 1. Information regarding the *Rhynchosporium secalis* isolates evaluated in the first and third experiment

Locality	District	No. of isolates in first experiment	No. of isolates in third experiment	Year of isolation
3	Riversdal	5	-	1993
4	Caledon	5	2	1993
5	Swellendam	5	2	1993
6	Bredasdorp	7	2	1993
7	Caledon	5	2	1993
8	Bredasdorp	5	2	1993
9	Bredasdorp	5	-	1993
10	Bredasdorp	5	1	1993
11	Caledon	2	1	1993
12	Heidelberg	3	1	1993
13	Heidelberg	3	-	1993
14	Swellendam	2	1	1993
15	Riversdal	5	1	1994
16	Caledon	7	4	1994
17	Bredasdorp	6	2	1994
18	Caledon	6	1	1994
19	Bredasdorp	3	-	1994
20	Caledon	3	-	1994
21	Bredasdorp	3	-	1994
22	Bredasdorp	8	1	1994
23	Bredasdorp	8	1	1994
24	Caledon	7	1	1994
25	Caledon	7	1	1994
26	Caledon	5	-	1994
27	Caledon	6	2	1995
28	Caledon	5	3	1995
29	Caledon	5	3	1995
31	Caledon	7	-	1995
32	Caledon	5	-	1995
33	Caledon	7	-	1995
34	Caledon	3	-	1995
36	Caledon	6	-	1995
37	Caledon	6	-	1995
38	Caledon	5	2	1995
39	Heidelberg	6	2	1995
40	Swellendam	6	-	1995
42	Caledon	7	1	1995
43	Caledon	7	4	1995

Table 2. Analysis of variance of *in vitro* sensitivity of *R. secalis* isolates towards propiconazole

Source	df	Sum of Squares	Mean Square	*F*- value	*P*
Between all types	3	55.2048	18.4016		
Wild-type vs rest	1	43.1740	43.1740	102.2018[a]	0.0000
1993,94 vs 95	1	11.3981	11.3981	8.66	0.0038
1993 vs 1994	1	0.6024	0.6024	0.46	0.4997
Error(a)	8	2.8991	0.3624		
Error(b)	148	202.4620	1.3680		
Total	159	260.5659			

[a] F = square of Cochran's t-like statistic

Table 3. Analysis of variance of *in vitro* sensitivity of *R. secalis* isolates towards flusilazole

Source	df	Sum of Squares	Mean Square	*F*- value	*P*
Between all types	3	56.7560	18.9187		
Wild-type vs rest	1	20.5882	20.5882	326.1790[a]	0.0000
1993,94 vs 95	1	32.3752	32.3752	39.00	0.0001
1993 vs 1994	1	3.664	3.664	4.41	0.0373
Error(a)	8	0	0		
Error(b)	146	127.8302	0.8755		
Total	157				

[a] F = square of Cochran's t-like statistic

Table 4. Analysis of variance of *in vitro* sensitivity of *R. secalis* isolates towards triadimenol

Source	df	Sum of squares	Mean square error	*F*-value	*P*
Between all types	5	54.2381	10.8476		
Wild-type vs rest	1	46.3822	46.3822	310.6077[a]	0.0000
Fields vs Years	1	1.5562	1.5562	2.9856	0.0854
Field A vs B	1	1.2671	1.2671	2.4311	0.1203
1993, 94 vs 95	1	3.9421	3.9421	7.5632	0.0064
1993 vs 1994	1	1.1483	1.1483	2.2031	0.1391
Error(a)	8	1.0728	0.1341		
Error(b)	227	118.3158	0.5212		
Total	240	173.6267			

[a] F = square of Cochran's t-like statistic

Table 5. Analysis of variance of *in vitro* sensitivity of *R. secalis* isolates towards tebuconazole

Source	df	Sum of squares	Mean square error	*F*-value	*P*
Between all types	5	76.7265	15.3453		
Wild-type vs rest	1	38.4384	38.4384	119.4241[a]	0.0000
Fields vs Years	1	18.4045	18.4045	15.7675	0.0001
Field A vs B	1	2.9902	2.9902	2.5617	0.1108
1993, 94 vs 95	1	12.7006	12.7006	10.8809	0.0011
1993 vs 1994	1	3.7048	3.7048	3.1739	0.0761
Error(a)	10	0.5073	0.0507		
Error(b)	240	280.1387	1.1672		
Total	255	359.5207			

[a] F = square of Cochran's t-like statistic

Table 6. Patterns of cross-resistance to triadimenol and tebuconazole in *Rhynchosporium secalis* populations from localities with 5-6 years of continuous fungicide applications, as indicated by the Pearson correlation coefficients

Locality	A	B
	(n^a = 41)	(n = 45)
r^b	0.64340	0.51783
P^c	0.0003	0.0001

[a] number of observations

[b] correlation coefficient

[c] significance level of r

Table 7. Patterns of cross-resistance to demethylation inhibitor (DMI) fungicides in the *Rhynchosporium secalis* population from the Rûens area, as indicated by the Pearson correlation coefficients

Fungicide	Bromuconazole	Triticonazole
Triticonazole		
r[a]	0.57449	----------
P[b]	0.0001	----------
n[c]	78	----------
Triadimenol		
r	0.07097	0.02332
P	0.5424	0.8342
n	76	83
Tebuconazole		
r	0.34014	0.44380
P	0.0023	0.0001
N	78	86
Flusilazole		
r	0.38517	0.32957
P	0.0008	0.0028
n	73	80
Propiconazole		
r	0.15849	0.15799
P	0.2524	0.2125
n	54	64

[a] correlation coefficient
[b] significance level of r
[c] number of observations

Table 8. Mean log MIC values and standard errors of *R. secalis* isolates evaluated for their *in vitro* sensitivity against four triazole fungicides in the first experiment

Source		Triadimenol	Tebuconazole	Propiconazole	Flusilazole
1993	Mean	2.14	0.49	2.12	0.49
	Standard Error	0.086	0.161	0.191	0.139
1994	Mean	2.36	0.87	1.97	0.88
	Standard Error	0.094	0.127	0.136	0.137
1995	Mean,	2.6	0.1	1.46	-0.30
	Standard Error	0.130	0.181	0.162	0.123
Wild-types	Mean	0.12	-1.19	-0.40	-1.2
	Standard Error	0.122	0.155	0.201	0

Table 9. Patterns of cross-resistance between triadimenol and three triazoles, tebuconazole, propiconazole and flusilazole in *Rhynchosporium secalis* populations collected during the 1993-1995 period, as indicated by the Pearson correlation coefficients

	Tebuconazole	Propiconazole	Flusilazole
r[a]	0.07097	0.00107	-0.08414
P[b]	0.4290	0.9914	0.3509
n[c]	126	105	125

[a] correlation coefficient
[b] significance level of r
[c] number of observations

5. Fitness on barley plants of *Rhynchosporium secalis* isolates differing in tebuconazole sensitivity

ABSTRACT

Leaf area affected and sporulation of *Rhynchosporium secalis* isolates, varying in minimum inhibitory concentrations *in vitro* (1, 3 or 10 µg/ml) towards tebuconazole, were investigated on tebuconazole treated and untreated barley plants. Regardless of their tebuconazole sensitivity, all *R. secalis* isolates included in this study were equally fit to induce lesions and to sporulate in the absence of tebuconazole. Lesions on untreated plants ultimately resulted in a total loss of turgor and collapse of the whole lamina. Thus no fitness cost was associated with the degree of tebuconazole sensitivity in the present study. All *R. secalis* isolates were able to induce lesions on tebuconazole treated leaves, but differed significantly with respect to the percentage leaf area affected. Isolates least sensitive (MIC = 10 µg/ml) towards tebuconazole were more adapted on tebuconazole treated leaves, being able to repeatedly cause larger lesions than sensitive *R. secalis* isolates (MIC = 1 µg/ml). Sporulation was not significantly different between isolates on lesions of untreated or tebuconazole treated leaves. Larger leaf areas affected and adequate sporulation suggest that a less sensitive population would result in more disease in tebuconazole treated fields. This would have a significant impact on the build-up of a resistant field population.

INTRODUCTION

Barley scald caused by *Rhynchosporium secalis* (Oudem.) Davis is the most important disease of barley in the Western Cape province of South Africa (Trench *et al.*, 1992). At present barley scald is primarily controlled by fungicide applications. Trials in the Western Cape province have shown that barley scald was responsible for yield reductions of up to 37% in the absence of effective spray programmes (Scott *et al.*, 1992).

Fungicides traditionally used against scald in the Western Cape province are triazoles and combinations of triazoles and carbendazim (Nel *et al.*, 1999). Triazole fungicides inhibit the C14 demethylation step in fungal ergosterol biosynthesis and are referred to as demethylation inhibitors (DMIs) (Copping *et al.*, 1984). Resistance

against DMIs develops gradually and a decrease in sensitivity of pathogen populations, as revealed by monitoring tests, manifest themselves progressively. This type of resistance is referred to as multi-step or directional resistance. The frequency of future resistant types depends on their fitness, which according to Hartl (1980) constitutes all factors favourable for the production of progeny. Thus some genetic changes, conferring resistance, are linked to lower fitness in absence of the fungicide, while other changes are not linked (Chin, 1987; Shaw, 1989).

A number of *in vitro* assays have been conducted world-wide to determine the sensitivity of different *R. secalis* populations towards triadimenol, propiconazole, carbendazim and prochloraz (Hollomon, 1984; Sheridan & Nendick, 1989; Jones, 1990; Kendall & Hollomon, 1990; Margot *et al.*, 1990; Kendall *et al.*, 1993). Only a few studies have investigated the pathogenicity of *R. secalis* field isolates which differed in fungicide sensitivity to determine which factor influenced fungicide resistance development (Hunter *et al.*, 1986; Kendall *et al.*, 1993; Kendall *et al.*, 1994).

In the past some studies have demonstrated fitness costs associated with DMI resistance (Nuninger-Ney *et al.*, 1989; De Waard & Van Nistelrooy, 1990), while others have not (Schepers, 1985; Hunter *et al.*, 1986; Peever & Milgroom, 1994). In a study by Schepers (1985), it was found that within a group of resistant *Sphaerotheca fuliginea* isolates, no relation existed between the degree of resistance to DMI's and the degree of fitness.

Race characterisation of the South African *R. secalis* population showed that the virulence spectrum varied considerably (Part 2). The population has therefore the potential to overcome host resistance genes. Barley scald control will consequently depend heavily on the durability of fungicides. Possible development of fungicide resistance in the *R. secalis* population of South Africa should therefore be anticipated.

In Part 4, *R. secalis* populations in the Western Cape province were monitored for their *in vitro* sensitivity to tebuconazole as well as other triazoles. The average minimum inhibitory concentration (MIC) of tebuconazole, determined over three years, was 3 µg/ml *in vitro*. *R. secalis* isolates with average MIC values were significantly less sensitive towards tebuconazole than wild-type isolates, indicating a shift in sensitivity *in vitro*. However, it is not known how a change in MIC from 1 µg/ml to 10 µg/ml *in vitro* will relate to or effect the pathogen's ability to infect and sporulate on tebuconazole treated leaves. It is also not known how this will influence

the pathogenicity (fitness) of the fungus in the absence of the fungicide. In the present study, the percentage lesion area and subsequent sporulation of isolates differing in fungicide sensitivity (MIC = 1 – 10 µg/ml), was investigated on treated and untreated barley plants. Ideally the behaviour of characterised isolates should be studied under field conditions in order to anticipate the change in effectiveness of fungicides towards less sensitive types under field conditions. Unfortunately, fluctuating weather conditions make it difficult to conduct conclusive field studies regarding *R. secalis* (Scott, 1994; Scott & Potgieter, 1995; Scott *et al.*, 1997). In order to have a more controlled environment, relationships between *in vitro* sensitivity and fitness were therefore investigated under glasshouse conditions.

MATERIALS AND METHODS

In vitro tebuconazole sensitivity assay

A subset of *R. secalis* isolates collected in 1995 from the Rûens area was selected, according to their sensitivity towards tebuconazole, based on results from a previous *in vitro* screening (Part 4). Each combination of isolate and tebuconazole concentration was evaluated again and replicated three times in this study.

For the *in vitro* fungicide sensitivity test, sterile lima bean agar (LBA) was amended with technical grade tebuconazole, provided by Bayer. The 97% technical grade fungicide was dissolved in a 70% ethanol solution to create a stock solution of 4000 µg ai/ml. Fungicide concentrations of 0, 0.03, 0.1, 0.3, 1, 3, 10 and 30 µg ai/ml were prepared by dilution of the stock solution into autoclaved LBA after being cooled to 50°C. Control plates consisted of LBA and ethanol (which never exceeded 1.1%). Medium was poured into Petri dishes (90 mm diam.), and a 27 mm^3 mycelial plug from the edge of an actively growing culture was inverted and placed in the centre of each plate. Fungal growth was assessed after 14 days of incubation at 17°C in the dark, and the minimum fungicide concentration required to inhibit growth (MIC) recorded.

In vivo tebuconazole sensitivity

Rhynchosporium secalis isolates were selected based on repeatable results obtained in the *in vitro* fungicide sensitivity assay. Two *R. secalis* isolates from each of three

different tebuconazole sensitivity classes (*in vitro* MICs of either 1, 3 or 10 µg/ml) were arbitrarily chosen for this study. Taking into account the average MIC values obtained during three seasons (1993 = 3.1 µg/ml; 1994 = 3.7 µg/ml and 1995 = 2.9 µg/ml) in the previous *in vitro* screening (Part 4), the sensitivity groups in the context of this study were classified as follows: MIC value of 1 µg/ml = sensitive class; MIC value of 3 µg/ml = intermediate class; MIC value of 10 µg/ml = less sensitive class. The less sensitive class did not necessarily imply total loss of control by the fungicide but the sensitivity groupings were so named for the sake of clarity when discussing results.

Experimental design and methodology for the *in vivo* tebuconazole sensitivity investigation were as follows: The percentage leaf area affected and sporulation ability of each isolate was measured on treated and untreated barley leaves. Susceptible barley plants (Clipper cultivar) were sprayed at the two leaf stage with Folicur 250EC (250g/l tebuconazole, Bayer) at a rate (125 g ai/ha) recommended to barley growers in South Africa. *Rhynchosporium secalis* cultures were incubated on LBA plates at 17 ±1°C in the dark for 2 weeks. Conidial suspensions were obtained from these cultures by adding sterile distilled water to the plates and scraping the conidia from the surface. The resulting conidial suspensions were adjusted to approximately 1 x 10^5 conidia/ml by adding sterile distilled water. Five days after the fungicide application, plants were sprayed with Tween 20 (0.1%) and inoculated with spore suspensions of each isolate. Inoculated seedlings were transferred to a dew chamber where plants were kept for 48h in the dark at approximately 17°C and a relative humidity of 95-98%. Seedlings were then transferred to a glasshouse. Fourteen days after inoculation the percentage lesion area of the second leaf (10 leaves per isolate per block) was determined by using a scanner (Hewlett Packard, Scan Jet 4c) and an image analysis computer programme (Image09, D. Martin, University of Cape Town, South Africa). Each treatment-isolate combination was replicated three times with pots arranged in a randomised block design and the whole experiment repeated three times.

After lesion percentages were determined, leaves were dried for two days in a paper press. Symptomatic leaves were subsequently cut into ten pieces (measuring 0.5 x 1 cm) per replicate. Leaf pieces were floated on 25 ml of sterile, distilled water in a Petri dish for 4 days in a 15°C incubator to induce sporulation. After incubation,

leaf disks and water were stirred vigorously for 3 min by means of a magnetic stirrer (Jackson & Webster, 1976). The concentration of the resulting spore suspensions was determined by using a haemacytometer for three replicates.

The data were processed using a randomised block analysis of variance and contrasts were used in mean separation. Statistical analysis was performed using SAS (Statistical Analysis System, Cary, NC).

RESULTS

In vivo tebuconazole sensitivity assay: leaf area affected

All isolates induced lesions on unsprayed, inoculated plants, which ultimately resulted in a total loss of turgor and collapse of the whole lamina. The mean percentages of lesion area on tebuconazole treated leaves over three trials were as follows: 23.8% caused by isolates sensitive towards tebuconazole, 32.0% caused by isolates in the intermediate class and 35.1% caused by isolates of the less sensitive class. In general, mean percentage lesion area differed significantly between sensitivity classes but not within sensitivity classes (Table 1). A significant difference was observed mainly between the sensitive and less sensitive class regarding the mean percentage lesion area on tebuconazole treated barley plants (Table 1). The less sensitive class was repeatedly responsible for a greater percentage lesion area than the sensitive class (Fig. 1). However, isolates with a MIC equal to 3 µg/ml, when compared to isolates with lower and higher *in vitro* sensitivities, varied significantly from their intermediate position in the *in vivo* trials.

In vivo tebuconazole sensitivity assay: degree of sporulation

Sporulation on lesions of untreated and tebuconazole treated leaves was not significantly different (Table 2, Fig. 2). There was also no significant difference in sporulation within sensitivity classes or between sensitivity classes on tebuconazole treated and untreated leaves (Table 2, Fig. 2). The average sporulation on tebuconazole treated leaves over three trials was as follows: 28 722 conidia/ml/cm^2 from isolates sensitive towards tebuconazole, 35 500 conidia/ml/cm^2 from isolates in the intermediate class and 40 233 conidia/ml/cm^2 from isolates least sensitive towards tebuconazole. The average sporulation on untreated leaves over three trials was as

follows: 58 611 conidia/ml/cm^2 from isolates sensitive towards tebuconazole, 31 522 conidia/ml/cm^2 from isolates in the intermediate class and 31 389 conidia/ml/cm^2 from isolates least sensitive towards tebuconazole. No significant fungicide-isolate interaction was observed regarding the amount of spores produced (Table 2).

DISCUSSION

All *R. secalis* isolates included in this study were equally fit to induce lesions and sporulate on barley plants in the absence of tebuconazole. Thus no significant fitness cost to the pathogen was associated with a decrease in tebuconazole sensitivity. These results were consistent with the behaviour of *R. secalis* isolates in the UK differing in triadimenol sensitivity *in vitro,* since triadimenol resistant strains were as virulent as sensitive ones (Kendall *et al.*, 1993). Furthermore, in a study dealing with carbendazim resistant field isolates of *R. secalis,* pathogenicity was not correlated with fungicide sensitivity (Kendall *et al.*, 1994). Based on results obtained in the present study, the *in vitro* shift in sensitivity may have been too small to cause a decrease in fitness with regard to the leaf area affected or sporulation ability.

All *R. secalis* isolates examined were able to cause infection on tebuconazole treated leaves, but differed significantly regarding their ability to cause disease on tebuconazole treated leaves. Isolates least sensitive towards tebuconazole *in vitro* were more adapted on tebuconazole treated leaves, being able to repeatedly cause larger lesions than the sensitive *R. secalis* isolates. *R. secalis* employs toxic metabolites capable of moving across cell walls to release nutrients from host cells (Wevelsiep *et al.*, 1993). It has been demonstrated that sterilised culture filtrates of *R. secalis* were able to induce visible symptoms and it was thus concluded that these metabolites are responsible for disease symptoms in infected barley plants (Ayesu-Offei & Claire, 1971). It is therefore possible, via the inhibitory action of tebuconazole, that less metabolites were produced by sensitive *R. secalis* isolates, resulting in smaller leaf areas affected by typical scald lesions.

According to the *in vitro* study, isolates with a MIC of 3 µg/ml were described as having an intermediate sensitivity towards tebuconazole, compared to isolates with a MIC of 1 µg/ml and 10 µg/ml. However, the leaf area affected by intermediate isolates varied from being less than those caused by sensitive isolates (MIC = 1 µg/ml) to being greater than those caused by less sensitive isolates (MIC = 10 µg/ml).

It thus seems that the fitness of intermediate isolates was variable with regards to percentage lesion area. However, the relationship of *in vitro* sensitive isolates and less sensitive isolates remained stable over three trials with regard to the leaf area covered by lesions on tebuconazole treated leaves. The shift in sensitivity that has been observed *in vitro* was therefore a significant and stable shift *in vivo*. This finding has implications for scald control employing tebuconazole. For example, if a tebuconazole sensitive population would shift to an average MIC of 10 µg/ml, the degree of scald control could decrease under field conditions. However, to make any predictions of control under field conditions, other factors influencing fitness and disease control need also to be taken into consideration.

Regarding sporulation, no significant treatment-isolate interaction was found which indicates that differences in sporulation between isolates were consistent when compared on treated and untreated leaves. This suggests that no specific isolate-treatment combination was significantly more favoured in producing conidia than any other combination. It should be noted that sporulation was measured as conidia/ml/cm^2, which means that it was measured from standardised leaf pieces excised from symptomatic leaf areas. Although spore density did not differ significantly between isolates, greater symptomatic leaf areas implicated more spores. Less sensitive isolates, being able to affect greater leaf areas, would therefore be able to outcompete sensitive isolates in producing more progeny for the next generation.

Sporulation ability did not seem to be influenced by tebuconazole sensitivity. An explanation for these results is that the difference in *in vitro* sensitivity towards tebuconazole may have been too small to significantly influence sporulation. To confirm results of the present study, the *in vivo* fitness of isolates with greater MIC values should also be investigated. Studying other factors such as the duration from inoculation until sporulation and symptom expression may reveal fitness costs associated with reduced tebuconazole sensitivity.

In conclusion, larger leaf areas affected and adequate sporulation suggest that a less sensitive population would result in more disease in tebuconazole treated fields. This might not have an immediate effect on disease control but would have a significant impact on the build-up of a resistant field population.

REFERENCES

Ayesu-Offei, E. N. & Claire, B. G. (1971). Symptoms of scald disease induced by toxic metabolites of *Rhynchosporium secalis*. *Australian Journal of Biological Sciences* **24**, 169-174.

Chin, K. M. (1987). A simple model of selection for fungicide resistance in plant pathogen populations. *Phytopathology* **77**, 666-669.

Copping, L. G., Birchmore, R. J., Wright, K. & Godson, D. H. (1984). Structure-activity relationships in a group of imidazole-1-carboxamides. *Pesticide Science* **15**, 280-284.

De Waard, M. A. & Van Nistelrooy, J. G. M. (1990). Stepwise development of laboratory resistance to DMI-fungicides in *Penicilium italicum*. *Netherlands Journal of Plant Pathology* **96**, 321-29.

Hartl (1980). *Principles of population genetics.* Sinauer Associates, Massachusetts.

Hollomon, D. W. (1984). A laboratory assay to determine the sensitivity of *Rhynchosporium secalis* to the fungicide triadimenol. *Plant Pathology* **33**, 65-70.

Hunter, T., Jordan, V. W. L. & Kendall, S. S. (1986). Fungicide sensitivity changes in *Rhynchosporium secalis* in glasshouse experiments. *British Crop Protection Conference - Pests and Diseases*, 523-530.

Jackson, L. F. & Webster, R. K. (1976). The dynamics of a controlled population of *Rhynchosporium secalis*, changes in race composition and frequencies. *Phytopathology* **66**, 726-728.

Jones, D. R. D. (1990). Sensitivity of *Rhynchosporium secalis* to DMI fungicides. *Brighton Crop Protection Conference - Pests and Diseases*, 1135-1140.

Kendall, S., Hollomon, D. W., Ishii, H. & Heany, S. P. (1994). Characterisation of benzimidazole-resistant strains of *Rhynchosporium secalis*. *Pesticide Science* **40**, 175-181.

Kendall, S. J. & Hollomon, D. W. (1990). DMI resistance and sterol 14a demethylation in *Rhynchosporium secalis*. *Brighton Crop Protection Conference - Pest and Diseases*, 1129-1134.

Kendall, S. J., Hollomon, D. W., Cooke, L. R. & Jones, D. R. (1993). Changes in sensitivity to DMI fungicides in *Rhynchosporium secalis*. *Crop Protection* **12**, 357-362.

Margot, P., Kirk, W. W. & Taylor, A. C. (1990). Sensitivity to propiconazole of *Rhynchosporium secalis* populations from individual barley fields in North East Scotland and East Anglia. *Brighton Crop Protection Conference - Pest and Diseases*, 1141-1146.

Nel, A., Krause, M., Ramautar, N. & Van Zyl, K. (1999). *A guide for the control of plant diseases.* National Department of Agriculture, Pretoria.

Nuninger-Ney, C., Schwinn, F. J. & Staub, T. (1989). *In vitro* selection of sterol-biosynthesis inhibitor (SBI)-resistant mutants in *Monilinia fructicola* (Wint.) Honey. *Netherlands Journal of Plant Pathology* **95**, 137-150.

Peever, T. L. & Milgroom, M. G. (1994). Lack of correlation between fitness and resistance to sterol biosynthesis-inhibiting fungicides in *Pyrenophora teres*. *Phytopathology* **84**, 515-19.

Schepers, H. T. A. M. (1985). Fitness of isolates of *Sphaerotheca fulginea* resistant or sensitive to fungicides which inhibit ergosterol biosynthesis. *Netherlands Journal of Plant Pathology* **91**, 65-76.

Scott, D. B. (1994). *Die bepaling van ekonomiese drumpelwaardes vir die toedienings van swamdoders by gars*. LNR-Kleingraaninstituut, Bethlehem.

Scott, D. B. & Potgieter, H. L. J. (1995). *Die bepaling van ekonomiese drumpelwaardes vir die toediening van swamdoders by gars*. LNR-Kleingraaninstituut, Bethlehem.

Scott, D. B., Potgieter, H. L. J. & Smit, H. A. (1997). *Die bepaling van ekonomiese drumpelwaardes vir die toediening op gars*. LNR-Kleingraaninstituut, Bethlehem.

Scott, D. B., van Niekerk, H. A. & Paxton, T. G. (1992). Effect of propiconazole on necrotrophic fungi and yield of barley genotypes differing in susceptibility to *Rhynchosporium secalis*. *Crop Protection* **11**, 243-247.

Shaw, M. W. (1989). A model of the evolution of polygenically controlled fungicide resistance. *Plant Pathology* **38**, 44-55.

Sheridan, J. E. & Nendick, D. K. (1989). Control of scald and net blotch of barley with DMI fungicides. *Proceedings of the 42nd New Zealand Weed and Pest Control Conference*, 221-224.

Trench, T. N., Wilkinson, D. J. & Esterhuysen, S. P. (1992). *South African Plant Disease Control Handbook.* Farmer Support Group, University of Natal, Pietermaritzburg.

Wevelsiep, L., Rupping, E. & Knogge, W. (1993). Stimulation of barley plasmalemma H+-ATPase by phytotoxic peptides from the fungal pathogen *Rhynchosporium secalis*. *Plant Physiology* **101**, 297-301.

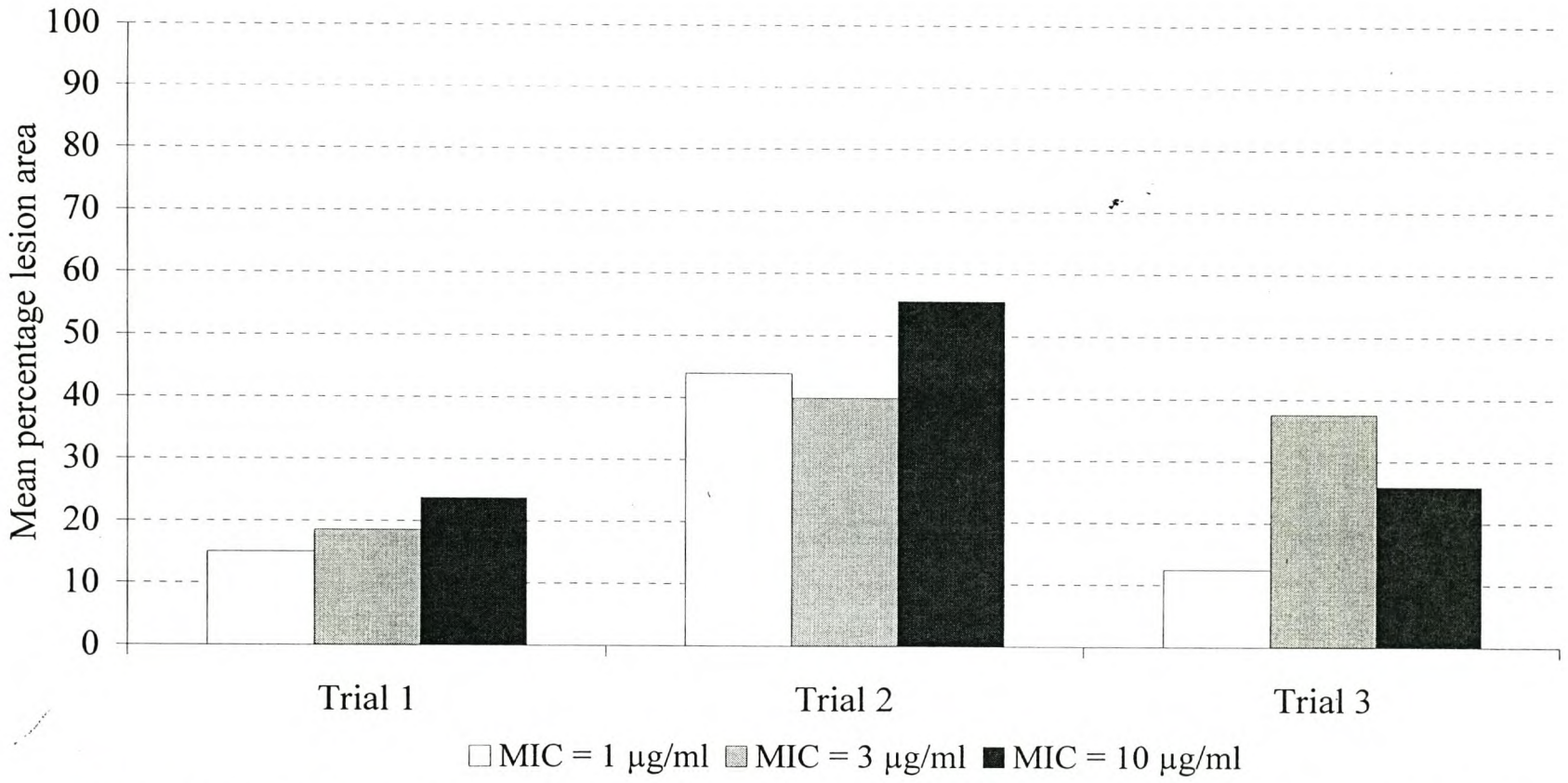

Fig. 1. Mean percentage lesion area induced on tebuconazole treated barley leaves by *Rhynchosporium secalis* isolates differing in tebuconazole sensitivity.

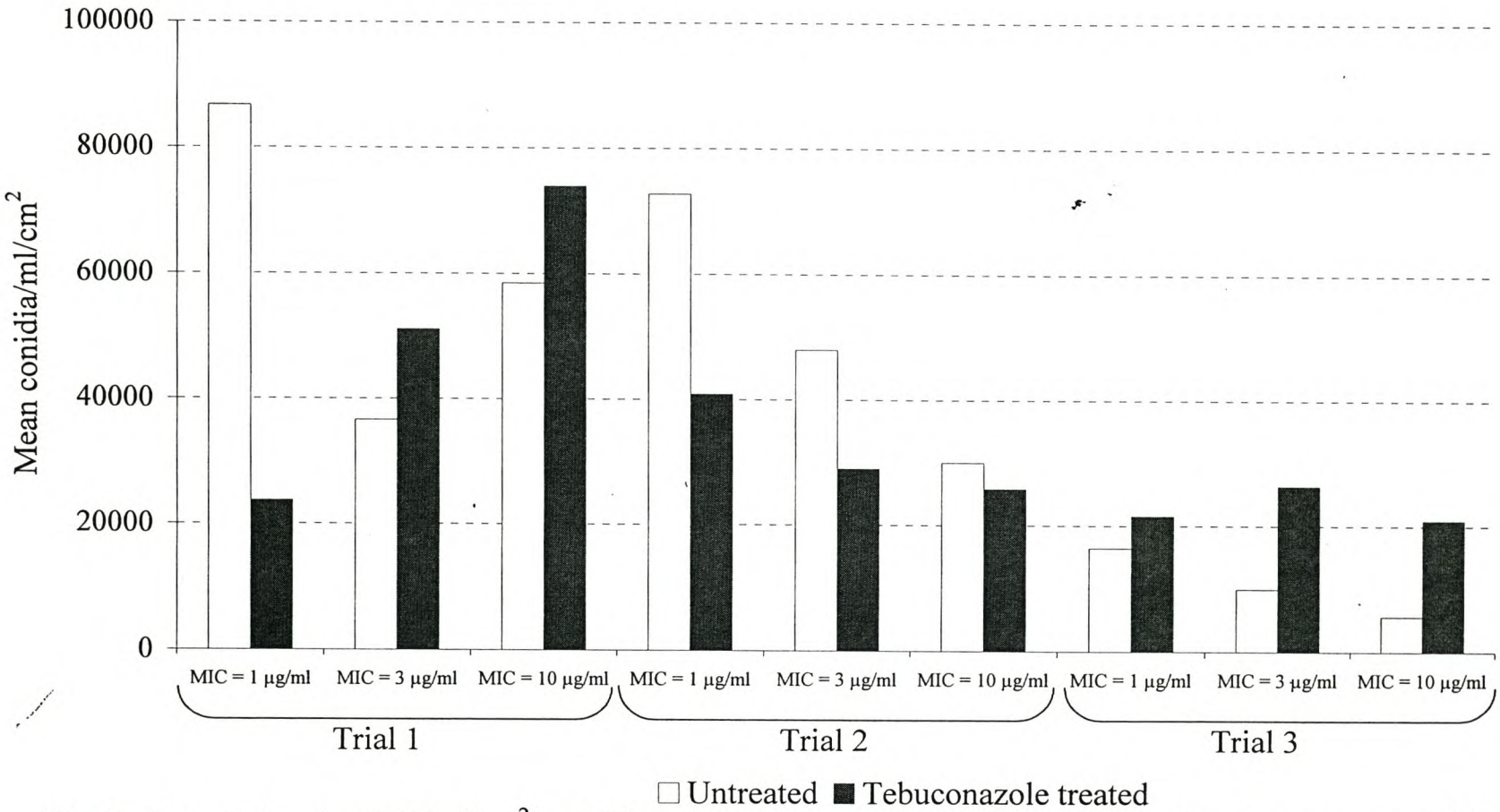

Fig. 2. Sporulation (conidia/ml/cm^2) of *Rhynchosporium secalis* isolates differing in tebuconazole sensitivity on tebuconazole treated and untreated barley leaves.

Table 1. Analysis of variance of mean percentage lesion area on barley leaves treated with tebuconazole and inoculated with *Rhynchosporium secalis* isolates differing in tebuconazole sensitivity

		Trial 1		Trial 2		Trial 3	
Source	df	Mean square	*P*-value	Mean square	*P*-value	Mean square	*P*-value
Block	2	40.9329	0.5622	65.9922	0.3130	190.0069	0.0842
Isolate	5	77.2992	0.3952	218.7375	0.0233	540.3667	0.0017
Between sensitivity classes	2	115.8663	0.2268	395.6428	0.0089	949.1277	0.0008
MIC1 vs MIC10	1	228.9880	0.0944	409.7345	0.0173	546.3451	0.0126
MIC3 vs (MIC1 + MIC10)/2	1	2.7445	0.8437	381.5511	0.0205	1351.9103	0.0008
Within sensitivity classes	3	51.5878	0.5370	100.8006	0.1784	267.8593	0.0301
Within MIC10	1	70.7954	0.3284	20.0934	0.5422	799.4913	0.0043
Within MIC3	1	67.2680	0.3402	137.8563	0.1294	3.1974	0.8211
Within MIC1	1	16.7000	0.6286	144.4523	0.1216	0.8894	0.9050
Error	10	67.0676		50.4733		59.3578	
Corrected Total	17						

Table 2. Analysis of variance on the degree of sporulation (conidia/ml/cm^2) of *Rhynchosporium secalis* isolates differing in tebuconazole sensitivity on tebuconazole treated and untreated barley leaves

		Trial 1		Trial 2		Trial 3	
Source	df	Mean square	*P*-value	Mean square	*P*-value	Mean square	*P*-value
Block	2	7.2569×10^{10}	0.1452	4.6106×10^{10}	0.0605	21.3774×10^{9}	0.1061
Fungicide	1	2.7115×10^{10}	0.3843	7.5717×10^{10}	0.0320	33.7947×10^{9}	0.0599
Isolate	5	3.9117×10^{10}	0.3707	3.7379×10^{10}	0.0548	3.0548×10^{9}	0.8731
Between sensitivity classes	2	3.7466×10^{10}	0.3541	6.4149×10^{10}	0.0450	2.8502×10^{9}	0.6086
MIC1 vs MIC10	1	1.8260×10^{10}	0.4740	12.5282×10^{10}	0.0075	4.8735×10^{9}	0.4593
MIC3 vs (MIC1 + MIC10)/2	1	5.6672×10^{10}	0.2127	0.3016×10^{10}	0.6521	0.8269×10^{9}	0.7593
Within sensitivity classes	3	4.0217×10^{10}	0.3442	1.9532×10^{10}	0.3736	3.1911×10^{9}	0.6415
Within MIC10	1	8.8580×10^{10}	0.1229	0.3169×10^{10}	0.6440	0.0016×10^{9}	0.9656
Within MIC3	1	1.9200×10^{10}	0.4630	0.2494×10^{10}	0.6817	0.0368×10^{9}	0.9484
Within MIC1	1	1.2871×10^{10}	0.5471	5.2934×10^{10}	0.0686	9.5203×10^{9}	0.3039
Fungicide*Isolate	5	6.8717×10^{10}	0.1189	1.7900×10^{10}	0.3243	5.6122×10^{9}	0.6622
Error	22	3.4411×10^{10}		1.4433×10^{10}		8.5906×10^{9}	
Corrected Total	35						