COMPARATIVE STUDIES ON GENETIC VARIABILITY AND FUNGICIDE RESISTANCE IN *TAPESIA YALLUNDAE*

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DECLARATION

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

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OPSOMMING

Hierdie studie ondersoek die genetiese variasie, reproduksie dinamika en fungisied weerstand in *Tapesia yallundae*. Elke hoofstuk handel oor spesifieke maar verwante onderwerpe. Oogvlek is 'n belangrike siekte van lentekoring (*Triticum aestivum* L.). Vier spesies van *Ramulispora* word geassosieer met die siekte, waarvan *Tapesia yallundae* en *T. acuformis* mees algemeen voorkom. *T. yallundae*, wat tans die enigste spesie is wat in Suid-Afrika aangeteken is, het al verliese van tot 50% veroorsaak. Om meer akkurate en effektiewe beheermaatreëls te implementeer, is dit noodsaaklik om die oorlewingsdinamika van die patogeen te verstaan. Van al die siektebeheermaatreëls soos kulturele praktyke, sanitasie, biologiese beheer ens., bly fungisiedbehandeling die mees algemene maatreël vir die beheer van oogvlek.

Fungisiedtoediening het egter ook verskeie probleme. Die patogeen kan weerstand opbou teen die fungisied. Die mees algemene fungisiedes wat vir oogvlekbeheer aangewend word sluit onder meer die benzimidasool karbendazim in, triasole soos flusilasool, tebukonasool, propikonasool, bromukonasool, flutriafol, fenbukonasool, triadimenol, en die imidasool, prochloraz. Weerstand is egter reeds teen hierdie middels bekend. Gedurige monitering vir weerstand is dus krities om die vermorsing van fungisied en besoedeling van die omgewing met oneffektiewe middels te beperk. In hoofstuk 2 van hierdie manuskrip word 300 isolate van *T. yallundae* van 15 lande geëvalueer vir weerstand teenoor karbendazim, flusilasool, tebukonasool, propikonasool, bromukonasool, flutriafol en fenbukonasool. Resultate dui daarop dat teen sommige van hierdie triasole, soos bv. fenbukonasool, daar reeds 'n hoë vlak van weerstand teenwoordig was in veldpopulasies.

In 'n seksueel reproduserende fungus soos *T. yallundae*, is dit noodsaaklik om te bepaal wat sy vermoë is om weerstandbiedenheid aan die nageslag oor te dra. Om die rede is paringstudies ook op ouers wat tekens van weerstand teenoor triasole getoon het uitgevoer. Drie generasies was gevolglik getoets vir weerstand teenoor vyf triasole, naamlik flusilasool, tebuconasool, propikonasool, brumukonasool en flutriafol. Resultate van die studie het 'n variasie in sensitiwiteit van die nageslag getoon, wat op 'n kwantitatiewe oorerwing van weerstand teen triasole dui. Alhoewel die teleomorf nog

nie in lande in Suid-Afrika opgemerk is nie, lê hierdie kennis die fondament vir die langtermyn vertolking van die populasie dinamika van hierdie fungus.

Die vermoë van 'n heterotalliese askomiseet populasie om seksueel voort te plant is afhanklik van die beskikbaarheid van sy twee paringstipes, MAT1-1 en MAT1-2, hul verpreiding, vroulike vrugbaarheid en ander faktore. Alhoewel die teleomorf algemeen in lande in die Verenigde Koninkryk opgemerk word, is dit in kontras met die situasie in Suid-Afrika, waar hierdie stadium nog slegs in die laboratorium geïnduseer kon word. 'n Studie is dus onderneem om die Suid-Afrikaanse en V.K. populasies met mekaar te vergelyk. Isolate van die twee populasies is dus gepaar met paringsisolate as beide sperm ontvangers en sperm donors. Hierdie prosedure het dit moontlik gemaak om die persentasie hermafrodiete te bepaal. Geen verskille in vroulike fertiliteit is tussen die Suid-Afrikaanse en V.K. populasies bespeur nie, en beide populasies het ook 'n lae effektiewe populasie getal getoon. Hierdie data het dus voorgestel dat die teleomorf ook meer algemeen in Suid-Afrika sou voorkom as die klimaat meer geskik was vir teleomorf vorming.

Die resultate van hierdie studie het tot die slotsom gelei dat oogvlek steeds deur fungisiedbehandeling in Suid-Afrika beheer kan word. Alhoewel daar 'n merkbare verskuiwing in sensitiwiteit teenoor fenbukonasool en flusilasool was, was geen weerstand teenoor karbendazim waargeneem nie. Laasgenoemde kan dalk toegeskryf word aan die afwesigheid van die teleomorf in die veld, gekombineer met die monosikliese natuur van die patogeen en gebruik van alternerende fungisiedes. Die afwesigheid van *T. acuformis* maak die plaaslike siektetoestand minder gekompliseerd in terme van fungisied aanwending en bestuur. Voortdurende opnames sal egter uitgevoer moet word om hierdie situasie ook in die toekoms te monitor.

SUMMARY

Eyespot is an important disease of spring wheat (*Triticum aestivum* L.). Four species of *Ramulispora* are associated with this disease, of which *Tapesia yallundae* and *T. acuformis*, are common. This thesis investigates the broader subjects of genetic variability, reproductive dynamics and fungicide resistance in *Tapesia yallundae*. Each of the chapters treats specific but related topics. *T. yallundae*, which is the only species thus far reported from South Africa, has been associated with yield losses of up to 50%. To enable the implementation of more accurate and effective control measures, understanding the dynamics of reproduction and the genetics of the pathogen is of utmost importance. Of the many plant disease control measures such as cultural practices, sanitation, biological control, etc., fungicide application is the most commonly resorted to measure in eyespot control. This thesis investigates the broader subjects of genetic variability, reproductive dynamics and fungicide resistance of *Tapesia yallundae*.

Fungicide application, however, is not without problems. The pathogen can build up resistance to fungicides. The most commonly used fungicides in eyespot control include the benzimidazole carbendazim, triazoles such as flusilazole, tebuconazole, propiconazole, bromuconazole, flutriafol, fenbuconazole, triademinol, and the imidazole, prochloraz. Cases of resistance to the groups listed above have been reported. Frequent monitoring for resistance is thus crucial to prevent wastage of fungicide and unnecessary impregnantation of the environment with potentially ineffective chemicals. In chapter 2 of this thesis 300 isolates of *T. yallundae* from 15 fields were evaluated for resistance against carbendazim, flusilazole, tebuconazole, propiconazole, bromuconazole, flutriafol and fenbuconazole. These results indicated that to some triazoles, such as fenbuconazole, a high level of resistance was already present in field populations.

In a sexually reproducing fungus such as *T. yallundae*, knowledge pertaining to its ability to pass resistance factors to offspring is equally important. Mating studies were, therefore, also conducted with parental strains that showed signs of triazole resistance. Three generations were subsequently tested for resistance to five triazoles, namely flusilazole, tebuconazole, propiconazole, bromuconazole and flutriafol. Results of this study showed variable sensitivity in progeny, which indicated quantitative inheritance of resistance to triazoles. Although the sexual stage

has not yet been observed in the field in South Africa, this knowledge lays the foundation for the long-term understanding of the population dynamics of the fungus.

The ability of a heterothallic ascomycete population to reproduce sexually is dependent on the availability of its two mating types, MAT1-1 and MAT1-2, their distribution, and female fertility amongst other factors. In the U.K. the teleomorph is commonly observed in the field, which is in contrast to the situation in South Africa, where it has only been induced in the laboratory. A comparative study between the South African and the U.K. populations was therefore undertaken. Isolates representative of the two populations were mated with tester strains as both sperm recipients and as sperm donors. This allowed the percentage of hermaphrodites to be determined. No difference in terms of female fertility was observed between the South African and the U.K. populations, with both populations showing low effective population numbers. These data suggested, therefore, that the teleomorph would also occur more frequently in South Africa if the climate was more indusive to its development.

The overall results of this study indicated that eyespot could still be controlled by means of fungicide application in South Africa. Although a shift in sensitivity was observed towards fenbuconazole and flusilazole, no resistance was detected towards carbendazim. The latter might be due to the absence of the sexual stage in the field, coupled by the monocyclic nature of the pathogen and sensible fungicide regimes. The absence of *T. acuformis* makes the disease situation less complicated in terms of fungicide application and management. Continuous surveys will have to be conducted, however, to monitor this situation in future.

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CONTENTS

1. THE REPRODUCTIVE CHARACTERISTICS OF ASCOMYCETES, WITH REFERENCE TO *TAPESIA YALLUNDAE* - AN OVERVIEW

ABSTRACT

Reproduction, either sexual or asexual, is crucial for a pathogen to survive in its environment. Favourable environmental conditions allow the pathogen to expand its biomass and pass its genes to succeeding generations. For a plant pathologist it is important to understand the strategies employed during infection, the relationship between the pathogen and other inhabitants of the niche, the possible occurrence of the sexual cycle and disease control. This review focuses on the vegetative and sexual aspects of the ascomycete, *Tapesia yallundae*, fungicide sensitivity, the role played by the environment in influencing sexual reproduction, and genetic control in reproduction and fungicide sensitivity.

INTRODUCTION

Eyespot, caused by *Tapesia yallundae* Wallwork & Spooner, is a chronic yield limiting disease of wheat (*Triticum aestivum* L.). The pathogen can persist in stubble residues for several years (Dyer and Lucas, 1995), where it produces conidia (Fig. 1) that are disseminated by rain splash to nearby plants from autumn through early spring (Murray, 1996). A critical stage in eyespot development is the initiation of lesions in the stem, before the adjacent leaf sheaths wither (Higgins *et al.*, 1986). Eyespot lesions only cause yield loss when there is substantial girdling of the stem, particularly if the stem is softened and predisposed to lodging (Fig. 2) (Jones, 1994). The eyespot fungus is an important component of the parasitic complex of stem-base rot fungi, occurring in association with *Rhizoctonia* and *Fusarium* spp. (Goulds and Polley, 1990). Economic damage does not occur until the fungus penetrates the stem and interferes with sap circulation (Thomas *et al.*, 1992). Yield losses in untreated fields can reach 30% of the total production, but vary greatly according to field location and season (Lagneau *et al.*, 1986). Brennan and Murray (1989) estimated the average loss from eyespot from southern New South Wales to be around $A 2 million/year.

The asexual stage, *Ramulispora herpotrichoides* (Fron) Arx, has been known since 1912, but the teleomorph remained unknown until it was recently found in Australia, and described as *Tapesia yallundae* (Wallwork and Spooner, 1988). Using newly developed molecular fingerprinting techniques, *T. yallundae* and *T. acuformis* (Boerema, Pieters & Hamers) Crous have been confirmed as the teleomorphs of the two major species (former pathotypes) of *Ramulispora herpotrichoides*, now known as *R. herpotrichoides* and *R. acuformis*. These species are routinely isolated from lesions in the field and may be differentiated on the basis of a variety of cultural characteristics (Fig. 3), biochemical tests (Daniels *et al.*, 1995) and molecular markers (Dyer *et al.*, 1993b). However, specific identification of *T. yallundae* is difficult because other stem-base pathogens may cause similar symptoms. The pathogen is slow growing in culture, and its presence is often masked by the other faster growing stem-base pathogens or saprophytes (Priestly and Dewey, 1993). The purpose of this review is to arm the reader with knowledge of the sexual reproductive dynamics of this fungus, and how this process influences its fungicide sensitivity.

EPIDEMIOLOGY AND RESISTANT CULTIVARS

Tapesia yallundae survives the inter-crop period on infected stubble and it has been shown to be infective even after burial for up to three years. This long period of saprophytism of the fungus has been associated with its low rate of cellulose decomposition, which would lead to conservation of substrate reserves. It is not, therefore, surprising that it has been demonstrated repeatedly that breaks of 1 to 2 years from wheat or barley cultivation do not lead to the elimination of eyespot. A break of 3 years will often give practical control without eliminating the fungus. After a serious attack, it is desirable to leave an interval of up to 5 years before replanting with wheat or barley. Eyespot has thus been found to occur in fields which have not carried a susceptible crop for 5 successive years. Experiments at Rothamsted showed that when wheat is grown continuously on the same land there is no decline with time in the incidence of eyespot (Western, 1971).

Whilst debris constitutes the main source of inoculum, volunteer wheat plants have also been shown to act as secondary hosts, but they are not as important as stubble in this context. The disease builds up under continuous cereal growing

systems, and is most common on heavy, wet soils. Large applications of nitrogen will also accentuate the disease by producing an abundance of tillers with an accompanying dense crop with high humidity beneath the canopy. The control of eyespot involves the use of resistant varieties and fungicide application. Furthermore, much can be done to reduce the risk of disease by appropriate cultural practices, especially in terms of stubble burning, rotation and correct manure practices.

Epidemiology concerns populations, not individuals, and not one population but two, namely the host and the parasite, and their interaction. *T. yallundae* is a monocyclic pathogen, as it completes all of its cycle of pathogenesis in one cropping season, from the germination of propagules to full disease occurrence or establishment in the host. As it survives in residue stubble, stubble burning and tillage are factors that reduce the initial inoculum for the following season (Q) in the formula:

$$x_t = QRt$$

from Vanderplank, (1982), where x_t = the amount of disease at time t, Q = initial inoculum, R = efficacy of the initial inoculum and t = the length of time of pathogen interaction with the host and the environment. Should the initial inoculum (Q) be reduced in the equation the amount of disease (x_t) is reduced. The use of resistant cultivars has the same effects as sanitation. Sowing a variety resistant to 99% of eyespot spores in the field is identical in effect with sanitation that destroys 99% of spores in the soil. Resistance and sanitation in this example each reduces x_t to 1/100 of what it otherwise would have been.

The short-straw and stiffer straw varieties are more resistant to lodging and should be used in high-risk situations. Partial control by the use of straw shortening and stiffening chemicals such as CCC (chloride-cholinechloride) can also be beneficial. Seedling tests for resistance to *T. yallundae* offer the advantages of shorter screening time and a more uniform environment than screening in the field. In wheat seedlings, the coleoptile and senescent tissues promote infection by *T. yallundae*, while epidermal cell responses including papilla and halo formation, cell wall thickening, and hypersensitive responses in the leaf sheaths serve as barriers to fungal penetration and colonization. These resistance responses occur in both resistant and susceptible cultivars but are more abundant and well-developed in resistant cultivars.

These responses also appear to establish resistance or susceptibility early in pathogenesis, because subsequent growth through leaf sheaths is reported to be independent of cultivar (Strausbaugh and Murray, 1989a).

Differences in variety reaction of wheat to *T. yallundae* have been reported, and plant breeders have been attempting to develop resistant cultivars. Macer (1966) found the most resistant cultivars were Cappelle-Desprez, Viking and Hybride du Joncquois, while *Triticum aethiopicum* showed a higher expression of resistance than any other species of *Triticum*. *Aegilops ventricosa* was almost immune in some tests, while King II rye also showed a high level of resistance.

Seedling tests for resistance to *T. yallundae* offer the advantages of a shorter screening time and a more uniform environment than screening plants in the field. However, the present seedling tests need to be improved to be able to differentiate highly and moderately resistant individuals. This inability to clearly differentiate progeny containing different levels of resistance leads to low heritability when these seedling tests are used to analyse segregating generations. The discovery of a new genetic locus for resistance raises the possibility of combining multiple genes for resistance to eyespot and eliminating the need for fungicide applications. In addition to yield loss, pathogenic specialization that could circumvent existing resistance genes and render them ineffective is of concern. Although strains of *T. yallundae* adapted to specific cultivars, i.e. races, have not been identified, strains adapted to wheat, rye, *Agropyron repens,* and some accessions of *Aegilops squarrosa* are known. As a result of specialization to host species, the durability of eyespot resistance introduced into wheat from other species is questionable. However, a broader genetic base of resistance by building a pyramid of multiple resistance genes into a single cultivar may prevent such changes in the pathogen and thus increase the chances of durable resistance (Murray *et al.,* 1994).

Plant breeders have tried to improve cultivar resistance without sacrificing yield and quality, but have had only limited success. Cappelle-Desprez contains resistance to *T. yallundae* that has remained durable for about 30 years despite widespread exploitation. Investigations have shown that an alien grass species, *Aegilops ventricosa* is highly resistant to *T. yallundae*. Most resistant cultivars are regionally adapted. For instance, two winter wheat cultivars were recently developed

specially for the Pacific Northwest region in the United States. These two cultivars contain the dominant resistance gene found on chromosome 7D in VPM-1 and suitable agronomic characteristics. In the future it will be important to incorporate other resistance genes into wheat cultivars while maintaining or improving grain yield and quality. Several studies that have focused on the inheritance of resistance to *T. yallundae* in winter wheat have indicated that the inheritance of resistance is complex (Law *et al.*, 1976; Jahier *et al.*, 1978; Bruehl, 1983; Murray, 1986), while several others have encountered less problems in this regard (Kimber, 1967; Doussinault *et al.*, 1983, 1983; Gale *et al.*, 1984; Saragoussi, 1986; Worland and Law, 1987).

TAXONOMY

The fungus commonly associated with eyespot disease symptoms of wheat was first described as *Cercosporella herpotrichoides* Fron (Sprague, 1936). Based on the unthickened and inconspicuous conidial scars, several *Cercosporella* species were reallocated to *Pseudocercosporella* Deighton (Deighton, 1973). Two varieties of *P. herpotrichoides* (Fron) Deighton were distinguished, namely *P. herpotrichoides* var. *herpotrichoides* and *P. herpotrichoides* var. *acuformis* Nirenberg (Nirenberg, 1981). In addition, *P. anguiodes* Nirenberg and *P. aestiva* Nirenberg were also described from eyespot lesions on cereals in Germany (Nirenberg, 1981). *P. herpotrichoides* var. *herpotrichoides* and var. *acuformis* were regarded comparable with the respective wheat (W-type) and rye (R-type) pathotypes of this fungus (King and Griffin, 1985; Sanders *et al.*, 1986, Julian and Lucas, 1990). Although these two varieties can be distinguished based on cultural characteristics, these characteristics are unstable (Fitt *et al.*, 1988), and pathogenicity tests are difficult and time consuming (Creighton *et al.*, 1989). Moreover, some isolates appear to be intermediate between the two types (Nicholson and Rezanoor, 1994). Spore morphology is widely used for classification, but spore characteristics, particularly shape and size, vary within individual isolates (Priestly and Dewey, 1992). Poupard *et al.* (1994) state that isolates of var. *herpotrichoides* are generally able to produce quantities of mycelia in a shorter time than var. *acuformis* isolates. This difference related to particular infection structures and processes, was demonstrated by means of electron microscopy. In a study by Walder-Zulauf (1991) investigating equal mixtures of the W- and R-strains on PDA, the R-strain completely dominated the population after two generations.

Differentiation of isolates according to growth speed corresponded better to genetic information than differentiation according to spore morphology (Frei and Wenzel, 1993).

Experiments with seedlings suggested that there were differences between W-type and R-type isolates in the rate of lesion development (Goulds and Fitt, 1991). However, comparative pathogenicity of isolates of *P. herpotrichoides* on seedlings did not relate well to their comparative pathogenicity on adult plants (Higgins and Fitt, 1985). In addition, isolates have also been differentiated by the colour of pigment produced in culture on maize-based agar under near-ultraviolet light (Nicholson *et al.*, 1993).

Analysis of biochemical markers such as isozyme polymorphisms, DNA restriction fragment length polymorphism or randomly amplified polymorphic DNA's (RAPD's) have all revealed consistent differences between groups of isolates, showing good correlation with the distinction between pathotypes (Moreau and Maraite, 1996).

Corlett (1991) listed several species of *Pseudocercosporella* which have teleomorphs in *Mycosphaerella*. The description of the telemorph of *P. herpotrichoides* as *T. yallundae* by Wallwork and Spooner (1988) further supports its separation from *Pseudocercosporella*.

New combinations for the varieties of *P. herpotrichoides* were subsequently introduced as *R. herpotrichoides* var. *herpotrichoides* and var. *acuformis* (Nirenberg) Boerema, Pieters and Hamers (Boerema *et al.*, 1992). Braun (1993) furthermore reduced *P. anguiodes* to an additional variety of *R. herpotrichoides*. Because of the uncertainty surrounding the generic placement of *P. aestiva*, the latter species was not included in *Ramulispora*. Based on data obtained from RAPD analyses, Robbertse *et al.* (1995) established the three varieties as separate species, namely *T. yallundae* [anamorph: *R. herpotrichoides* (Fron) Arx], *T. acuformis* (Boerema, Pieters and Hamers) Crous [anamorph: *R. acuformis* (Nirenberg) Crous], and *R. anguioides* (Nirenberg) Crous. For the remainder of this text the names *T. yallundae* and *T. acuformis* will be used to refer to the two prominent eyespot fungi.

VEGETATIVE COMPATIBILITY

The presence of vegetative incompatibility systems has several practical implications for plant pathologists. Vegetative compatibility groups (VCG's) have been used to study population dynamics of plant pathogenic fungi as well as non-pathogenic species. VCG identification has been useful in determining the sources of races new to a particular geographic area and increasing numbers of VCG's are an indication of genetic diversity within a population (Glass and Kuldau, 1992). Many examples of vegetative incompatibility in filamentous ascomycetes are known. In several species a large number of VCG's occur. Within such groups heterokaryon formation and the exchange of genetic material are possible, but not between groups. Kohn *et al.* (1991) analysed VCG's of *Sclerotinia sclerotiorum* in two neighbouring fields of canola. In this study they found the diversity of VCG's to be high, and demonstrated with molecular data that the VCG's represented genotypically different strains. Lori *et al.* (1996), using genetic complementation tests, demonstrated the existence of single VCG's among *Fusarium oxysporium* f. sp. *dianthi* strains causing carnation wilt in Argentina. Finding a single VCG indicated that the genetic diversity of this pathogen in Argentina was narrow, and that only one race was present in carnation growing areas.

Sources of VCG diversity

In ascomycetes vegetative incompatibility can prevent the somatic exchange of genetic material between cospecifics. Mycelium consists of hyphae, vegetative filaments that are constructed of separated segments, which usually contain several nuclei each. During growth mycelia may encounter cospecifics. If the two cospecifics are vegetatively compatible, they will fuse by a process called anastomosis and form a heterokaryon (Nauta and Hoekstra, 1994). Many fungi produce a barrage reaction at the point where two incompatible strains meet (Rizet, 1952; Rizet and Schecroun, 1959). This barrage is characterised by degenerating, dying hyphae and aborted heterokaryotic cells. In *Podospora anserina*, the barrage is a clear zone where heterokaryotic cells undergo self-lysis as a result of an incompatible reaction (Rizet, 1952; Rizet and Schecroun, 1959; Bernet, 1963). Protection of the genetic integrity of the individual seems the most plausible selection mechanism favouring vegetative incompatibility (Todd and Reyner, 1980). This can

be interpreted as the prevention of conflict between two different nuclear genomes in a heterokaryon (Hartl *et al.*, 1975), and the prevention of an invasion by harmful cytoplasmic elements such as plasmids, viruses and mitochondria (Day, 1968; Caten, 1972). Alternatively, Davis (1966) and Bernet (1992) proposed that the incompatibility reaction is an unselected by-product of genes regulating cell death and a consequence of genetic divergence of natural isolates.

In many species studied the incompatibility reactions are always mediated by many nuclear loci (Puhalla and Spieth, 1985), with two and occasionally with multiple alleles. Since one allelic difference can cause incompatibility, large numbers of VCG's can exist (Nauta and Hoekstra, 1994). Genetic analysis of *P. anserina* has revealed the presence of both allelic and nonallelic incompatibility systems (Esser, 1962; Bernet, 1967; Rossignol and Picard, 1991). *P. anserina* has 17 incompatibility loci, four of which participate in allelic interactions (Rossignol and Picard, 1991), and some of these have several alleles (Bernet, 1967). Studies of allelic interactions in different VCG isolates in *Neurospora crassa* have also provided evidence for the role of proteinaceous cytoplasmic factors in incompatibility (Wilson *et al.*, 1961; Williams and Wilson, 1966).

A large number of VCG's of *Aspergillus flavus* were identified within a cotton field, and the population was unique in comparison to other local field populations (Glass and Kuldau, 1992). It was also noted that the distribution of VCG's changed from year to year, suggesting a high incidence of genetic efflux within the population. The high incidence of VCG diversity was somewhat surprising since *Aspergillus flavus* only reproduces asexually. The authors suggested that the most likely source of VCG diversity was from the influx of spores from other sites and differential changes in the number of strains (Glass and Kuldau, 1992). In *A. nidulans* vegetative incompatibility can be overcome through the use of protoplast fusion and the subsequent generation of heterozygous diploids. This suggests that the cell wall plays a role in vegetative self / nonself recognition.

Protoplast fusion

Protoplast fusion is the underlying process in sexual reproduction and is followed by cell differentiation, which cannot take place without it. Protoplasts of incompatible strains can be fused artificially. The use of protoplast fusion to initiate heterokaryosis

and stimulate interspecific recombination via the parasexual cycle has proved to be a fruitful method for hybrid formation in fungi. Protoplast fusion can overcome many of the non-sexual incompatibility barriers that preclude normal sexual fusion between species (Hocart and McNaughton, 1994), resulting in viable progeny.

The formation of viable fusion products between incompatible strains has been interpreted as evidence that the expression of incompatibility in some species occurs primarily at the level of the cell wall, rather than at the intracellular level. The lower fusion frequencies in incompatible strain combinations found by Di San Lio *et al.* (1994) in a study in *T. yallundae* may indicate that in this species part of the vegetative incompatibility is expressed also at the cytoplasmic level in the fused protoplast. Alternatively, the lower fusion frequency may result from a need for karyogamy following protoplast fusion to generate a viable cell in incompatible crosses, whereas fully compatible strains are able to grow as heteryokaryotic colonies following fusion. Reduced fusion frequency has been reported in other cases where fusion of protoplasts has been used to cross vegetatively incompatible strains. Protoplast fusion can be detected by the use of molecular techniques such as isozyme analysis.

Isozymes are different proteins with distinct genetic origins that catalyse the same reaction. The proteins differ in one or more amino acids, which reflects a difference in the DNA which specifies them. Their effects may vary quantitatively, that is, one isozyme may be more efficient than the other. The product, however, stays the same, namely there is no qualitative difference between products of two isozymes (Vanderplank, 1978). Protoplast fusion determines that the two strains are compatible. In the laboratory, isozyme analysis has been used to show that protoplast fusion has occurred between two parental strains. There has been confirmation of protoplast fusion between *T. yallundae* and *T. acuformis* using esterase isozymes (Hocart *et al.*, 1993).

REPRODUCTIVE CHARACTERISTICS OF ASCOMYCETES

The Ascomycetes represent the largest sub-division of the fungi, containing *ca.* 28650 species in 2720 genera, with the characteristic feature of sexually produced spores being borne in a sac or ascus, typically containing eight ascospores which are

explosively ejected (Dyer *et al.*, 1993b). The asci are contained in different ascocarps such as cleistothecia, apothecia, or perithecia (Moore-Landecker, 1982). Ascomycetes reproduce sexually or asexually, depending on whether conditions favouring plasmogamy, the fusion of cytoplasms of protoplasms of sexual nature prevail. In such a case sexual reproduction takes place. There are clear disadvantages to sexual reproduction. These include the metabolic costs of producing pheromones and forming structures related to sex, the risk of failing to find a compatible partner, and the possibility that recombination may separate a successful combination of alleles (Metzenberg and Glass, 1990). Despite this many fungi retain the ability to reproduce sexually, even if this is rarely manifested in nature. Given that sexuality is easily lost in laboratory cultures, the selective forces for sex in nature must be very strong (Andrew, 1991).

Effective population size, and the role of hermaphrodites and female sterility

The effective population number is a critical parameter used to estimate the effects of drift and inbreeding, and to compare field populations to an idealized population. An idealized member of a heterothallic population of filamentous ascomycetes is a self-sterile hermaphrodite that is capable of producing male gametes and elaborating the female reproductive structure. The effective population number may be based on the number of stains of different mating types or the relative frequency of hermaphrodites. The female-sterile mutants are at a selective disadvantage every time sexual reproduction occurs, and must have an advantage during vegetative propagation to persist at significant frequency. When high frequency of female sterile strains is observed in field populations, it indicates that vegetative propagation is a significant component of the fungus's natural history (Leslie and Klein, 1996).

Selection either for hermaphrodites during sexual reproduction or for female sterile strains during vegetative propagation can drive the population close to an extreme (all hermaphrodites or all female sterile strains). If, however, the environment is such that alternating selection favours first the hermaphrodites and then the female sterile strains, then a sort of equilibrium should ensue (Leslie and Klein, 1996). Female sterility also limits genetic exchange in field populations and reduces the inbreeding population size. The inbreeding effective population size is a property of the proportion of the population in the different classes, as well as the size

of the population. Leslie and Klein (1996) considered two factors that impact the inbreeding effective population size: mating type and female sterility. Mating types are expected at a 1: 1 ratio in heterothallic ascomycetes, since the trait is known to be under the control of single Mendelian locus.

Sexual compatibility

Sexual compatibility is the ability of two isolates of different mating types to form reproductive structures with viable progeny. In the case of *T. yallundae* the formation of apothecia (Figs 4-6) indicates sexual compatibility between crossed isolates. The apothecia bear asci (Fig. 7) that contain ascospores (Fig. 8).

The mating type genes of fungi determine sexual compatibility between different haploid individuals. Mating type genes from several species have now been cloned and sequenced and alternative forms within the same species are generally found to be unrelated or very dissimilar in sequence (Kuees and Casselton, 1992).

Sexual compatibility is largely genetically prescribed, therefore variation within ascospores in an apothecium might favour sexual compatibility depending on whether the variation involves mating type genes. However, it has been found that within an ascus the ratio of male to female ascospores is Mendelian. The availability of a sexual stage permits assortment of genes and facilitates combinations in fungicide sensitivity genes as well.

Homothallic, pseudohomothallic and heterothallic strains

Within the Ascomycetes, sexually reproducing species usually follow one of three basic sexual reproductive strategies, namely homothallic, pseudohomothallic (also called secondary homothallic), and haploid heterothallic, with each species limited to a single reproductive mode. Harrington and McNew (1997), however, found uni-directional mating type switching in *Ceratocystis coerulescens* isolates. This entails self-fertility in this well-known heterothallic species. Individual strains of homothallic fungi and individual haploid, heterokaryotic strains of pseudohomothallic fungi may be self-fertile or may cross with other individual strains of their species to complete their sexual portion of their life cycles. In heterothallic species, the parents of a cross must be of different, usually termed opposite, mating types. Mating type in these fungi is controlled by a single locus with two alleles termed "*A*" / "*a*", "*a*" / "∝",

"+" / "-" , or " *mat 1-1* " / " *mat 1-2* " depending on the organism. All of these loci appear to function as regulators of complex genetic pathways containing numerous genes, many of which are poorly characterised. For population purposes mating type in filamentous ascomycetes can be considered to be determined by one locus with two functional alleles (Moore-Landecker, 1982).

Mating type

Sexual reproduction in heterothallic and pseudohomothallic filamentous ascomycetes requires the participation of the opposite mating type. Individual homokaryotic isolates exhibit the mating behaviour of one or two possible mating types. This one-locus two-allele determination of sexual compatibility is called bipolar (Fincham *et al.*, 1979), and is the characteristic mating system of ascomycetes. Four ascomycetes used extensively as model organisms are the filamentous fungi *Neurospora crassa*, *A. nidulans*, and the yeasts *Saccharomyces cerevisae* and *Schizosaccharomyces pombe.* These eukaryotes have relatively small genomes (about 100 times smaller than the human genome), with little repeated DNA which simplifies molecular studies. Efficient transformation systems for these model fungi allow detailed analysis of gene structure, function and expression. Characterisation of the ascomycete mating system has been carried out largely in *N. crassa*, *S. cerevisiae* and *S. pombe*, because sexual development of these fungi can be precisely controlled in the laboratory, and large numbers of mutant strains are available for study (Nelson, 1996).

Neurospora crassa has two mating types that have been designated A and a (Perkins *et al.*, 1976, 1982). The fusion between a trichogyne and a cell of the opposite mating type initiates the events leading to meiosis and ascospore formation (Backus, 1939; Dodge, 1935; Raju, 1980), a process that is mediated by mating-type specific pheromones (Bistis, 1981, 1983). *N. crassa* forms unitunicate asci borne in superficial perithecia, a characteristic of the Sordariaceae in the series Pyremycetes (Alexopoulos, 1962). Although mating type regulates sexual reproduction in *N. crassa*, it also has a function in the vegetative growth phase. If hyphae of A and a strains fuse, an incompatibility reaction is elicited that results in the death of cells at the fusion point (Beadle and Coonradt, 1944; Sansome, 1946; Gross, 1952; Garnjobst, 1953; Pittenger, 1957). Glass and Kuldau (1992) demonstrated that a single gene

product in a strain confers mating type during sexual reproduction and vegetative incompatibility during asexual growth.

The importance of ascospores in disease occurrence

Asexual reproduction generally occurs by either mycelial propagation or the production of numerous conidia (Alexopoulos, 1962). Conidia may be dispersed by wind or rain to initiate clonal populations of new individuals. Hyphal fusion readily occurs within an individual colony during vegetative growth, maintaining the physiological continuity of the organism. Sexual reproduction increases the possibility that the pathogen will develop new pathotypes through genetic recombination that may overcome host resistance or have increased resistance to fungicides (Wilson and Kaiser, 1995). Ascospores can function as over-wintering structures or infection propagules and can be an important component of the disease cycle (Glass and Kuldau, 1992). Studies with *Guignardia bidwelli*, causal agent of black rot of grapevine, revealed that young bunches under attack wither quickly and show numerous, point-like fructifications. These are spermagonia and pycnidia, that later develop into perithecia that differentiate in spring to form asci whose ascospores induce the primary infection on leaves (Jailoux, 1991). In another study in eastern Washington and northern Idaho, *Didymella rabiei,* the telemorph of *Ascochyta rabiei,* showed an overlap in the period of ascospore discharge with the vegetative stage of the chickpea crop, indicating that ascospores may serve as primary inoculum for epidemics of Aschochyta blight in that region (Wilson and Kaiser, 1995). Frei and Ginrat (1995b) demonstrated that inoculations of wheat and barley stems with ascospores of *T. yallundae* were unsuccessful, while inoculations with conidia and mycelia resulted in eyespot lesions. On the other hand, Daniels *et al.* (1995) demonstrated successful infection of wheat seedlings using ascospores of *T. yallundae*. Elucidation of environmental conditions required for the formation of the teleomorph is essential for understanding and establishing the potential importance of the sexual state.

Environmental requirements for sexual reproduction

Environmental requirements are among the criteria that must be satisfied before fungi are able to undergo sexual reproduction. Many fungi require an environmental trigger

to induce a competent mycelium to initiate sexual reproduction. There is no single set of optimum conditions for sex, and significant variation occurs between species (Moore-Landecker, 1982). In the study of fungal reproduction, possible simultaneous interactions between temperature, light and the nutrient medium must always be taken into consideration. It has been noted that in particular the role of temperature and light in conidiogenesis and sexual reproduction is often different (Jailoux, 1991). Kaiser (1973) as well as Nene and Reddy (1987) speculated that certain environmental condition requirements may explain why the sexual stage rarely occurs in chickpea-growing regions of the world, and why the teleomorph may be important only in regions of the world where chickpea residues between crops are exposed to cool, moist climates. None of these factors act independently, with sex being induced only if the sum of the influences is favourable. The effects of these various factors on fungal sexual reproduction have been reviewed by Moore-Landecker (1982). The environmental triggers are often of ecological significance to ensure that sex occurs only under suitable conditions, such as after a reduction in the nutrient supply following previous exploitation of a substratum by vegetative growth, or light being required to ensure spore production and dispersal on the surface of a substrate. Sexual reproduction and sexual morphogenesis are important phenomena in ascomycetes, it is necessary to understand the molecular and genetic factors in control. Exciting progress has been made in recent years in defining the molecular basis of action of the genes located at the mating type loci. Such research has indicated that mating type genes may not only control the initial compatibility of crosses involving heterothallic ascomycete species, but that they may also be involved with the later regulation of ascocarp development. This would be induced through the formation of hybrid regulatory products in the sexual dikaryon, providing a link between the sexual cycle and ascocarp formation (Dyer *et al.*, 1992).

Genetic requirements for sexual reproduction in fungi

Genetic factors at the mating type locus are not the only factors that determine sexual compatibility, and the ultimate productivity of sexual crosses in heterothallic ascomycetes. When investigated, studies of ascospore progeny have indicated that differences in the degree of compatibility, as judged by the number of fertile ascocarps produced, are under genetic control independent of mating type. Genetic

factors other than those at the mating type locus may thus determine the productivity of sexual crosses, although this fact is rarely considered (Burnett, 1975). Explanation for this polygenic control of compatibility and fertility include the following.

- Genes encoding for functions necessary for successful sexual reproduction, such as the development of ascocarp initials might be expressed at different levels in different isolates or have multiallelic forms.

- Certain isolates may have reduced ability to synthesize the metabolites required for mating (Nelson, 1970; Dyer, 1991; Dyer *et al.*, 1993a).

- True secondary sexual incompatibility systems may operate. In contrast to the above factor a system of "heterogenic incompatibility" may be present in some fungi. To achieve full fertility, it is necessary that mating partners carry similar alleles at all relevant incompatibility loci. Such an incompatibility mating system might be important in mating isolation between separate races of an otherwise compatible species. Evidence for heterogenic incompatibility has come from an analysis of crossing relationships in *Coprinus bisporus* (Kemp, 1989) and *P. anserina,* in which at least nine loci were identified (Esser and Kuenen, 1967; Fincham, *et al.*, 1979).

- Evolutionary changes may lead to subgroup fertility barriers, as observed in *C. ulmi,* with incompatibility arising from slight differences in pheromone structure or differences in cell-surface recognition molecules (Brasier, 1984).

- Cytoplasmic factors may determine sexual potential. The degree of compatibility between different cell nuclei and mitochondria may be a key factor governing the success of sexual fusions (Rayner and Ross, 1990).

- There may be partial karyotype mismatch between isolates due to structural incompatibilities in chromosome architecture. Differences in electrophoretic karyotype have been revealed amongst isolates of various ascomycetes. This may lead to genetic isolation, with meiosis being a process to enforce chromosome standardization. It is also noted that the method of crossing isolates of heterothallic ascomycetes *in vitro* may determine the success of a sexual cross (Maddock and Ingram, 1981; Ilott *et al.*, 1984).

MOLECULAR GENETIC CONTROL OF SEXUAL MORPHOGENESIS

Given favourable environmental and genetic conditions, sexual reproduction may be initiated, leading to the formation of novel structures not seen during vegetative growth. This process is termed "sexual morphogenesis." Classical genetic studies have clearly demonstrated that ascus, ascocarp and ascospore formation involves the sequential expression of a series of developmentally regulated genes, i.e. sexual morphogenesis is under polygenic control.

Evidence has been put forward to support the hypothesis that specific chemical factors are involved with the regulation and nutrition of sexual morphogenesis. However, the question then arises as to what genetic processes initially trigger the production of novel sexual signals themselves. Very little is known of such processes, although certain conclusions may be drawn based on observations linking the sexual cycle to ascocarp development, and recent discoveries about the action of genes at the mating type locus. An unsubstantiated regulatory mechanism involving translational ambiguity has also been proposed (Picard-Bennoun, 1982).

The sexual cycle and ascocarp development

Sexual reproduction in ascomycetes involves the growth of a series of potentially independent hyphal systems which may be divided into ascogenous hyphae involved in ascus formation in the sexual cycle, i.e. dikaryon formation, karyogamy, meiosis, and somatic hyphae involved with primordium and ascocarp formation. An important question to ask therefore is whether the processes controlling the growth of these hyphal systems are intrinsically linked, or whether ascocarp formation may occur independently of the sexual cycle. Evidence is available to support both viewpoints.

Evidence supporting the fact that the events of the sexual cycle and ascocarp development are intrinsically linked include the following.

- The results of mating between closely related species. It is occasionally possible to cross closely related species and get a partial sexual response even leading to production of few viable ascospores. However, the ascocarps formed in such crosses are normally poorly developed and lack features characteristic of normal intra-species crosses. Thus although ascocarp formation is triggered, it would appear to require specific signal(s) from the functional ascogenous system of the sexual cycle to

proceed further. Such observations have been observed in crosses between heterothallic *Neurospora* species and subgroups of *Ophiostoma ulmi.* Normal perithecia were produced in an interspecific cross between *Sordaria heterothallis* and *S. thermophila,* but in this case development had proceeded as far as the delimination of immature ascospores (Lewis, 1969).

- It has been observed that mutants blocked at some point in dikaryon formation or karyogamy characteristically form only immature ascocarps, again suggesting signal(s) from the functional ascogenous hyphae is required for further development. This has been well-documented in *N. crassa,* with male and female mutants blocked at various stages of perithecial development. Similar mutants have been identified in *Nectria haematococca* (Dyer, 1991; Dyer *et al.*, 1992). Crosses with rare heterokaryon self-incompatible strains of *Gibberella fujikuroi* developed only as far as the formation of immature perithecia, with completion of the sexual cycle required for the production of mature perithecia (Correll *et al.*, 1987).

Evidence suggesting that the events of the sexual cycle and ascocarp development are independent include the following.

- The phenomenon of homokaryon fruiting. This has been observed mainly in basidiomycetes, in which single basidiospores of certain species have been shown to form morphologically mature basiodiocarps despite a lack of plasmogamy, although the basidia may develop only two spores. Homokaryotic fruiting has also been reported in the ascomycete *Podospora anserina.* Mutant *vacua* (*va*) strains formed mature perithecia in culture, only lacking ostiolar hairs. However, these perithecia were sterile and contained only a gelatinous mass of proliferating paraphyses. In some ascomycetes it has been possible to induce the initial stages of ascocarp formation in culture, independent of the sexual cycle, by applying erogenous chemical factors. However, sex morphogenes rarely induce the formation of fully mature ascocarps.

- Overall, evidence currently available for the ascomycetes supports a middle viewpoint, namely that the initial stages of ascocarp development can occur independently of the events of the sexual cycle, but that a signal(s) from the ascogenous hyphae is required for mature ascocarp formation. This is not unexpected, as one key function of fruit bodies is the dispersal of sexual spores, so it

would be expected that the developing spores would exert some kind of effect on their formation (Dyer *et al.*, 1992).

Chemical factors

Studies in a wide range of fungi have shown that prominent chemical changes occur in species during sexual reproduction, often involving the appearance of novel sex-related compounds. It is now becoming apparent that some of these chemical factors have important regulatory or nutritional roles in sexual morphogenesis. Of particular interest are certain diffusible factors or 'sex hormones' which appear to be important in controlling, differentiation and switching on sexual morphogenesis. Here a sex hormone is defined as any diffusible chemical substance that at low concentrations can induce a physiological response forming part of the sexual process, from the initial attraction of gametes to final fruit body formation. These sex hormones are of great interest as they may be used as key 'switching' signals to alter growth from an asexual to a sexual mode of reproduction, given asexual and sexual reproduction seem to be largely mutually exclusive in many fungi.

Sex hormones may be involved with sexual morphogenesis at two different stages.

- In the interaction between genetically compatible strains, with pheromone-like activity, inducing gametangial formation, chemotactic growth and plasmogamy.
- In co-ordinating and triggering fruit bodies. The later group of morphogenetically active compounds are termed 'sex morphogens'.

Sex hormones and sexual growth substances may jointly be termed chemical 'sex factors'. Various classes of chemicals have been identified as sex factors. These include peptides, proteins, terpenoids, sterols, sphingolipids, phospholipids and fatty acids. It is of interest to note the different specificity of some of these chemicals. Those involved in initial gamete interaction are invariably very specific to a given species or genus. This specificity is presumed to be advantageous as it prevents wasteful activation of sexual reproduction unless a compatible partner is present. In contrast, certain chemicals involved in fruit body formation have been shown to influence growth and sexual reproduction in a broader range of fungal species. Linoleic acid, phosphatidicholine, SF and zearalenone have all been shown to

promote sexual reproduction in a variety of fungi, whilst cyclic AW and derivatives have been shown to act as signaling molecules in slime moulds, ascomycetes and basidiomycetes (Dyer *et al.*, 1992).

Enzymatic processes and morphogenesis

One way in which chemical factors may control morphogenesis is by directly or indirectly triggering the production of enzymes that are responsible for catalysing hyphal differentiation and aggregation. Hyphal aggregation is clearly critical in fruit body formation. Mutants of *Gibberella fujikuroi* with a reduced ability to undergo hyphal fusion were completely female sterile (Correll *et al.*, 1987). Enzymes are also likely to regulate the degree of hyphal branching, itself a key factor in differentiating structures. Phenoloxidases, which oxidise polyphenolic compounds leading to pigment and melanin formation, have often been correlated with the formation of fruit bodies. Enzymes tend to be produced in relatively large amounts, particularly during starvation conditions. Phenoloxidases may catalyse the formation of phenolic polymers between adjacent hyphae in developing fruit bodies, thereby causing these hyphae to adhere, resulting in hyphal aggregates (Rayner and Ross, 1990; Ainsworth and Rayner, 1991).

REPRODUCTIVE CHARACTERISTICS OF *TAPESIA YALLUNDAE*

As mentioned earlier, the sexual stage of the eyespot fungus was discovered as recently as 1987 in Australia (Wallwork, 1987). Since then, apothecia of *T. yallundae* have been found at field sites in five more countries, suggesting that the sexual stage may be an intrinsic part of the life cycle of the pathogen in the field (Dyer *et al.*, 1994). *T. yallundae* reproduces sexually by the formation of apothecia that bear asci and ascospores which can be harvested under laboratory conditions and be inoculated into a medium to form pure cultures. Variability in colony morphology and benomyl sensitivity among progeny from a single apothecium suggested that *T. yallundae* is heterothallic (Moreau and Maraite, 1995). In a study conducted by Nicholson *et al.* (1991) no apothecia were produced on straws with one isolate only, which suggested that at least two strains were required for apothecial formation. A similar study by Frei and Ginrat (1995a) confirmed this finding.

Field occurrence of apothecia

In the Western Cape province of South Africa apothecia were observed on wheat stubble incubated in the laboratory for 8 months at 10°C under near-ultraviolet light. Single ascospore isolates produced colonies typical of *T. yallundae* in culture (Robbertse *et al.*, 1994).

In Belgium in March 1989, apothecia were found on lower leaf sheaths of wheat stubble in a field that had never been sprayed against eyespot. Their major characteristics corresponded with those reported for *T. yallundae* (Moreau *et al.*, 1989).

Apothecia of *T. yallundae* were reported for the first time in the U.K. in January 1989. They were observed on decaying stems and leaf sheaths of unharvested wheat near Bristol. Isolates from single ascospores produced cultures typical of the W-type form (*R. herpotrichoides*) of the anamorph. Pathogenicity on wheat was confirmed by inoculation and re-isolation from glasshouse-grown wheat seedlings (Hunter, 1989).

Wallwork (1987) placed straws colonized by *T. yallundae* in the dark at 10°C with short exposures to light at room temperature, and observed apothecia on the straws after 8 weeks. In a study conducted by King *et al.* (1990) in Germany, apothecia were first found on stubble in a field plot at Gottingen. At the same time, apothecia formed on incubated stubble collected from the field 3 weeks earlier. Apothecia formed on 5% of stems both in the field and in the incubator, and production continued until the end of March. In many cases apothecia formed on incubated stems in two or three flushes. A survey of standing stubble was undertaken throughout the month of March. During the survey, apothecia were found on stubble standing in the field at nine of 37 sites on approximately 1% of stems. Apothecia were not found on either green plants or secondary stubble.

In a study by Bateman *et al.* (1995), plots treated with carbendazim and prochloraz to select for *T. yallundae* and *T. acuformis,* respectively, the incidence of apothecia correlated with *T. yallundae,* and plots which had predominantly *T. acuformis* yielded few apothecia. This suggests that the sexual stage of *T. yallundae* occurs more frequently in nature than that of *T. acuformis*. It may also explain why *T.*

acuformis exhibits less genotypic variation than *T. yallundae*, as *T. yallundae* and *T. acuformis* are sexually incompatible (Dyer and Lucas, 1995).

Ascospore discharge

In vitro. Apothecial formation is induced by crossing different isolates of the fungus. Either McCartney bottles or plates can be used for the crosses. The isolates are crossed by bringing stubble colonized with different isolates into contact with one another. In the case of bottles, sterile, distilled water must be added to permit the free-flow of mycelia that must come into contact with either partner. If the end result of the experiment is to harvest ascospores, plates are preferable to bottles, as the free water in the bottles can allow premature ascospore discharge to occur. For the discharge of ascospores, apothecia are attached to a Petri dish lid, and covered with water for an hour or two. When the water is removed and the lid inverted, apothecia dry out. The subsequent change in humidity and osmotic potential induce a release of ascospores.

In vivo. Ascospore liberation was thought to be achieved by instantaneous rapture of the asci that had attained a critical state of turgidity. It was proposed that during a prolonged still period, asci become highly turgid and unstable, and some environmental stimulus such as strong light, dry air, or a temperature fluctuation could then trigger ascospore discharge or "puffing". An extracellular mucilaginous matrix appears to be involved in the generation of this high water potential, with a possible antidessicant role (Daniels *et al.*, 1995). The stimulus for this event is unlikely to be a drying out process as in the case of laboratory conditions. Under the correct atmospheric conditions, ascospores undoubtedly become airborne (Saunderson and King, 1988). Following dispersal by wind, ascospores provide inoculum with a wide range of genetic variation.

FUNGICIDE SENSITIVITY

Fungicide applications are usually seen as a control measure to protect plants against fungal diseases (Scott, 1996). Variability in sensitivity to fungicides, e.g. methyl-benzimidazole carbamate-generating (MBC) fungicides or inhibitors of sterol biosynthesis currently used for the control of eyespot, is also quite evident in *T. yallundae*. Isolates resistant to MBC fungicides have been reported for *T. yallundae*

as well as *T. acuformis*. Isolates of *T. acuformis* exhibit natural tolerance to triazole compounds, whereas isolates of *T. yallundae* can be sensitive or resistant to them (Leroux and Gredt, 1988). The effectiveness of the fungicide is measured by the sensitivity of fungal isolates to the fungicide. The application of a fungicide selects against the sensitive subpopulation and provides a selective advantage for the resistant subpopulation, which subsequently increases in proportion. The important question is not whether a certain fungus is resistant to a certain fungicide, but rather is, what must be done for the fungicide to be effective? For each fungicide we need to understand the biochemical mode of action which can give us an indication as to how a fungicide can regain its effectiveness after it has been found ineffective (Dekker, 1985).

The genetic and reproductive basis of fungicide resistance

A fungus can acquire fungicide resistance either in one step, due to mutation of a major gene, or in multiple steps, by the interaction of several mutant genes, each with a small individual effect. Several studies have focused on the inheritance of resistance to *T. yallundae* in winter wheat. Some indicate that the inheritance of resistance is complex, while others show resistance to be simply inherited. In both cases the genetic potential for overcoming the fungicide toxicity must be present in the fungus. In one-step resistance, mutation of a single gene is all that is required for the organism to acquire the highest resistance possible. If such a highly resistant strain is crossed to a sensitive wild-type, a Mendelian ratio of distinct phenotypes is obtained in the progeny (Strausbaugh and Murray, 1989b).

In the polygenic control of resistance, the effect of individual genes is generally small and may even be impossible to measure. Mutagenesis and first step selection can recognize only small changes in sensitivity. A highly resistant strain may be obtained only from many mutant genes in the same nucleus, either by crossing first step mutants among themselves or by stepwise selection. If such a resistant strain is crossed with a sensitive one, no Mendelian segregation will be observed, but rather a continuous range of sensitivities characteristic of a quantitative response (Georpoulos, 1985).

Development of fungicide resistance through recombination

Recombination is the production of new combinations of existing parental characters. It probably occurs in all organisms and re-assorts existing genetic variation to give new genotypes and phenotypes, on which selection and chance can act to give adaptation and evolution (Lamb, 1996). Present studies show that recombination during the sexual cycle can lead to greater variation within populations of *T. yallundae*. This is relevant to the epidemiology and control of eyespot in that it provides the pathogen with the flexibility to respond to selection pressures such as fungicide treatment (Dyer *et al.*, 1993a). Sexual reproduction, therefore, might hasten the breakdown of fungicide control by facilitating the integration and spread of fungicide resistant genes in the pathogen gene pool.

Cases of fungicide resistance and sensitivity

Since the early 1980s, field populations of the pathogen have become predominantly resistant to MBC fungicides, and control of the fungicide relies to a large extent on the sterol biosynthesis inhibitor (SBI) prochloraz, which acts by inhibiting the C-14 demethylation of lanosterol (Julian *et al.*, 1994). In a study published by Murray (1996), isolates of *T. yallundae* resistant to benzimidazole fungicides were reported to have been detected in commercial winter wheat fields in the Pacific Northwest region (Washington, Oregon, and Idaho) of the U.S.A. for the first time in the spring of 1989. Benzimidazole resistant isolates were found in nine of 62 fields sampled in 1989 and 17 of 167 fields sampled in 1990, which respectively represents 24 and 19% of those fields yielding benzimidazole resistant isolates. Ninety-six and 70% of all isolates collected from fields showing signs of benzimidazole resistance during 1989 and 1990, respectively, were resistant.

Strains of *Tapesia* collected on winter wheat in France by Leroux *et al.* (1988), gave either fast growing mycelial colonies with regular margins (*T. yallundae*), or slow growing mycelial colonies with irregular margins (*T. acuformis*). Most of the *T. yallundae* isolates were sensitive to triadimenol (EC_{50} below 2mg/l), but some of them were resistant to triazoles, which inhibit the sterol C-14 demethylation. In contrast, all the *T. acuformis* strains were highly resistant to triadimenol (EC_{50} greater 100 mg/l). From a specific field in the U.K., Hoare *et al.* (1986) made isolations from 880 lesions during July 1983, and from 440 lesions during July 1984 and 1985. Of the

1983 collections 3% or less of the isolates from any treatment were classified as carbendazim resistant. By the end of the second season in July 1984, 65-75% of isolates recovered from plots that had received carbendazim sprays alone were classified as carbendazim resistant, whereas from plots sprayed with a carbendazim-prochloraz mixture, 35% of the isolates were carbendazim resistant. Incidence of resistance remained small (8% or less) in untreated or prochloraz treated plots. Isolates resistant to prochloraz were not detected in any treatment in any year.

In a survey conducted in South Africa during the 1991-1992 seasons by Robbertse *et al.* (1996), isolates of the eyespot fungus from the Western Cape province were characterised and tested for sensitivity to carbendazim and sterol biosynthesis inhibiting fungicides. The 100 isolates tested were all fast growing, even marginate, and designated as *T. yallundae.* Fungal growth was completely inhibited on PDA amended with carbendazim (1 ppm), indicating that the local population is still at baseline sensitivity to benzimidazoles. This finding clearly indicates the importance of conducting research to monitor the build-up of fungicide resistance.

Agricultural crops are under constant attack by plant pathogens. To safeguard food production, crop protection measures are indispensable. When the use of resistant varieties, crop rotation and other cultural practices, or alteration of the environment are inadequate to suppress pathogens sufficiently, the use of chemicals becomes essential. To date chemicals play a predominant role in plant protection. However, control of diseases with chemicals may also encounter problems, for example when the causal organism becomes resistant to the toxicant. This resistance involves one of the most fundamental properties of living matter, namely, the ability of organisms to adapt to changing environmental conditions and to survive under new, often adverse, circumstances. The evolution of organisms would have not been possible without this property, as illustrated by natural history of life on earth. The application of a fungicide also constitutes an adverse change in the environment for an organism that is sensitive to such a compound, and it may adapt to the new situation by becoming resistant (Georpoulos, 1985).

Until recently foot rot of cereals has not been considered very extensively by plant breeders, and only a few programmes have been designed to improve the resistance against fungi attacking the lower internodes of the culms, e.g. *Tapesia*

yallundae, Fusarium graminearum, F. culmorum and *Microdochium nivale* (Lind, 1992). The findings of the present review clearly indicate, however, that a more integrated approach will have to be followed in future. Control regimes should not only incorporate a mixture of fungicides, but also consider that these compounds are generally wide spectrum fungicides, the application of which influences the sensitivity of a range of different disease causing organisms.

REFERENCES

Ainsworth, A.M. and Rayner, A.D.M. (1991). Ontogenetic stages from coenocyte to basidiome and their relation to phenoloxidase activity and colonization processes in *Phanerochaete magnoliae. Mycological Research* **95**, 1414-1422.

Alexopoulos, C.J. (1962). Introductory mycology, pp. 217-240. Wiley: New York.

Andrews, J.H. (1991). Comparative ecology of microorganisms and macroorganisms. Sprnger: New York.

Backus, M.P. (1939). The mechanics of conidial fertilization in *Neurospora sitophila. Bulletin of the Torrey Botanical Club* **66**, 63-76.

Bateman, G.L., Dyer, P.S. and Manzhula, L. (1995). Development of apothecia of *Tapesia yallundae* in contrasting populations selected by fungicides. *European Journal of Plant Pathology* **101**, 695-699.

Beadle, G.W. and Coonradt, V.L. (1994). Heterokaryosis in *Neurospora crassa. Genetics* **29**, 291-308.

Bernet, J. (1967). Les systemés d' incompatibilité chez le *Podospora anserina. Comptes Rendus de l' Academie des Sciences Paris Serie D* **265**, 1330-1333.

Bernet, J. (1992). In *Podospora anserina*, protoplasmic incompatibility genes are involved in cell death control via multiple gene interaction. *Heredity* **68**, 79-87.

Bistis, G.N. (1981). Chemotropic interactions between trichogynes and conidia of opposite mating-type in *Neurospora crassa. Mycologia* **73**, 959-975.

Bistis, G.N. (1983). Evidence for diffusible, mating-type-specific trichogyne attractants in *Neurospora crassa*. *Experimental Mycology* **7**, 292-295.

Boerema, G.H., Pieters, R. and Hammers, M.E.C. (1992). Check-list for scientific names of common parasitic fungi. Supplement Series 2b (additions and corrections): Fungi on field crops: cereals and grasses. *Netherlands Journal of Plant Pathology* **98**, 1-32 (Supplement 1).

Brasier, C.M. (1984). Inter-mycelial recognition systems in *Ceratocystis ulmi*: their physiological properties and ecological importance. In *The Ecology and Physiology of the Fungal Mycelium*. (ed D.H. Jennings, A.D.M. Rayner), pp. 451-498. Cambridge University Press: Cambridge.

Braun, U. (1993). Studies on *Ramularia* and allied genera (VI). *Nova Hedwigia* **56**, 423-454.

Brennan, J.P. and Murray, G.M. (1989). Australian wheat disease, assessing their economic importance. *Agricultural Science* **2**, 26-35.

Bruehl, G.W. (1983). Nonspecific genetic resistance to soilborne fungi. *Phytopathology* **73**, 948-951.

Burnett, J.H. (1975). *Mycogenetics. An Introduction to the General Genetics of Fungi*. John Wiley: London.

Caten, C.E. (1972). Vegetative incompatibility and cytoplasmic infection in fungi. *Journal of General Microbiology* **72**, 221-229.

Corlett, M. (1991). An annotated list of the published names in *Mycosphaerella* and *Sphaerella*. *Mycological Memoir* **18**, 1-328.

Correll, J.C., Klittich, C.J.R. and Leslie, J.F. (1987). Heterokaryon self-incompatibility in *Gibberella fujikuroi* (*Fusarium moniliforme*). *Mycological Research* **93**, 21-27.

Creighton, N.F., Cavelier, N. and Fitt, B.D.L. (1989). Pathogenicity to wheat and rye of *Pseudocercosporella herpotrichoides* isolates from France and the UK. *Mycological Research* **92**, 13-17.

Daniels, A., Papaikonomou, M., Dyer, P.S. and Lucas, J.A. (1995). Infection of wheat seedlings by ascospores of *Tapesia yallundae*: Morphology of the

infection process and evidence for recombination. *Phytopathology* **85,** 918-927.

Davis, R.H. (1966). Heterokaryosis. In *The fungi: an advanced treatise* (ed. G.C. Ainstworth and A.S. Sussman), pp. 567-588. Academic Press: New York.

Day, P.R. (1968). The significance of genetic mechanism in soil fungi. In *Root diseases and soilborne pathogens* (eds. T.A.Tousson, R.V. Bega, and P.E. Nelson), pp 69-74. University of California Press: Berkeley.

Deighton, F.C. (1973). Studies on *Cercospora* and allied genera. IV. *Cercospora* Sacc, *Pseudocercosporella* gen. nov. *Mycological Papers* **133**, 1-62.

Dekker, J. (1985). The development of resistance to fungicides. Progress in Pesticide Biochemistry. *Toxicology* **4**, 165-218.

Di San Lio, G.M., Hocart, M.J., Lucas, J.A. and Peberdy, J.F. (1994). Overcoming vegetative incompatibility within and between pathotypes of *Pseudocercosporella herpotrichoides* by protoplast fusion. *Mycological Research* **98,** 653-659.

Dodge, B.O. (1935). The mechanisms of sexual reproduction in *Neurospora. Mycologia* **27**, 418-436.

Doussinault, G., Delibes, A., Sanchez-Monge, R. and Garcia-Olmedo, F. (1983). Transfer of a dominant gene for resistance to eyespot disease from a wild grass to hexaploid wheat. *Nature* (*London*) **303**, 698-700.

Doussinault, G., Dosba, F. and Jahier, J. (1983). New results on the improvement of the level of resistance to eyespot in wheat. In *Proceedings of the 6th International Wheat Genetics Symposium,* pp. 193-198. Kyoto: Japan.

Dyer, P.S., Ingram, D.S. and Jonestone, K. (1992). The control of sexual morphogenesis in the Ascomycotina. *Biological Reviews* **67,** 421-458.

Dyer, P.S. and Lucas, J.A. (1995). Incidence of apothecia of *Tapesia yallundae* at set-aside sites in England and sensitivity of the ascospore offspring to the fungicides benomyl and prochloraz. *Plant Pathology* **44,** 796-804.

Dyer, P.S., Ingram, D.S. and Jonestone, K. (1993a). Evidence for the involvement of linoleic acid and other endogenous lipid factors in perithecial development of *Nectria haematococca* mating population VI. *Mycological Research* **97**, 485

Dyer, P.S., Nicholson, P., Rezanoor, H.N., Lucas, J.A. and Peberdy, J.F. (1993b). Two-allelle heterothallism in *Tapesia yallundae,* the teleomorph of the cereal eyespot pathogen *Pseudocercosporella herpotrichoides. Physiological and Molecular Plant Pathology* **43,** 403-414.

Dyer, P.S., Papaikonomou, M., Lucas, J.A. and Peberdy, J.F. (1994). Isolation of R-type progeny of *Tapesia yallundae* from apothecia of wheat stubble in England. *Plant Pathology* **43,** 1039-1044.

Dyer, P.S. (1991). Perithecial development in *Nectria haematococca* mating population IV. Ph.D Thesis, University of Cambridge.

Esser, K. (1962). Die genetik de sexuelen Fortpflenzing bie den Pilsen. *Biologisches Zentral Blatt* **881**, 161-172.

Esser, K and Kuenen, R. (1967). *Genetics of Fungi.* Springer-Verlag: Berlin.

Fincham, J.R.S., Day, P.R. and Radford, A. (1979). Fungal genetics. University of California Press: Berkeley/Los Angeles.

Fitt, B.D.L., Goulds, A. and Polley, R.W. (1988). Eyespot (*Pseudocercosporella herpoirichoides*) epidemiology in relation to prediction of disease and severity and yield loss in winter-wheat — a review. *Plant Pathology* **37,** 311-329.

Frei, P. and Ginrat, D. (1995a). Diversite morphologique en culture, sensibilite au carbendazim et pouvoir pathogen du *Tapesia yallundae* (anamorphe: *Pseudocercosporella herpotrichoides). Canadian Journal of Botany* **73,** 1379-1384.

Frei, P. and Ginrat, D. (1995b). The complete cycle of eyespot disease of wheat (*Tapesia yallundae*) under controlled environmental conditions. *Botanica Helvetica* **105**, 233-237.

Frei, U. and Wenzel, G. (1993). Differentiation and diagnosis of *Pseudocercosporella herpotrichoides* (Fron) Deighton with genomic DNA probes. *Phytopathology* **139,** 229-237.

Fron, G. (1912). Contribution a 1' étude de la maladie de `pied noir des céréales' ou `maladie du piétin. *Annales de la Science Agronomique Francaise (et Etrangere) 4 ser*, **1**, 3-29.

Gale, M.D., Scott, P.R., Law, C.N., Ainsworth, C.C., Hollins, T.W. and Worland, A.J. (1984). An alpha-amylase gene from *Aegilops ventricosa* transferred to bread wheat together with a factor for eyespot resistance. *Heredity* **52**, 431-435.

Garnjobst, L. (1953). Genetic control of heterokaryosis in *Neurospora crassa. American Journal of Botany* **40**, 607-614.

Georpoulos, S.G. (1985). The genetic basis of classification of fungicides according to resistance risk. *EPPO Bulletin* **15**, 513-517.

Glass, N.L. and Kuldau, G.A. (1992). Mating type and vegetative incompatibility in filamentous ascomycetes. *Phytopathology* **30**, 201-224.

Goulds, A. and Fitt, B.D.L. (1991). The development of eyespot on stems of winter wheat and winter barley in crops inoculated with W-type or R-type isolates of *Pseudocercosporella herpotrichoides. Journal of Plant Disease and Protection* **98**, 490-502.

Goulds, A. and Polley, R.W. (1990). Assessment of eyespot and other stem base diseases of winter wheat and winter barley. *Mycological Research* **94**, 819-822.

Gross, S.R. (1952). Heterokaryosis between opposite mating types in *Neurospora crassa. American Journal of Botany* **39**, 574-577.

Harrington, T.C. and McNew, D.L. (1997). Self-fertility and uni-directional mating type switching in *Ceratocystis coerulescens*, a filamentous ascomycete. *Current Genetics* **32**, 52-59.

Hartl, D., Dempter, E.R. and Brown, S.W. (1975). Adaptive significance of vegetative incompatibility in *Neurospora crassa. Genetics* **81**, 553-569.

Higgins, S. and Fitt, B.D.L. (1985). Pathogenicity of *Pseudocercosporella herpotrichoides* isolates to wheat seedlings and adult plants. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz* **92**, 176-185.

Higgins, S., Fitt, B.D.L. and White, R.P. (1986). The development of eyespot (*Pseudocercosporella herpotrichoides*) lesions in wheat crops. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz* **93,** 210-220.

Hoare, F.A., Hunter, T. and Jordan, V.W.L. (1986). Influence of spray programmes on development of fungicide resistance in the eyespot pathogen of wheat, *Pseudocercosporella herpotrichoides. Plant Pathology* **35,** 506-511.

Hocart, M.J., Lucas, J.A. and Peberdy, J.F. (1993). Characterization of the parasexual cycle in the eyespot fungus, *Pseudocercosporella herpotrichoides. Mycological Research* **97,** 967-976.

Hocart, M.J. and McNaughton, J.E. (1994). Interspecific hybridisation between *Pseudocercosporella herpotrichoides and P. anguioides* achieved through protoplast fusion. *Mycological Research* **98,** 47-56.

Hunter, T. (1989). Occurrence of *Tapesia yallundae,* teleomorph of *Pseudocercosporella herpotrichoides,* on unharvested wheat culms in England. *Plant Pathology* **38,** 598-603.

Ilott, T.W., Ingram, D.S. and Rowlinson, C.J. (1984). Heterothallism in *Pyrenopeziza brassicae,* cause of light leaf spot of Brassicas. *Transactions of British Mycological Society* **82,** 477-483.

Jahier, J., Doussinault, G., Dosba, F. and Bourgeois, F. (1978). Monosomic analysis of resistance to eyespot in the variety "Roazon". In *Proceedings of the 5th International Wheat Genetics Symposium* pp. 437-440. New Delhi, India.

Jailloux, F. (1991). *In vitro* production of the teleomorph of *Guignardia bidwellii,* causal agent of black rot of grapevine. *Canadian Journal of Botany* **70,** 254-257.

Jones, D.R. (1994). Evaluation of fungicides for control of eyespot disease in yield loss nationships in winter wheat. *Plant Pathology* **43,** 831-846.

Julian, A.M., Hardy, J.E. and Lucas, J.A. (1994). The induction and characterization of isolates of *Pseudocercosporella herpotrichoides* with altered sensitivity to prochloraz. *Pesticide Science* **41,** 121-128.

Julian, A.M. and Lucas, J.A. (1990). Isozyme polymorphism in pathotypes of *Pseudocercosporella herpotrichoides* and related species from cereals. *Plant Pathology* **39**, 178-190.

Kaiser, W.J. (1973). Factors affecting growth, sporulation, pathogenicity, and survival of *Aschochyta rabiei. Mycologia* **65**, 444-457.

Kemp, R.F.O. (1989). Incompatibility in the Basidiomycetes. The heterogenic pentax. *Fork Publications* **1**,1-19.

Kimber, G. (1967). The incorporation of the resistance of *Aegilops ventricosa* to *Cercosporella herpotrichoides* into *Triticum aestivum. Journal of Agricultural Science Cambridge* **68**, 373-376.

King, J.E. and 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.

King, A.C., Bemdt, H. and Fehrmann, H. (1990). Aspects of eyespot control. *Journal of Plant Disease and Protection* **97**, 237-249.

Kohn, L.M., Stasovski, E., Carbone, I. and Anderson, J.B. (1991). Mycelial incompatibility and molecular markers identify genetic variability in field populations of *Sclerotinia sclerotiorum. Phytopathology* **81**, 480-485.

Kuees, U. and Casselton, L.A. (1992). Fungal mating type gene-regulators of sexual development. *Mycological Research* **96,** 993-1006.

Lagneau, C., Dandois, J., Bastin, V., Poncelet, J. and Maraite, H. (1986). Evolution et severite des maladies sur froment d'hiver et escourgeon en 1985. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* **51,** 603-616.

Lamb, B.C. (1996). Ascomycete genetics: the part played by ascus segregation phenomena in our understanding of the mechanisms of recombination. *Mycological Research* **100**, 1025-1059.

Law, C.N., Scott, P.R., Worland, A.J. and Hollins, T.W. (1976). The inheritance of resistance to eyespot (*Cercosporella herpotrichoides*) in wheat. *Genetical Research* **25**, 73-79.

Leroux, P., Gredt, M. and Boeda, P. (1988). Resistance to inhibitors of sterol biosynthesis in field isolates or laboratory strains of the eyespot pathogen *Pseudocercosporella herpotrichoides*. *Pesticide Science* **23**, 119-129.

Leroux, P. and Gredt, M. (1988). Caractérisation des souches de *Pseudocercosporella herpotrichoides*, agent du piétin-verse des céréales, résistantes à des substances antimitotiques et à dse inhibiteurs de la biosynthése des stérols. *Agronomie* **8**, 719-729.

Leslie, J.F. and Klein, K.K. (1996). Female fertility and mating type effects on effective population size and evolution in filamentous fungi. *Genetics* **144**, 557-567.

Lewis, L.A. (1969). Genetic evidence for hybridisation in an interspecific cross in the genus *Sordaria*. *Journal of General Microbiology* **59**, 359-367.

Lind, V. (1992). Measuring the severity of eyespot disease induced by *Pseudocercosporella herpoirichoides* (Fron) Deighton in wheat cultivars at different growth stages. *Plant Breeding* **108**, 202-209.

Lori, G., Wolcan, S. and Alippi, H. (1996). Genetic diversity of *Fusarium oxysporum* f. sp. *dianthi* in Argentina. *Plant Disease* **80**, 821 (Abstr.).

Macer, R.C.F. (1966). Resistance to eyespot disease (*Cercosporella herpotrichoides* Fron) determined by a seedling test in some forms of *Triticum*, *Aegilops*, *Secale*, and *Hordeum*. *Journal of Agricultural Science, Cambridge* **67**, 389-396.

Maddock, S.E. and Ingram, D.S. (1981). Studies of the perfect stage of light leaf spot pathogen of Brassicas, *Pyrenopeziza brassicae*. *Transactions of the British Mycological Society* **71**, 207-210.

Metzenberg, R.L. and Glass, N.L. (1990). Mating and mating strategies in *Neurospora*. *Bioessays* **12**, 53-59.

Moore-Landecker, E. (1982). Fundamentals of the fungi, Second edition, Glassboro State College, Prentice-Hall, Inc., Englewood Cliffs: New Jersey.

Moreau, J.M. and Maraite, H. (1995). Bipolar heterothallism in *Tapesia yallundae for* two varieties of the anamorph *Pseudocercosporella herpotrichoides. Mycological Research* **99,** 76-80.

Moreau, J.M. and Maraite, H. (1996). Evidence for heterothallic mating system in *Tapesia acuformis* using benomyl sensitivity and esterase isozyme profiles. *Mycological Research* **100**, 1227-1236.

Moreau, J.M., Schingen, J.C. and Maraite, H. (1989). Detection of *Tapesia yallundae* on wheat stubbles in Belgium. *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* **54,** 255-259.

Murray, T.D., De La Pena, R.C., Yildirim, A. and Jones, S.S. (1994). A new source of resistance to *Pseudocercosporella herpotrichoides,* cause of eyespot disease of wheat, located on chromosome 4V of *Dasypyrum villosum. Plant Breeding* **113,** 281-286.

Murray, T.D. (1986). Effects of host resistance to *Pseudocercosporella herpotrichoides* and foot rot severity on yield and yield components in winter wheat. *Plant Disease* **70**, 851-856.

Murray, T.D. (1983). Resistance in winter wheat to *Pseudocercosporella herpotrichoides*. PhD. dissertation. Washington State University, Pullman.

Murray, T.D. (1996). Resistance to benzimidazole fungicides in the cereal eyespot pathogen, *Pseudocercosporella herpotrichoides,* in the Pacific Northwest 1984 to 1990. *Plant Disease* **80,** 19-23.

Nauta, M.J. and Hoekstra, R.F. (1994). Evolution of vegetative incompatibility in filamentous ascomycetes I. Deterministic models. *Evolution* **48**, 979-995.

Nelson, R.R. (1970). Variation in mating capacities among isolates of *Cochliobolus carbonum. Canadian Journal of Botany* **48**, 261-263.

Nelson, M.A. (1996). Mating systems in ascomycetes: a romp in the sac. *Trends in Genetics* **12**, 69-74.

Nene, Y.L. and Reddy, M.V. (1987). Chickpea diseases and their control. In *The chickpea,* pp.233-270 (eds. M.C.Saxena, and K.B. Singh). CAB International: Oxon, United Kingdom.

Nicholson, P., Rezanoor, H.N. and Hollins, T.W. (1993). Classification of a world-wide collection of isolates of *Pseudocercosporella herpotrichoides* by RFLP analysis of mitocondrial and ribosomal DNA and host range. *Plant Pathology* **42,** 58-66.

Nicholson, P., Rezanoor, H.N. and Hollins, T.W. (1991). Occurrence of *Tapesia yallundae* apothecia on field and laboratory-inoculated material and evidence for recombination between isolates. *Plant Pathology* **40,** 626-634.

Nicholson, P. and Rezanoor, H.N. (1994). The use of random amplified polymorphic DNA to identify pathotype and detect variation in *Pseudocercosporella herpotrichoides. Mycological Research* **98,** 13-21.

Nirenberg, H.I. (1981). Differezirerung der Erreger der Halmbruchkrankheit. I. Morphologie. *Zeitschrift fur Pflanzenkrankheiten Pflanzenpathologie und Pflanzenschutz* **88**, 241-248.

Perkins, D.D., Turner, B.C. and Barry, E.G. (1976). Strains of *Neurospora crassa* collected from nature. *Evolution* **30**, 281-313.

Perkins, D.D. and Radford, A., Newmeyer, D. and Bjorkman, M. (1982). Chromosomal loci of *Neurospora crassa. Microbiological Reviews* **46**, 426-570.

Picard-Bennoun, M. (1982). Does translational ambiguity increase during cell differention? *FEBS Letters* **149**, 167-170.

Pittenger, T.H. (1957). The mating type alleles and heterokaryon formation in *Neurospora crassa. Microbial Genetics Bulletin* **15**, 21-22.

Poupard, P., Grare, S., Cavelier, N. and Lind, V. (1994). Development of *Pseudocercosporella herpotricoides* (Fron) Deighton var. *herpotrichoides* and var. *acuformis* on wheat plants measured by ELISA. *Phytopathology* **140,** 301-311.

Priestly, R.A. and Dewey, F.M. (1992). Comparison of isozyme and DNA markers for differentiating W-, R- and C-pathotypes of *Pseudocercosporella herpotrichoides. Plant Pathology* **41,** 591-599.

Priestly, R.A. and Dewey, F.M. (1993). Development of a monoclonal antibody immunoassay for the eyespot pathogen *Pseudocercosporella herpotrichoides. Plant Pathology* **42,** 403-412.

Puhalla, J.E. and Spieth, P.T. (1985). A comparison of heterokaryosis and vegetative incompatibility among varieties of *Gibberella fujikuroi. Experimental Mycology* **9**, 39-47.

Raju, N.B. (1980). Meiosis and ascospore genesis in *Neurospora. European Journal of Cell Biology* **23**, 208-223.

Rayner, A. and Ross, I. (1990). Sexual politics in the cell. *New Scientist* **129**, 30-33.

Rizet, G. (1952). Les phénomènes de barrage chez *Podospora anserina*: analyse génétique des barrages entre souche *s* and *S. Revue de Cytologie et de Biologie Vegetales* **13**, 51-92.

Rizet, G. and Schecroun, J. (1959). Sur les facteurs associés au couple de gènes *S-s* chez le *Podospora anserina. Comptes Rendus de l' Academie des Sciences Paris Serie D* **249**, 2392-2394.

Robbertse, B., Campbell, G.F. and Crous, P.W. (1995). Revision of *Pseudocercosporella*-like species causing eyespot disease of wheat. *South African Journal of Botany* **61,** 41-46.

Robbertse, B., Crous, P.W. and Holz, G. (1994). *Tapesia yallundae* collected from wheat stubble in South Africa. *Mycopathologia* **125,** 23-28.

Robbertse, B., Holz, G. and Crous, P.W. (1996). Sensitivity of South African *Ramulispora herpotrichoides* isolates to carbendazim and ergosterol biosynthesis inhibitors. *Plant Pathology* **45,** 270-275.

Rossignol, J. and Picard, M. (1991). *Ascobolus immersus* and *Podospora anserina*: Sex, recombination, silencing, and death. In *More Gene Manipulations in Fungi* (Eds. J.W. Bennet and L.A. Lasure), pp. 266-290. San Diego.

Sanders, P.L., Waarde, de, M.A. and Loerakker, W.M. (1986). Resistance to carbendazim in *Pseudocercosporella herpotrichoides* from Dutch wheat fields. *Netherlands Journal of Plant Pathology* **92,** 15-20.

Sansome, E.R. (1946). Heterokaryosis, mating-type factors, and sexual reproduction in *Neurospora. Bulletin of the Torrey Botanical Club* **73**, 397-409.

Saragoussi, M. (1986). Study of the genetic composition of the resistance to *Pseudocercosporella herpotrichoides* (Fron) Deighton in four varieties of bread wheat (*Triticum aestivum* L.) by a diallelic cross. *Revista Brasilera de Genetica* **9**, 625-636.

Saunderson, F.R. and King, A.C. (1988). Field occurrence of *Tapesia yallundae,* the teleomorph of *Pseudocercosporella herpotrichoides. Australasian Plant Pathology* **17,** 20-21.

Scott, D.B. (1996). Disease improvement and yield improvement obtained with fungicide applications to spring wheat in South Africa. *Crop Protection* 15, 167-170.

Sindberg, S., Munk, L. and Schulz, H. (1994). *Tapesia yallundae,* the teleomorph of *Pseudocercosporella herpotrichoides* observed for the first time in Denmark. *Journal of Plant Disease and Protection* **101**, 316-320.

Sprague, R. (1936). Relative susceptibility of certain species of graminaceae to *Cercosporella herpotrichoides. Journal of Agricultural Research* **53**, 659-670.

Strausbaugh, C.A. and Murray, T.D. (1989a). Use of epidermal cell responses to evaluate resistance of winter wheat cultivars to *Pseudocercosporella herpotrichoides. Phytopathology* **79**, 1043-1047.

Straubaugh, C.A. and Murray, T.D. (1989b). Inheritance of resistance to *Pseudocercosporella herpotrichoides* in three cultivars of winter wheat. *Phytopathology* **79**, 1048-1053.

Thomas, D.T., Maraite, H. and Boutry, M. (1992). Identification of rye- and wheat-types of *Pseudocercosporella herpotrichoides* with DNA probes. *Journal of General Microbiology* **138**, 2305-2309.

Todd, T.K. and Rayner, A.D.M. (1980). Fungal individualism. *Science Progress* (Oxford) **66**, 331-354.

Vanderplank, J.E. (1978). Genetic and molecular basis of plant pathogenesis. In *Advanced Series in Agricultural Sciences*. Berlin: Springer.

Vanderplank, J.E. (1982). Host-pathogen interactions in plant disease. Academic Press: New York.

Walder-Zulauf, M. and Gisi, U. (1991). Population changes in *Pseudocercosporella herpotrichoides* with and without fungicide selection. *Phytopathology* **132,** 89-98.

Wallwork, H. (1987). A *Tapesia* teleomorph for *Pseudocercosporella herpotrichoides,* the cause of eyespot of wheat. *Australasian Plant Pathology* **16,** 92-93.

Wallwork, H. and Spooner, B. (1988). *Tapesia yallundae* the teleomorph of *Pseudocercosporella herpotrichoides. Transactions of the British Mycological Society* **91**, 703-705.

Western, J.H. (1971). Diseases of crop plants. (Ed. J.H. Western). McMillan: London.

Williams, C.A. and Wilson, J.F. (1966). Cytoplasmic incompatibility in *Neurospora crassa. Annals of the New York Academy of Science* **129**, 853-863.

Wilson, A.D. and Kaiser, J. (1995). Cytology and genetics of sexual compatibility in *Didymella rabiei. Mycologia* **87**, 795-804.

Wilson, J.F., Garnjobst, L. and Tatum, E.L. (1961). Heterokaryon incompatibility in *Neurospora crassa* micro-injection studies. *American Journal of Botany* **48,** 299-305.

Worland, A.J. and Law, C.N. (1987). Resistance to eyespot. In *Annual Report of the Plant Breeding Institute*, pp. 62-63. Trumpington: Cambridge.

2. FUNGICIDE SENSITIVITY OF *TAPESIA YALLUNDAE* POPULATIONS COLLECTED FROM 15 DIFFERENT FIELDS IN THE WESTERN CAPE PROVINCE OF SOUTH AFRICA

ABSTRACT

In the Western Cape province of South Africa, eyespot disease (*Tapesia yallundae*) of wheat is primarily controlled by fungicide applications. Previous studies have shown, however, that isolates of *T. yallundae* vary in their response to various fungicides. In the present study, 20 isolates per field (1 isolate per plant) from 15 fields with different fungicide histories were screened against carbendazim at 1 µg/ml, propiconazole, tebuconazole and flutriafol each at 0.1, 0.5, 1 and 2 µg/ml, flusilazole and bromuconazole each at 0.05, 0.1, 0.5 and 1 µg/ml and fenbuconazole 0.05, 0.1, 0.5, 1, 5, 10 µg/ml. No isolates were identified as resistant to carbendazim. Fenbuconazole was least effective in inhibiting mycelial growth *in vitro,* whereas flusilazole proved to be most effective of all the triazole fungicides tested. Based on the analysis of EC_{50} values compared to that of a field at Gouda that is at baseline sensitivity, shifts in sensitivity and cross-resistance were detected to all triazoles except bromuconazole. The latter can possibly be explained by the similar mode of action of these fungicides. These results suggest, therefore, that the continuous use of one triazole could also induce resistance to other, similar triazoles. However, products comprising a carbendazim/triazole mixture should still be effective in combating disease. Application of such products should also reduce the build-up of resistance, in contrast to products containing triazoles only.

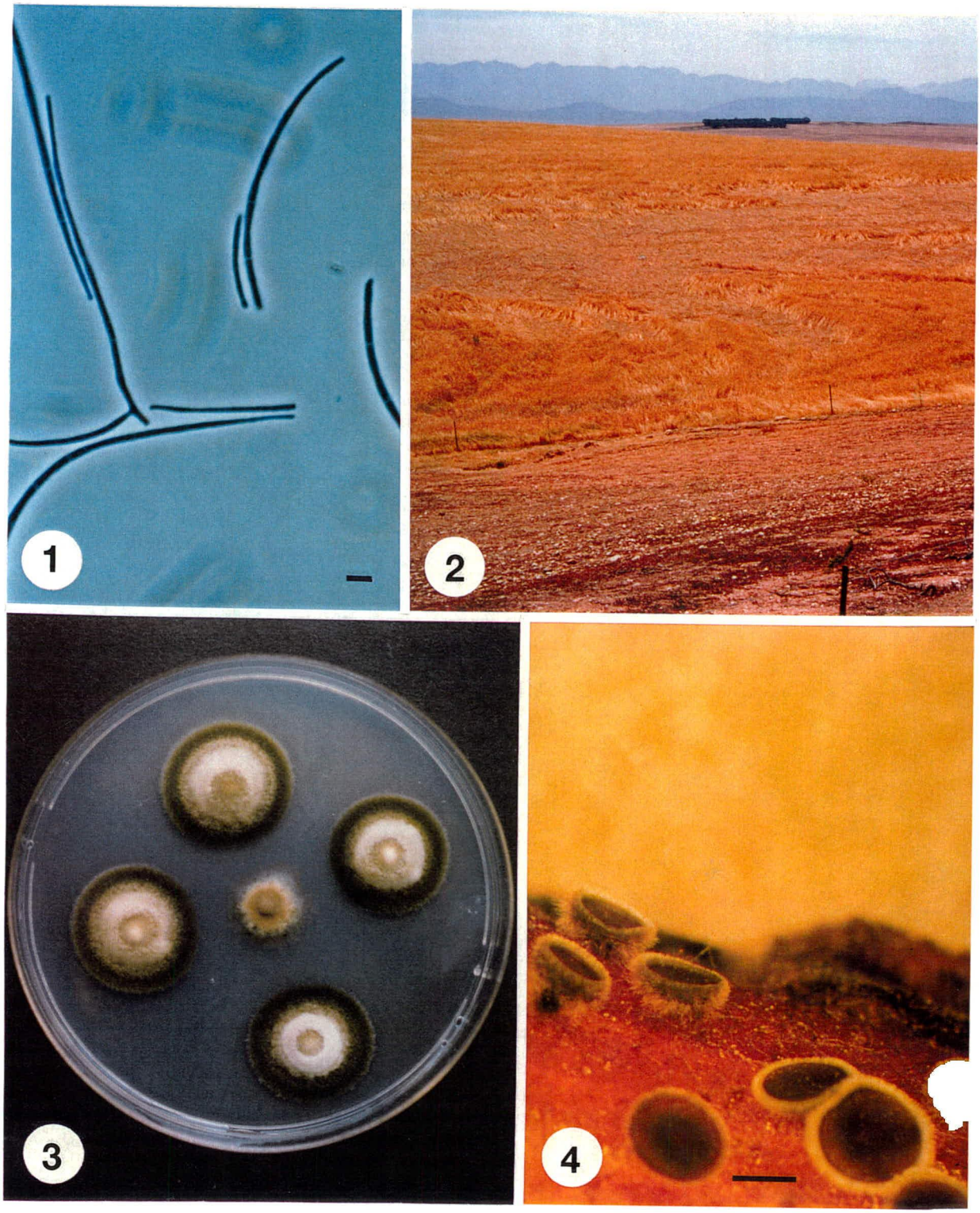
INTRODUCTION

Eyespot is an economically important disease of cereals in all temperate regions of the world, including the Swartland and Koeberg areas of the Western Cape (Welgemoed, 1987; Lochner, 1989; Scott, 1990). The disease is characterised by the presence of damaging stem base lesions which can cause lodging, and render plants difficult to harvest with a consequent loss of yield. Four species have been linked to the disease, of which two are common, namely *Tapesia yallundae* Wallwork and Spooner (W-type) and *T. acuformis* Boerema, Pieters and Hamers (R-type). However, to date only one species, *T. yallundae,* has been associated with the disease in South Africa. The

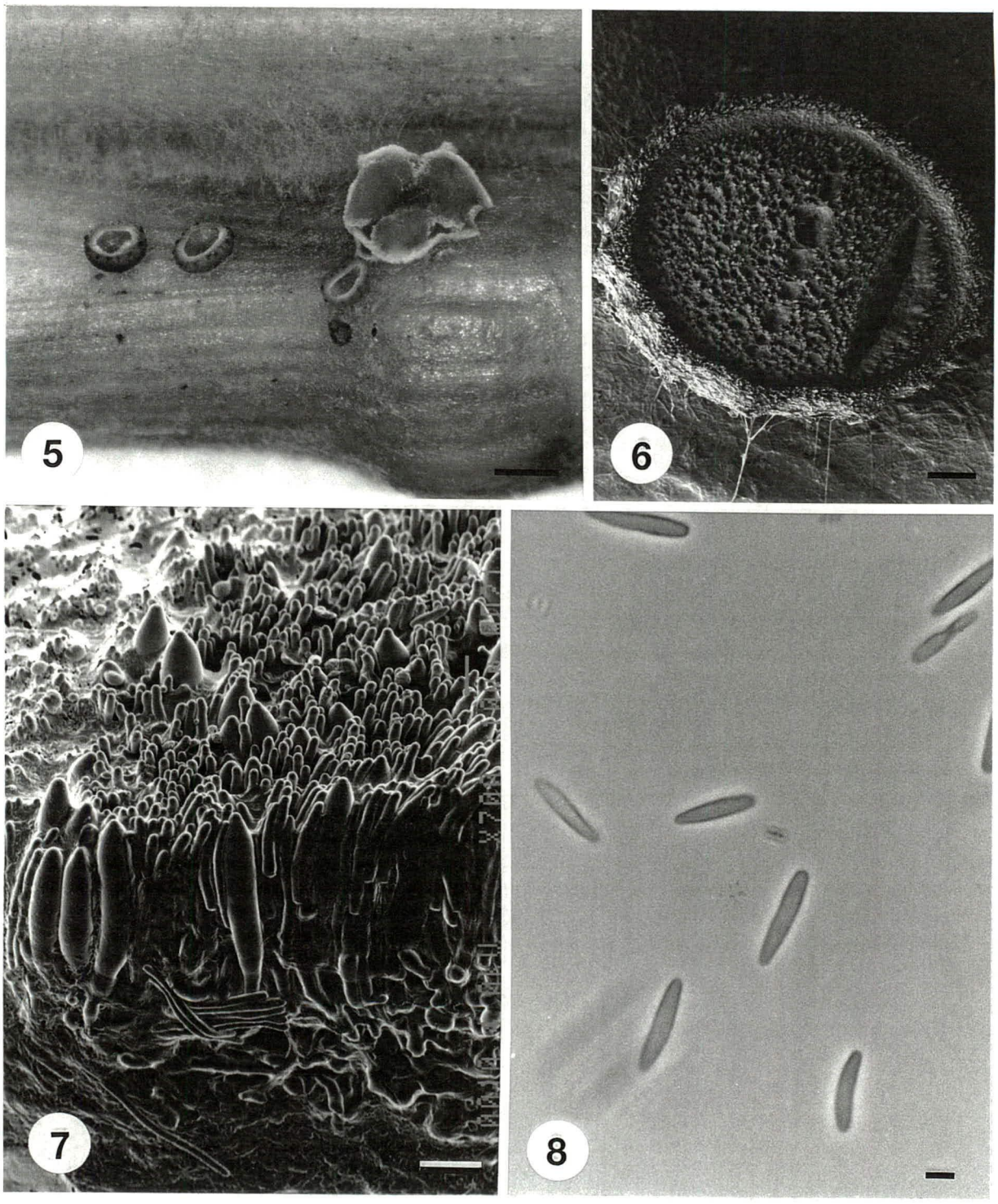
pathogen survives primarily on wheat stubble left in fields from the previous harvest, where it produces spores that are disseminated by wind driven rain droplets to nearby healthy plants. In the Western Cape province, eyespot is primarily controlled by fungicide application (Trench *et al.*, 1992). Previous studies have shown, however, that isolates of *T. yallundae* show variable responses to the various fungicides (Robbertse *et al.*, 1996).

Until 1982, carbendazim and carbendazim-generating fungicides (benomyl and thiophanate-methyl) were the principal control agents for eyespot (Griffin and Yarham, 1983). However, resistance of this pathogen to carbendazim-generating fungicides, originally reported by Rashid and Schlösser (1977) in the Federal Republic of Germany (1977), has been recognized as a problem in the U.K. since 1981 (Griffin and Yarham, 1983; Brown *et al.*, 1984). In 1986, prochloraz and a combination of dichlobutrazol and prochloraz, were the first fungicides recommended in South Africa for eyespot control (Bot *et al.*, 1987). Prochloraz and triazole fungicides inhibit the C14 demethylation step in the fungal ergosterol biosynthesis (Copping *et al.*, 1984), whereas benzimidazole fungicides (methyl-benzimidazole-carbamate-generating [MBC] fungicides) act by affecting tubulin synthesis (Davidse, 1973). Evidence was obtained in Germany that strains of the pathogen were present that were resistant to MBC fungicides even before these fungicides had been used (Rashid and Schlösser, 1975, 1977). In 1980 the low frequency of resistance was, however, not considered to be of any significance in practice (Fehrmann *et al.*, 1982).

The establishment of baseline sensitivities in different populations is important to enable plant pathologists to monitor gradual shifts in sensitivity. This would enable the timely implementation of alternative fungicide strategies before the commercial application of a fungicide becomes ineffective. The present study reports on the screening of 15 different populations of *T. yallundae* collected from farms with different fungicide histories. The aims of this study were to determine the fungicide sensitivity of the eyespot populations present in specific wheat fields, and to contrast each of these fields with a population from a field which had not been subjected to any fungicide application.



Figs 1-4. Field symptoms, cultural and morphological characters of *Tapesia yallundae*. **Fig. 1.** Straight and curved conidia produced on stubble (bar = 0.5 µm). **Fig. 2.** Lodging of wheat in the Swartland. **Fig. 3.** Single conidial colonies on potato dextrose agar. **Fig. 4.** Apothecia produced *in vitro* on a wheat stubble (bar = 375 µm).



Figs 5-8. Apothecia, asci and ascospores of *Tapesia yallundae*. **Fig. 5.** Apothecia forming on an incubated piece of wheat stubble (bar = 750µm). **Fig. 6.** A scanning electron micrograph of an apothecium viewed from above (bar = 100 µm). **Fig. 7.** A scanning electron micrograph of a vertical section through an apothecium showing cylindrical asci with attenuated apices (bar = 10 µm). **Fig. 8.** Ascospores dispersed on water agar (bar = 10 µm).

MATERIALS AND METHODS

Sampling

In 1996 samples were collected from 15 fields to which fungicides had regularly been applied. These fields were compared to one at Gouda (sampled in 1992), regarded to be at baseline sensitivity, as it had not previously been exposed to any fungicide applications (Table 1). Data pertaining to the fungicide regimes employed at the different fields were obtained from the respective farmers and agrochemical companies involved. Plants collected were past the pseudostem erection stage of growth (stage 30+) (Zadoks *et al.*, 1974). Each of the 16 samples comprised 30-50 stems randomly collected. Samples were washed, stems separated, and those with symptoms retained for further study.

Isolation and identification

Symptomatic stubble were dipped in 70% ethanol for 30 sec to dissolve the waxy layer, and surface-sterilized in 1% NaOCl for 1 min. The NaOCl was removed by dipping stubble into 70% ethanol for a further 30 sec, and the stubble rinsed in sterile distilled water. Stubble were placed on moistened filter paper in Petri dishes and incubated for 2 weeks at 10°C under near-ultraviolet light to enhance sporulation. After sporulation conidia were suspended in sterile water, dispersed on water agar (12 g Biolab agar) in Petri dishes and left under a laminar flow hood to germinate. Conidia were located under a stereo microscope and single conidial colonies were established. Isolates were grown on potato dextrose agar (PDA) (Biolab) at 25°C in the dark. One colony was obtained for each stem, with 20 colonies taken as representative of each field, resulting in 320 isolates tested per fungicide.

Fungicide sensitivity

Mycelial plugs were removed from the edges of mycelial mats with a cork borer (3.5 mm diam.), and placed (mycelium down) on PDA amended with fungicide (technical grade) in Petri dishes. This was done in triplicate, with three different plates per isolate per fungicide concentration. Fungicide sensitivity was determined at a range of concentrations. Carbendazim was tested at 1 µg/ml, because it is generally accepted that insensitivity towards carbendazim is only present when growth occurs at this concentration. Furthermore, previous studies (Robbertse *et al.*, 1996;

unpublished research reports) employed a wider range of carbendazim concentrations, and failed to find any indication of resistance towards this compound. Propiconazole, tebuconazole and flutriafol were each screened at 0.1, 0.5, 1, 2 µg/ml, fenbuconazole at 0.05, 0.1, 0.5, 1, 5, 10 µg/ml, flusilazole and bromuconazole each at, 0.05, 0.1, 0.5 and 1 µg/ml.

Plates were incubated at 20°C in the dark for 10 days, and colony growth assessed. Linear growth of each isolate was determined by calculating the mean of the two perpendicular diameters for each of the three colonies at each concentration. The fungicide concentration which inhibited the colony growth of the isolates by 50% (EC_{50} value), compared to the control, was determined by regression analysis of the log inhibition. The mean EC_{50} values of each eyespot population in the 15 fields were compared to the wild-type population using Dunnett's T test (SAS Institute Inc., 1989) was used. According to the test, if the interval between the lower confidence limit and the upper confidence limit for a particular field with the wild-type field includes 0, then there is no significant difference ($P = 0.05$) in the mean EC_{50} value between the experimental population and the wild-type population.

RESULTS AND DISCUSSION

As reported by Robbertse *et al.* (1994, 1996), only *T. yallundae* (W-type) was found to be present in South Africa. This was highly peculiar, as both species seemed to occur in most other countries where eyespot disease was a problem. However, although eyespot had been reported to occur in African countries such as Ethiopia, Morocco, Tanzania and Tunisia (Anonymous, 1997), no record of the *T. acuformis* (R-type) is presently known from this continent. Continuous monitoring of this situation would be required, however, to ensure that agrochemical companies and farmers were alerted the moment *T. acuformis* was found in South Africa. The latter pathogen differs from *T. yallundae* with regard to its fungicide sensitivity and mode of pathogenicity (Hoare, *et al.*, 1986; Bateman, *et al.*, 1990).

Carbendazim

No isolates from any of the fields were found to grow at 1 µg/ml. In South Africa, carbendazim was never used as a sole agent for eyespot control, but was always applied in combination with prochloraz or other triazoles (Bot *et al.*, 1988; Vermeulen

et al., 1990, 1992; Nel *et al.*, 1993). In similar situations elsewhere, it was reported that carbendazim resistance was delayed when used in combination with prochloraz and triazoles (Hoare *et al.*, 1986). Bateman and Fitt (1991) found carbendazim to enhance the activity of prochloraz. When the latter was applied with carbendazim, prochloraz always decreased eyespot incidence and severity. Application of both fungicides proved to be most efficient against eyespot, especially where both *T. yallundae* and *T. acuformis* were present. Prochloraz selected more consistently for *T. acuformis* and carbendazim for *T. yallundae* (Hoare, *et al.*, 1986; Bateman, *et al.*, 1990). Gindrat *et al.* (1993) reported a mixture of carbendazim and flusilazole to be highly efficient in reducing eyespot. Yield was significantly increased in fields where a flusilazole/carbendazim mixture was applied, compared to fields where only flusilazole was applied. A further advantage of combining a triazole with carbendazim was shown by Korbas *et al.* (1994), who achieved a yield increase of 9-13% by using two applications of a flusilazole/carbendazim mixture. In a survey by Robbertse *et al.* (1996), all South African isolates tested were sensitive to the discriminatory carbendazim concentration of 1 µg/ml, suggesting that the local population of *T. yallundae* was still at baseline sensitivity to MBC fungicides.

The triazoles included in the study by Robbertse *et al.* (1996) were tebuconazole, flusilazole and propiconazole, which had EC_{50} values in the same category as that found in the present study, and by Leroux *et al.* (1988) in France. According to Robbertse *et al.* (1996), South African isolates of *T. yallundae* remained sensitive to these fungicides. Isolates were found to differ, however, in their sensitivity towards triademinol and tebuconazole, with some isolates having EC_{50} values greater than 2 µg/ml. The mean EC_{50} values obtained by Robbertse *et al.* (1996) were 0.1 µg/ml for flusilazole, 0.31 µg/ml for propiconazole and 0.7 µg/ml for tebuconazole, thus suggesting reasonable effectivity.

Bromuconazole

None of the fields tested had any history of bromuconazole application. There was no significant difference ($P = 0.05$) among the mean EC_{50} values of fields 7, 12 and 13 with that of the wild-type field, while all other fields had mean EC_{50} values significantly different to that of the field at baseline sensitivity (Fig. 1). Fields 12 and 13 were exposed to a mixture of tebuconazole and carbendazim for 3 years, while

field 7 had a 9-year-history of prochloraz application. Field 4 had the highest significantly different mean EC_{50} value compared to that of the baseline sensitivity. It had a similar fungicide history to that of fields 12 and 13, but also had been exposed to tebuconazole for 6 years. The results of the present study identified several examples that suggested cross-resistance to be present between different triazoles. None of the fields tested had any history of bromuconazole application. However, fields with a history of tebuconazole application (fields 1, 2, 4; Fig. 1) had mean EC_{50} values that were significantly different to that of the wild-type population, suggesting that tebuconazole applications could have reduced the sensitivity of isolates towards bromuconazole. Irregular efficacies because of acquired bromuconazole resistance to *T. yallundae* in France had also been reported from areas where other triazoles were previously used (Duvert *et al.*, 1997; Leroux and Gredt, 1997). These findings indicated that it would be advisable to determine the sensitivity towards bromuconazole in South African *T. yallundae* populations prior to incorporating this triazole into local products aimed at control of eyespot.

Propiconazole

Isolates from fields 1, 3-5, 10, 12 and 13 had mean EC_{50} values that showed no significant difference ($P = 0.05$) with the mean EC_{50} value of the wild-type isolates 0.42 µg/ml/0.087 (Fig. 2). Propiconazole was never applied to any of these fields. It was applied to all other fields, except field 7, which had been exposed to prochloraz only. Robbertse *et al.* (1996) found South African isolates of *T. yallundae* to be sensitive to propiconazole where the mean EC_{50} value of the isolates tested was 0.31 µg/ml. The mean EC_{50} values found in the present study (Fig. 2) were much higher than those reported by Robbertse *et al.* (1996), indicating a shift towards insensitivity to this fungicide. Although the results of the present survey indicated that propiconazole could still control eyespot, prolonged exposure to this fungicide seem to lead to the build-up of resistance, as had been found in France (Leroux, *et al.*, 1988).

Flutriafol

Fields 1, 2, 5, 8 and 9 had mean EC_{50} values that were significantly different to those found in the wild-type isolates (Fig. 3). The mean EC_{50} value of the wild-type isolates was 0.64 µg/ml and the rest of the eyespot isolates evaluated had mean EC_{50} values

that varied from 0.47-1.15 µg/ml. The mean EC_{50} values on fields 6 and 11 which had a history of flutriafol application are in the same range as those of fields 3, 4, 7, 10, 12, 13, 14 and 15, which had no history of flutriafol application. These results failed to indicate any correlation between fungicide history and sensitivity, which might be due to the fact that exposure to one triazole could lead to reduced sensitivity to another (Leroux, *et al.*, 1988).

Tebuconazole

The eyespot isolates of fields 1-4, 6 and 8-10 had mean EC_{50} values that were significantly different ($P = 0.05$) from the mean EC_{50} value for the wild-type isolates (Fig. 4). The mean EC_{50} values determined for the various samples of eyespot isolates from the 15 fields varied from 0.39-1.04 µg/ml, compared to the wild-type population which had a mean EC_{50} value of 0.43 µg/ml. The mean EC_{50} values of isolates from fields 12-14 revealed the greatest inhibition by tebuconazole in this survey. In fields 12 and 13 tebuconazole was applied in combination with carbendazim for three years, proving that such a mixture of fungicides delays the build-up of resistance. In field 9 where tebuconazole had been applied as a single product for five years, inhibition was at its lowest level relative to other fields. In contrast however, field 10, which reportedly had a 9 year history of tebuconazole application, had a mean EC_{50} value of 0.6578µg/ml, being lower than that of field 9 (1.0492µg/ml). Although several fields had EC_{50} values that were significantly different from that of the wild-type population towards tebuconazole, no correlation between years of application and the level of sensitivity was apparent. However, given the other examples of cross-resistance found in this study, it is possible that other triazoles such as flutriafol, fenbuconazole and propiconazole applied to these fields (Table 1) could have clouded the picture and led to the build-up of resistance to tebuconazole. At present this resistance does not seem to influence disease control, as Scott (1996) reported that tebuconazole still gave good control and a significant yield increases when applied against eyespot.

Flusilazole

The mean EC_{50} values of all 15 fields showed significant differences ($P = 0.05$) to that of the wild-type field (Fig. 5). The highest values were close to 0.3 µg/ml, compared to that of the wild-type field that was 0.07748 µg/ml. Furthermore, in none of the

fields did the interval between the lower confidence limit and the upper confidence limit include "0" according to Dunnett's T test, thus indicating a shift in flusilazole sensitivity. In experiments conducted on eyespot in the U.K. by Jones (1994), prochloraz and flusilazole gave significant reductions of eyespot disease and increases in yield. Leroux and Gredt (1997) reported irregular efficacies of flusilazole because of acquired resistance following years of application in France. Further research will now have to be conducted to determine if flusilazole is still effective under field conditions.

Fenbuconazole

The mean EC_{50} values of all 15 fields showed significant to highly significant differences compared to the mean EC_{50} value of the wild-type field (Fig. 6). Most fields had mean EC_{50} values above 5 µg/ml, compared to that of the wild-type population (0.043 µg/ml), which resulted in the mean resistance factors (EC_{50} value of resistant isolate/ EC_{50} of wild-type isolate) to range from 98.606 to 164.42. A shift of this magnitude indicated resistance towards fenbuconazole, as could be seen from field 2 (Fig. 7) compared to the wild-type population (Fig. 8). In field 7, a gradual shift in sensitivity (Fig. 9) was evident towards fenbuconazole, relative to the wild-type population (Fig. 8). Fields 1, 5, 7, 12 and 13 still had some isolates that had EC_{50} values lower than 1 µg/ml. The EC_{50} values of these isolates ranged from 0.16-0.7 µg/ml. Fields 2 and 7 had no history of fenbuconazole application. The only fields with fenbuconazole history were 8, 9, 14 and 15. However, their levels of sensitivity were indifferent from those of the rest of the fields. The best inhibition was found in fields 12 and 13 where a mixture of carbendazim and tebuconazole had been applied for 3 years.

Further research is required, however, to determine if the resistance *in vitro* is also indicative of field resistance. Findings obtained in the present study had clearly shown differences among the triazoles regarding their efficacy in controlling eyespot disease. Furthermore, evidence presented here of apparent cross-resistance between triazoles also underline the importance of planning fungicide strategies to monitor shifts in sensitivity towards triazoles in an attempt to guard against the build-up of resistance against a wider spectrum of these fungicides than that currently applied.

REFERENCES

Anonymous. (1997). CAB International, Wallingford, Oxon OX10 8DE, UK.

Bateman, G.L., Fitt, B.D.L., Creighton, N.F and Hollomon, D.W. (1990). Changes in populations of *Pseudocercosporella herpotrichoides* in successive crops of winter wheat in relation to initial populations and fungicide treatments. *Crop Protection* **9**, 135-142.

Bateman, G.L. and Fitt, B.D.L. (1991). Effects of fungicides on eyespot in five successive crops of winter wheat in plots with different initial or fungicide-selected populations of *Pseudocercosporella herpotrichoides*. *Crop Protection* **10**, 479-484.

Bot, J., Sweet, S. and Hollings, N. (1987). A guide to the use of pesticides and fungicides in the Republic of South Africa. Pretoria, South Africa: Department of Agriculture, 31 ed.

Bot, J., Sweet, S., Krause, M. and Hollings, N. (1988). A guide to the use of pesticides and fungicides in South Africa. Pretoria, South Africa: Department of Agriculture, 32 ed.

Brown, M.C., Taylor, G.S. and Epton, H.A.S. (1984). Carbendazim resistance in the eyespot pathogen, *Pseudocercosporella herpotrichoides*. *Plant Pathology* **33**, 101-111.

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

Crop Protection Compendium. (1997). CAB International, Wallingford, Oxon OX10 8DE, UK.

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

Duvert, P., Aubert-Giquea ıx, C. and Lacombe, J.P. (1997). Combination bromuconazole-prochloraz. Unintentional effects on physiological lodging. *Phytoma* **49**, 32-34.

Fehrmann, H., Hostern, J. and Siebrasse, G. (1982). Five years' results from long-term field experiment on carbendazim resistance of *Pseudocercosporella herpotrichoides*. *Crop Protection* **1**, 165-168.

Gindrat, D., Frei, P. and Maillard, A. (1993). Experiments on the control of diseases of winter wheat in French speaking Switzerland (1987-1991) I. Eyespot (*Pseudocercosporella herpotrichoides*). *Revue-Suisse-d' Agriculture* **25**, 53-60.

Griffin, M.J. and Yarham, D.J. (1983). Fungicide resistance-MBC resistance in the eyespot fungus. *Agrospray. FBC Limited, Technical Information* **6**, 2-4.

Hoare, F.A., Hunter, T. and Jordan, V.W.L. (1986). Influence of spray programmes on development of fungicide resistance in the eyespot pathogen of wheat, *Pseudocercosporella herpotrichoides*. *Plant Pathology* **35**, 506-511.

Jones, D.R. (1994). Evaluation of fungicides for control of eyespot disease and yield loss nationships in winter wheat. *Plant Pathology* **43**, 831-846.

Korbas, M., Janczak, C. and Remlein, D. (1994). Effect of a correct decision for controlling the fungus *Pseudocercosporella herpotrichoides* (Fron.) Deighton, the pathogen causing eyespot. *Materialy-Sesji-Instytutu-Ochrony-Roslin* **34**, 132-138.

Leroux, P., Gredt, M. and Boeda, P. (1988). Resistance to inhibitors of sterol biosynthesis in field isolates or laboratory strains of the eyespot pathogen *Pseudocercosporella herpotrichoides*. *Pesticide Science* **23**, 119-129.

Leroux, P. and Gredt, M. (1997). Evolution of fungicide resistance in the cereal eyespot fungi *Tapesia yallundae* and *Tapesia acuformis* in France. *Pesticide Science* **51**, 321-327.

Lochner, H. (1989). Omlê word onderskat. *Landbouweekblad* **591**, 36-37.

Nel, A., Krause, M., Hollings, N., Greyling, J. and Dreyer, M. (1993). A guide to the use of pesticides and fungicides in South Africa. Pretoria, South Africa: Department of Agriculture.

Rashid, T. and Schlösser, E. (1975). Resistenz von *Cercosporella herpotrichoides* gegenüber benomyl. *Zeitschrift für Pflanzenkrakheiten und Pflzenschutz* **82**, 765-766.

Rashid, T. and Schlösser, E. (1977). Cereal foot-rot pathogens. II. Occurrence and distribution of benomyl-tolerant strains. *Mededelingen van de Faculteit Landbouwetenschappen Rijksuniversiteit Gent* **42**, 1057-1065.

Robbertse, B., Crous, P.W. and Holz, G. (1994). *Tapesia yallundae* collected from wheat stubble in South Africa. *Mycopathologia* **125**, 23-28.

Robbertse, B., Holz, G. and Crous, P.W. (1996). Sensitivity of South African *Ramulispora herpotrichoides* isolates to carbendazim and ergosterol biosynthesis inhibitors. *Plant Pathology* **45**, 270-275.

SAS Institute Inc. (1989). *SAS/STAT® User's Guide, Version 6.12, Fourth Edition, Volume 2,* Cary, NC: SAS Institute Inc.

Scott, D.B. (1990). Wheat diseases in South Africa. Republic of South Africa, Department of Agricultural Research, Technical Communication **220**, 17-19.

Scott, D.B. (1996). Disease control and yield improvement obtained with fungicide applications to spring wheat in South Africa. *Crop Protection* **15**, 167-170.

Trench, T.N., Wilkinson, D.J. and Esterhuysen, S.P. (1992). South African plant disease control handbook. Pietermaritzburg, South Africa. Farmer Support Group, University of Natal, Pietermaritzburg.

Vermeulen, J.B., Sweet, S., Krause, M. and Hollings, N. (1990). A guide to the use of pesticides and fungicides in Republic South Africa. Pretoria, South Africa: Department of Agriculture.

Vermeulen, J.B., Krause, M., Nel, A., Hollings, N. and Greyling, J. (1992). A guide to the use of pesticides and fungicides in the Republic of South Africa. Pretoria, South Africa: Department of Agriculture.

Welgemoed, Z. (1987). Keer omvalsiekte by koring betyds. *Landbouweekblad* **463**, 16-19.

Zadoks, J.C., Chang., T.T. and Konzak, C.F. (1974). A decimal code for the growth stages of cereals. *Weed Research* **14**, 415-421.

Table. 1. Localities in the Western Cape province where samples of *Tapesia yallundae* were collected and the history of fungicides applied.

Field	Locality	Fungicides applied	Product	Years in use
1	Malkopsvlei	Tebuconazole; Carbendazim/ Tebuconazole	Folicur; Libero	6; 3
2	Malkopsvlei	Tebuconazole; Carbendazim/ Tebuconazole	Folicur; Libero	6; 3
3	Malkopsvlei	Carbendazim/ Tebuconazole	Libero	6
4	Malansdam	Tebuconazole; Carbendazim/ Tebuconazole	Folicur; Libero	6; 3
5	Tweefontein	Flusilazole; Flusilazole/ Carbendazim	Capitan; Punch C	6; 3
6	Bakenfontein	Carbendazim/ Flutriafol; Flusilazole	Early Impact; Capitan	4; 5
7	Spes Bona	Prochloraz	Sportak	9
8	Klipfontein	Fenbuconazole; Propiconazole	Indar; Tilt	3; 4
9	Bloemendal Trust	Propiconazole; Fenbuconazole; Tebuconazole	Tilt; Indar; Folicur	2; 3; 5
10	Dasdrif	Tebuconazole	Folicur	9
11	Grensplaas	Flutriafol	Impact	?
12	Papkuilsfontein	Carbendazim/ Tebuconazole	Libero	3 (untreated 1996)
13	Papkuilsfontein	Carbendazim/ Tebuconazole	Libero	3
14	Klipfontein	Fenbuconazole; Prochloraz	Indar; Sportak	3; 4 (untreated 1996)
15	Klipfontein	Fenbuconazole; Prochloraz	Indar; Sportak	3; 4
16	Gouda	Untreated	—	—

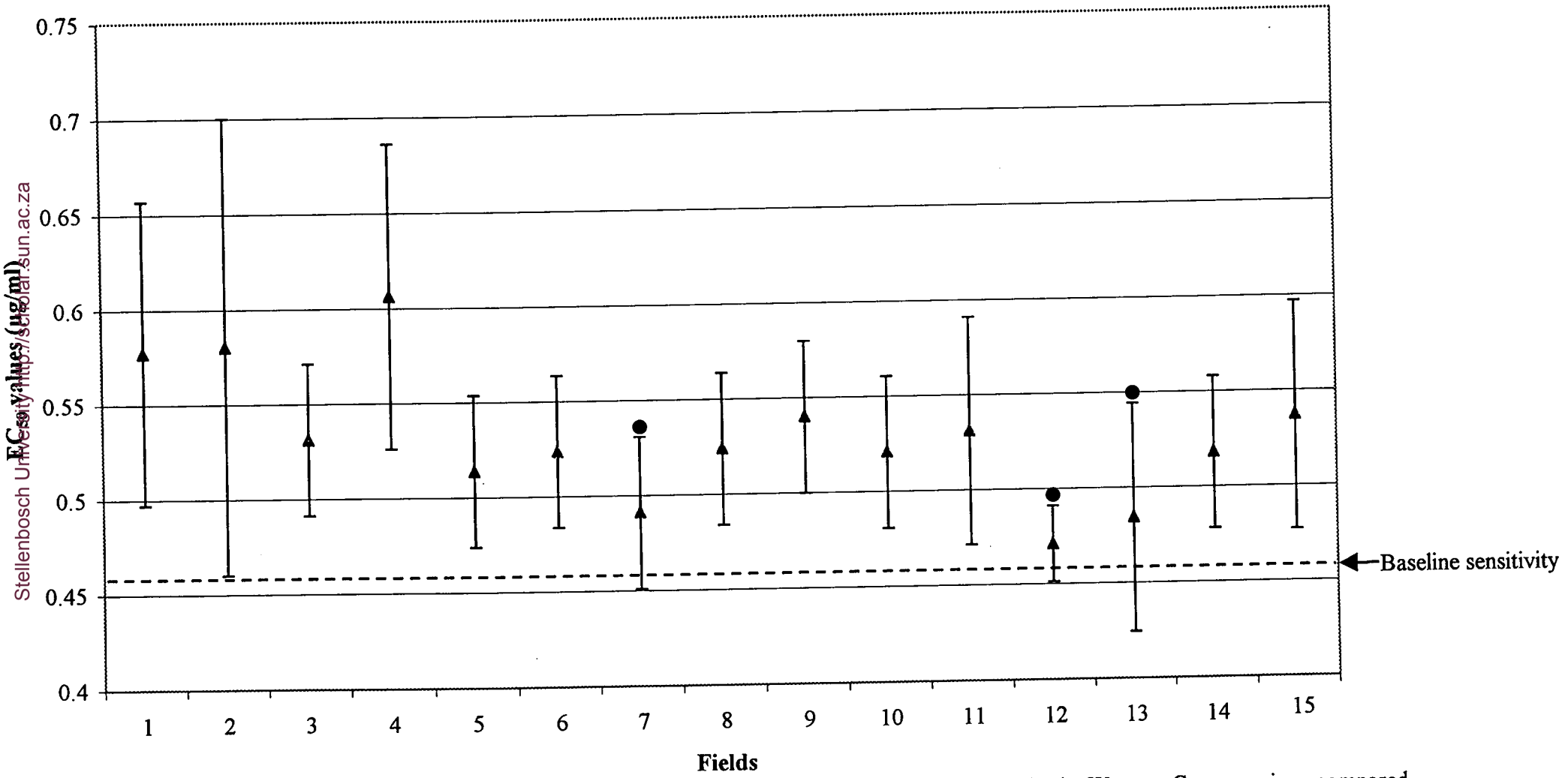


Fig. 1. Range in bromuconazole sensitivity of eyespot (*Tapesia yallundae*) populations from 15 fields in the Western Cape province compared to the mean EC_{50} value of a wild-type population at baseline sensitivity (field 16). Bar indicates standard deviation. Dot indicates fields where the mean EC_{50} value is not significantly different ($P = 0.05$) from baseline sensitivity.

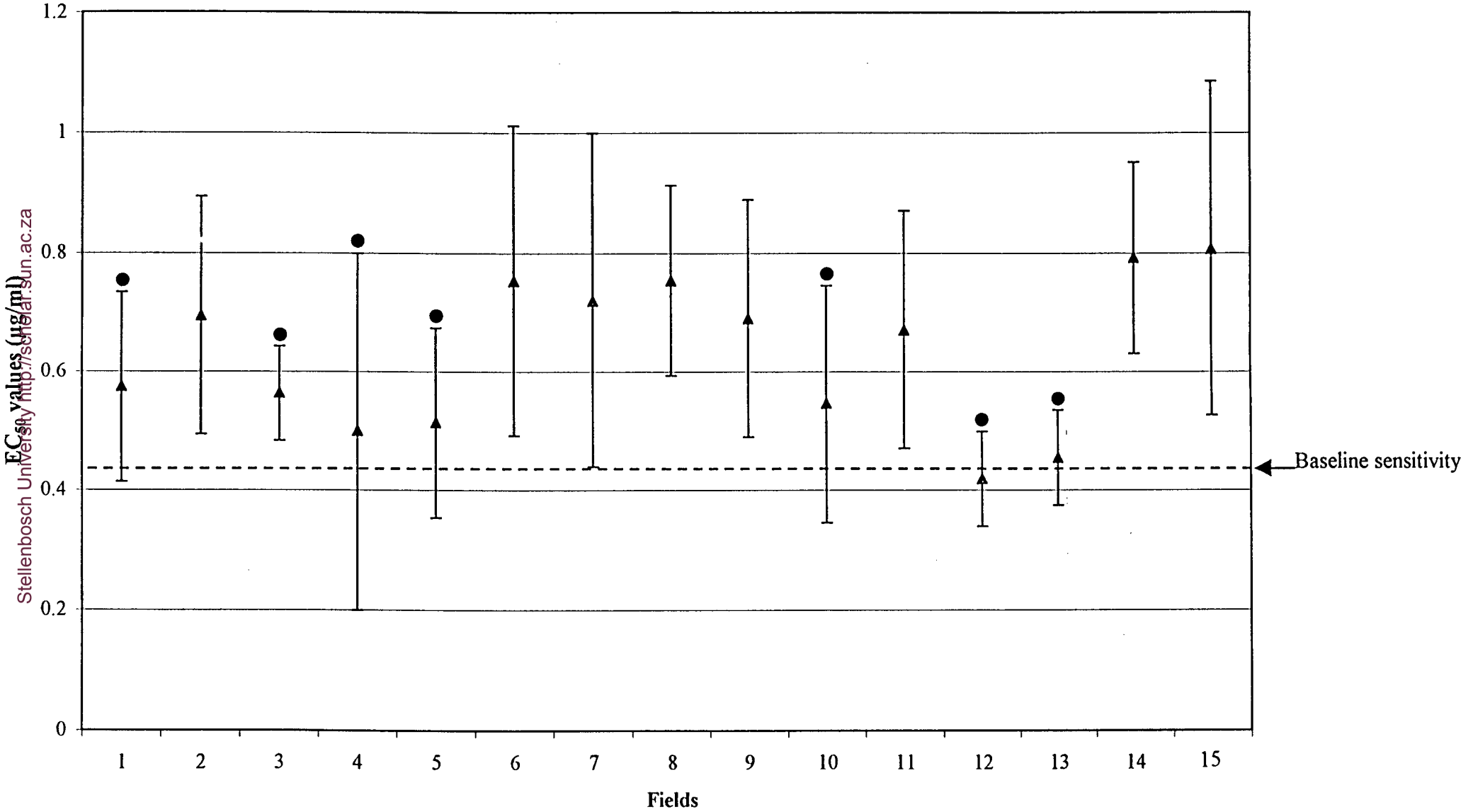


Fig. 2. Range in propiconazole sensitivity of eyespot (*Tapesia yallundae*) populations from 15 fields in the Western Cape province compared to the mean EC_{50} value of a wild-type population at baseline sensitivity (field 16). Bar indicates standard deviation. Dot indicates fields where the mean EC_{50} value is not significantly different ($P = 0.05$) from baseline sensitivity.

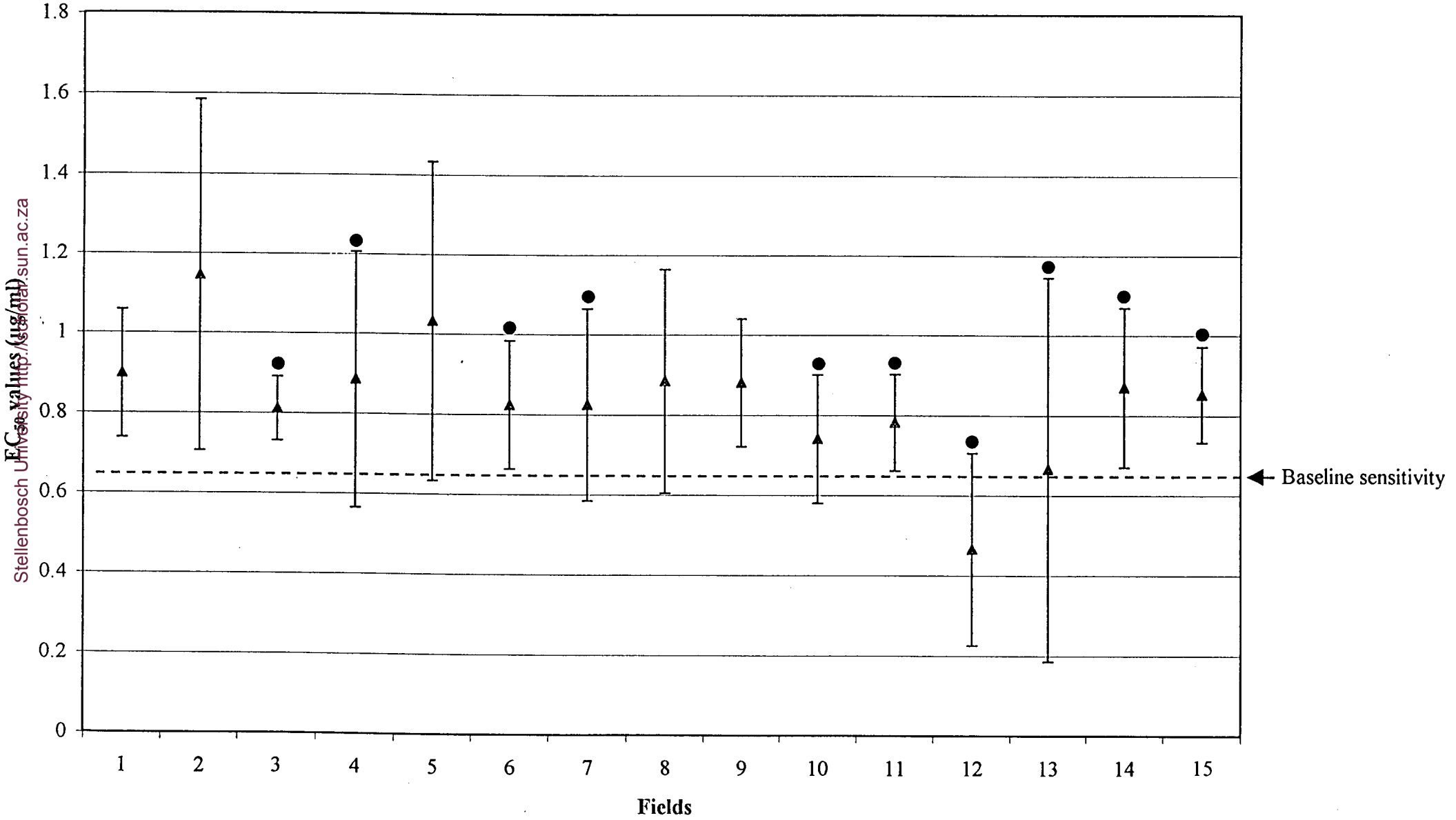


Fig. 3. Range in flutriafol sensitivity of eyespot (*Tapesia yallundae*) populations from 15 fields in the Western Cape province compared to the mean EC_{50} value of a wild-type population at baseline sensitivity (field 16). Bar indicates standard deviation. Dot indicates fields where the mean EC_{50} value is not significantly different ($P = 0.05$) from baseline sensitivity.

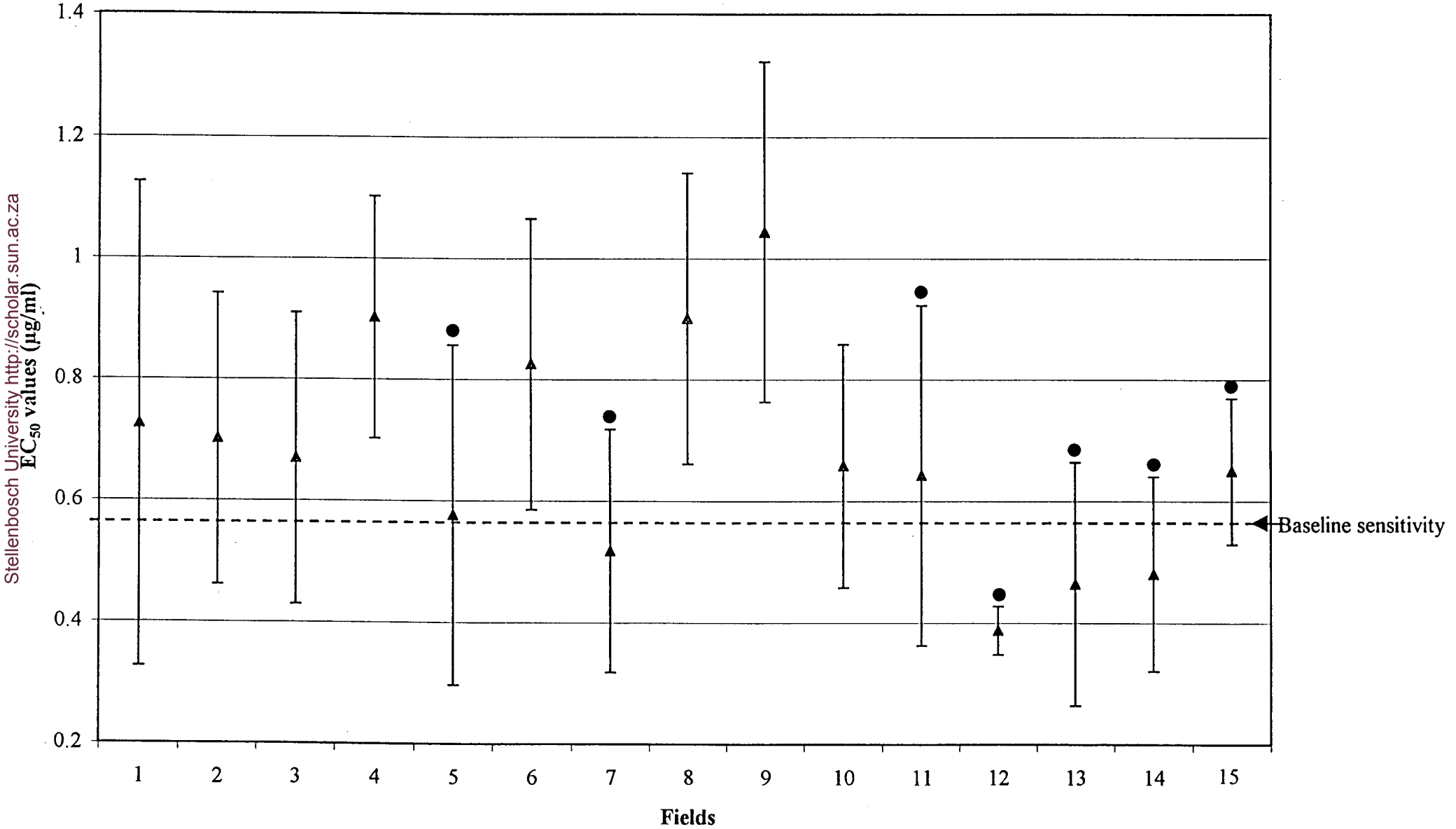


Fig. 4. Range in tebuconazole sensitivity of eyespot (*Tapesia yallundae*) populations from 15 fields in the Western Cape province compared to the mean EC_{50} value of a wild-type population at baseline sensitivity (field 16). Bar indicates standard deviation. Dot indicates fields where the mean EC_{50} value is not significantly different ($P = 0.05$) from baseline sensitivity.

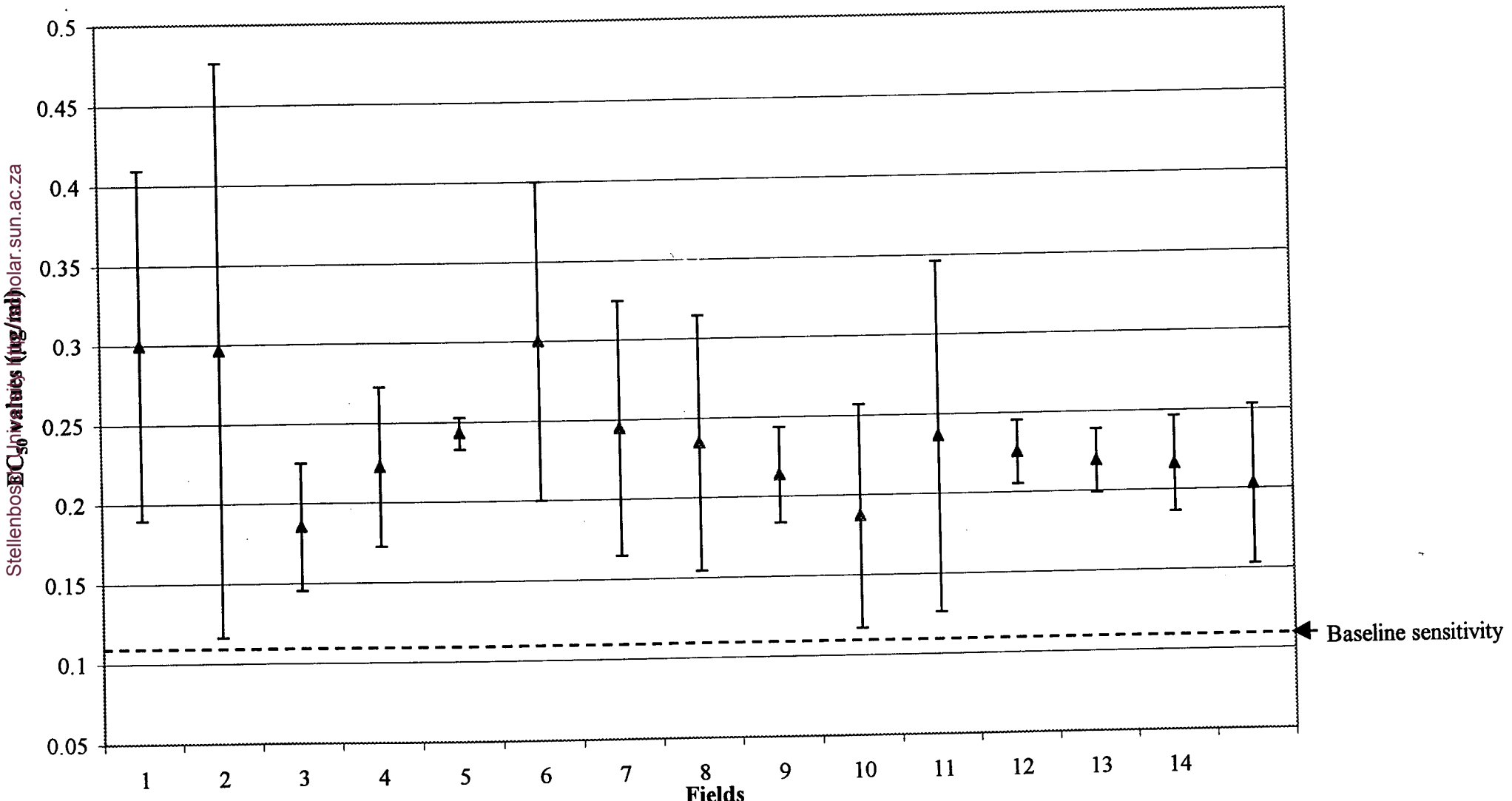


Fig. 5. Range in flusilazole sensitivity of eyespot (*Tapesia yallundae*) populations from 15 fields in the Western Cape province compared to the mean EC_{50} value of a wild-type population at baseline sensitivity (field 16). Bar indicates standard deviation. All fields have mean EC_{50} values significantly different ($P = 0.05$) from baseline sensitivity.

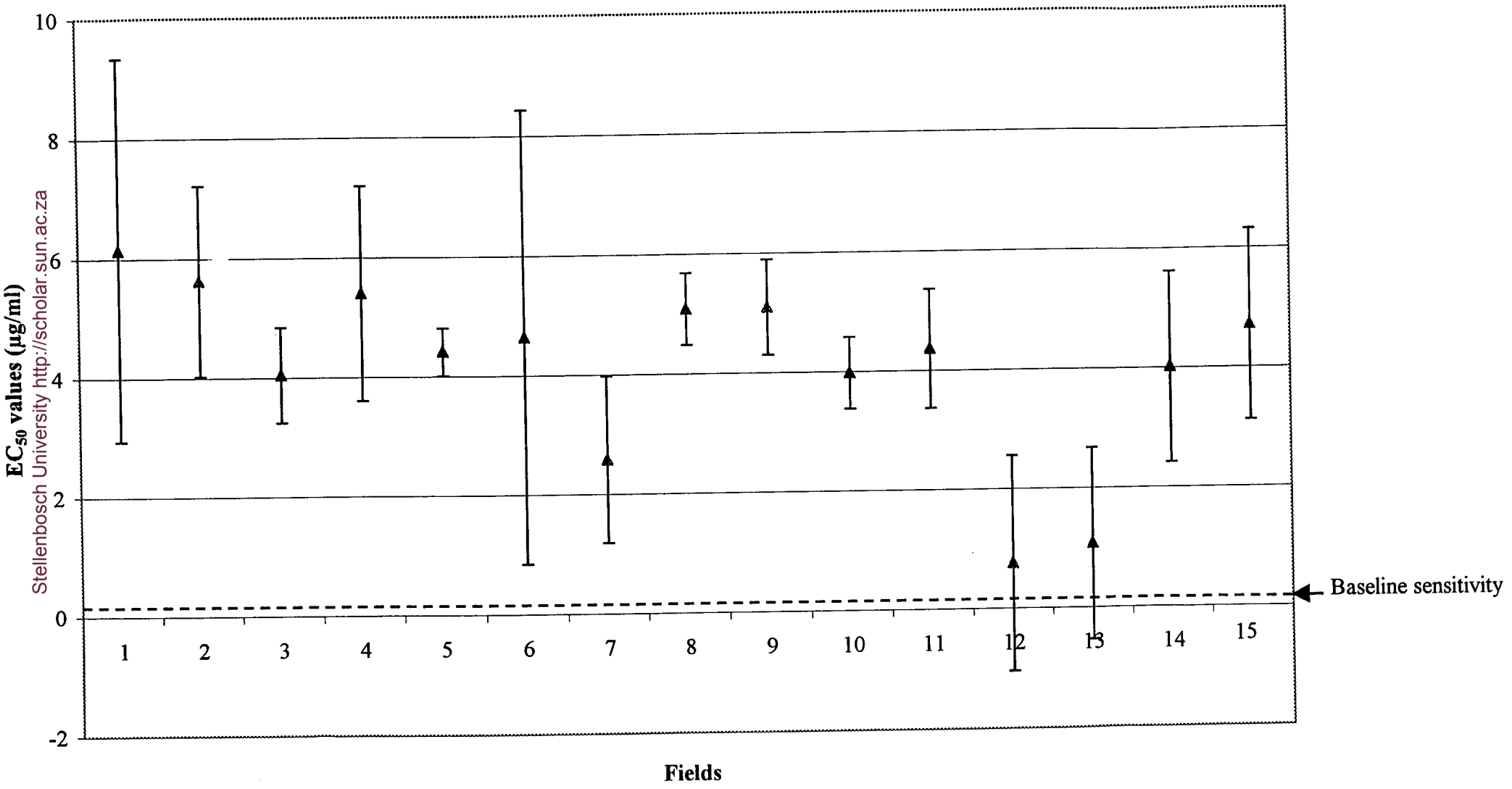


Fig. 6. Range in fenbuconazole sensitivity of eyespot (*Tapesia yallundae*) populations from 15 fields in the Western Cape province compared to the mean EC_{50} value of a wild-type population at baseline sensitivity (field 16). Bar indicates standard deviation. All fields have mean EC_{50} values significantly different ($P = 0.05$) from baseline sensitivity.

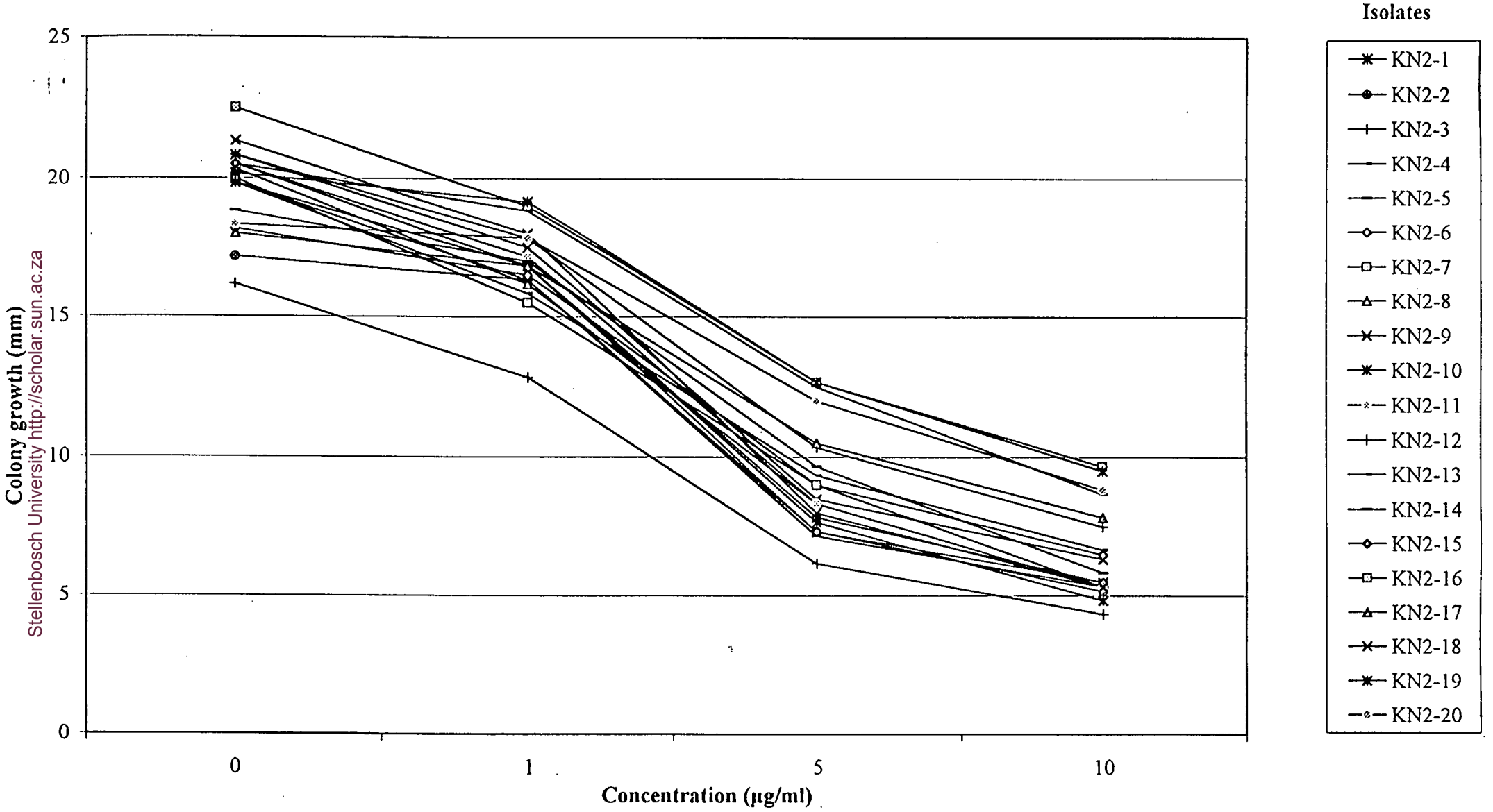


Fig. 7. Growth response of *Tapesia yallundae* isolates from field 2 on potato dextrose agar amended with fenbuconazole.

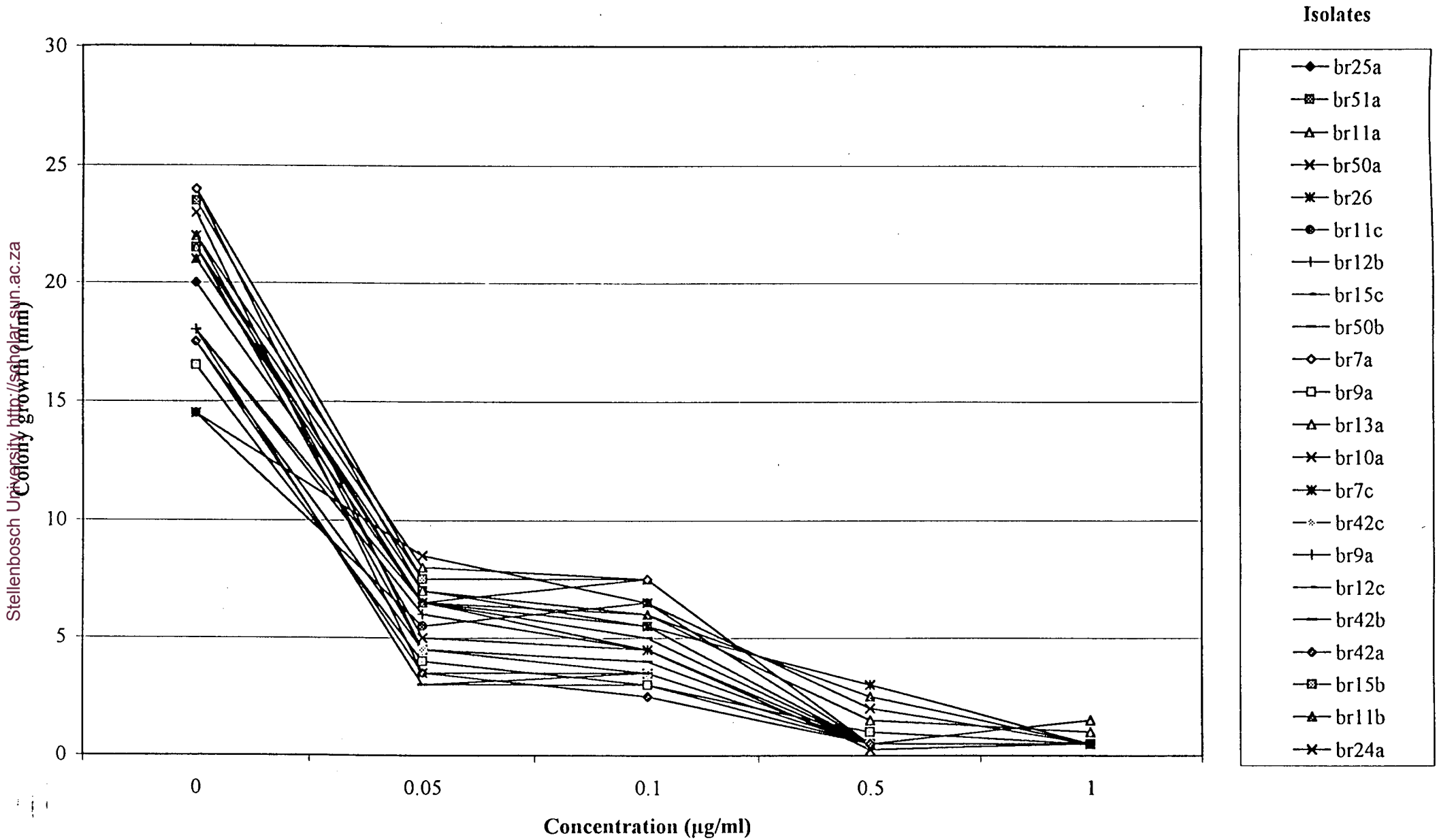


Fig. 8. Growth response of *Tapesia yallundae* isolates from a field with a wild-type population on potato dextrose agar amended with fenbuconazole.

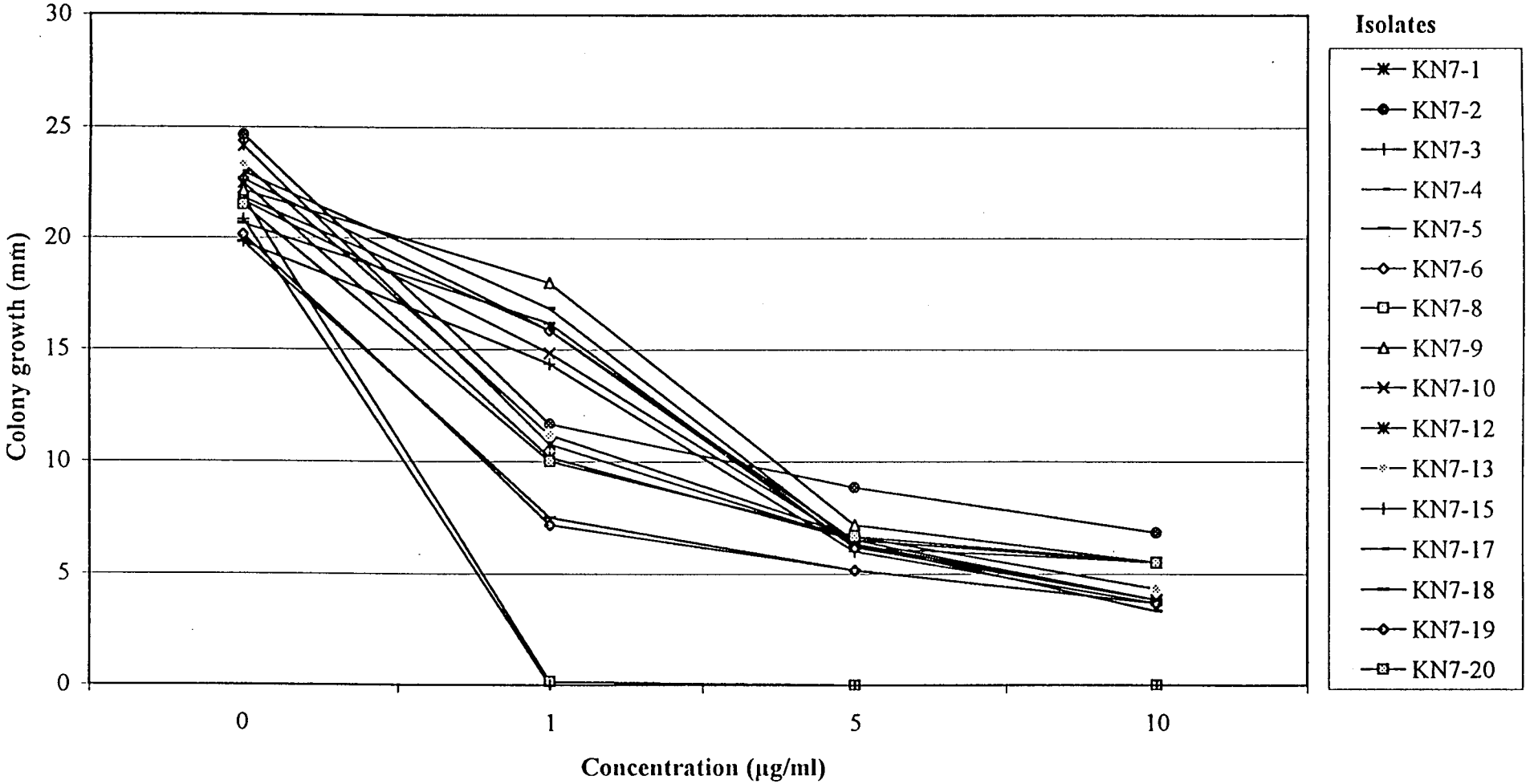


Fig. 9. Growth response of *Tapesia yallundae* isolates from field 7 on potato dextrose agar amended with fenbuconazole.

3. EFFECT OF RECOMBINATION ON SENSITIVITY TO TRIAZOLE FUNGICIDES IN ASCOSPORE PROGENIES OF *TAPESIA YALLUNDAE*

ABSTRACT

The relative contribution of sexual and asexual reproduction in a population determines how rapidly new combinations of virulence genes appear in the field, and also influences the development of fungicide resistance. Populations that undergo regular sexual reproduction, continually have progeny with new combinations of genes, whereas populations with strict asexual reproduction possess a more limited number of different gene combinations, and have to rely on mutation, genetic drift or the parasexual cycle to induce genetic variation. In the present study, two parental isolates, and the resulting F1 and F2 progeny were tested for sensitivity towards the triazole fungicides bromuconazole, propiconazole, flutriafol, tebuconazole and flusilazole to determine the effect of recombination on fungicide sensitivity. A specific RAPD primer defining mating type was identified, and used to segregate MAT1-1 and MAT1-2 isolates to investigate the possible correlation between fungicide sensitivity and mating type. No correlation was found between these two characters with any of the triazoles tested, which suggests different loci or genes controlling mating type and fungicide sensitivity towards triazoles. Furthermore, a continuous range of sensitivities characteristic of a quantitative response was observed between the triazoles in the F1 and F2 progenies, producing greater variation towards the fungicides after recombination.

INTRODUCTION

Eyespot, caused by *Tapesia yallundae* Wallwork & Spooner, is a serious disease of wheat in various temperate wheat producing regions of the world (Fitt *et al.*, 1988), including the Swartland and Koeberg areas of the Western Cape province (Welgemoed, 1987; Lochner, 1989; Scott, 1990). Control of eyespot disease has hitherto been dominated by the use of chemicals. Fungicide treatments are, and will remain, essential for maintaining healthy crops and reliable, high quality yields. They form a key component of integrated crop management, and their effectiveness must be sustained as much as possible. Pathogen resistance to fungicides is widespread. The

performance of most of the modern, systemic fungicides has been affected to some degree (Georpoulos, 1985).

The importance of selection in overcoming host-plant resistance and in the development of resistance to fungicides is well-known in plant pathology. Migration, genetic drift, mutation and recombination may be equally important evolutionary forces shaping the genetic structure of pathogen populations, but these processes have received relatively little attention from plant pathologists (Peever and Millgroom, 1993). Recombination creates novel genotypes in pathogen populations and may be especially important in terms of the evolution of fungicide resistance and overcoming host resistance genes (Wilson and Kaiser, 1995). Populations of plant pathogens that reproduce sexually are often genetically more variable than those that reproduce asexually (Burdon and Roelfs 1985; Goodwin *et al.*, 1992). Estimation of genetic correlations requires an experimental design in which genetic and environmental sources of variation in phenotypes can be partitioned (Ennos and Swales, 1988, 1991).

Genetic correlation has been proposed as a method for studying the evolution of pesticide resistance (Via, 1986). One of the relevant factors controlling resistance evolution may also be the way in which resistance is inherited. Resistance to chemicals in fungi is often controlled by several major genes (Grindle, 1987). Barriers to gene exchange among different populations, including vegetative compatibility and several incompatibility factors are quite frequent in fungi (Burnett, 1983; Brasier, 1987). Resistance to several systemic fungicides has been shown to be determined by a single major genetic locus (Stanis and Jones, 1984; Smith *et al.*, 1991). Resistance to other fungicides appears to be determined quantitatively (Hollomon 1981; Hollomon *et al.*, 1984). The mode of inheritance of resistance has also been thought to correlate to the fungicide rather than the pathogen (Skylakakis, 1985; Skylakakis and Hollomon, 1987).

The mode of inheritance of the fungicide resistance may play a role in how rapidly resistance evolves in a pathogen population, and anecdotal field observations have supported this generality (Georpoulos, 1985). One way this may be explained is that resistance conferred by single genetic loci, such as resistance to benomyl or metalaxyl, confer very high levels of resistance, which result in greater additive genetic variance relatively to quantitatively determined resistance (Van Tuyl, 1977;

Stanis and Jones, 1984, 1985). This is because single gene resistances are distributed into a limited number of discrete phenotypic classes, whereas quantitatively inherited resistances are distributed continuously. The rate at which resistance evolves in a population is directly proportional to the amount of additive genetic variance in resistance, and thus all other factors being equal, single gene resistances will have greater additive genetic variance and will evolve faster (Milgroom *et al.*, 1989). In biallelic, heterothallic ascomycetes, sexual progeny (ascospores) are obtained by crossing strains of opposite mating type carrying the MAT 1-1 and MAT1-2 alleles (Faretra *et al.*, 1988).

Tapesia yallundae is a well-known heterothallic ascomycete (Moreau and Maraite, 1995; Dyer *et al.*, 1993) that exhibits resistance to several triazole fungicides (Leroux *et al.*, 1988; Leroux and Gredt, 1997). The sexual state of the eyespot fungus has been reported from Australia (Saunderson and King, 1988), Europe (King, 1991; Dyer *et al.*, 1994; Sindberg *et al.*, 1994; Dyer and Lucas, 1995) and induced in the laboratory in South Africa (Robbertse, *et al.*, 1994). Dekker (1993) stated that resistance to triazole fungicides develops quantitatively. The aim of the present study was to determine how recombination in ascospore progeny would influence resistance towards the various triazoles commonly used in fungicide compounds to control eyespot disease. Furthermore, the possible correlation between mating type and fungicide sensitivity was also investigated.

MATERIALS AND METHODS

Isolation and identification

Symptomatic stubble were dipped in 70% ethanol for 30 sec to dissolve the waxy layer, and surface sterilised in 1% NaOCl for 1 min. The NaOCl was removed by dipping the stubble into 70% ethanol for a further 30 sec, and the stubble rinsed in sterile distilled water. Stubble were placed on moistened filter paper in Petri dishes and incubated for 2 weeks at 10°C under near-ultraviolet light to enhance sporulation. After sporulation conidia were suspended in sterile water on water agar (12 g Biolab agar), and left under a laminar flow hood to germinate. Conidia were located under a stereo microscope and single conidial colonies were established. Isolates were grown on potato dextrose agar (PDA) (Biolab) at 25°C in the dark. One colony was obtained

for each piece of stubble. Isolates obtained from a field in the Western Cape province with a known fungicide resistance towards tebuconazole were selected, and the two identified in mating experiments as MAT1-1 and MAT1-2, respectively.

Mating system and the recovery of ascospore isolates

Straw segments (5 cm in length) were autoclaved twice (15 min at 121°C) with a 24 h interval. Stubble were colonized with either the MAT1-1 or MAT1-2 conidial isolates of the parental strains (P1) on potato dextrose agar (PDA) (Biolab) plates for 4 weeks at 25°C. The colonized stubble were mated (MAT1-1 x MAT1-2) on water agar (WA), and incubated at 10°C under near-ultraviolet light for 3 months. Mature apothecia were removed from successful matings, stuck to the lids of Petri dishes with petroleum jelly, and submerged with sterile, distilled water for 2 h. After the water was drained, Petri dish lids with swollen apothecia were placed on fresh WA plates to allow ascospore discharge. Single ascospores of the first filial generation (F1) were isolated and plated onto PDA. Two F1 isolates were chosen as parents for the second generation (P2), and the same procedure repeated for the recovery of isolates of the second filial generation (F2). Mycelial plugs of the F1 and F2 progeny were inoculated onto malt extract agar (MEA) (Biolab) slants in 15 ml bottles for long term preservation. Eight ascospores were also obtained from one ascus to assist with screening of primers to identify one that could distinguish mating type.

Testing isolates for fungicide sensitivity

Mycelial plugs were removed from the edges of mycelial mats with a cork borer (3.5 mm diam.), and placed (mycelium down) on PDA amended with fungicide (technical grade) in Petri dishes. Isolates were screened in triplicate, with three different plates per isolate per fungicide concentration. Fungicide sensitivity was tested at the following concentrations: propiconazole, tebuconazole and flutriafol, each at 0.1, 0.5, 1, and 2 µg/ml; flusilazole, bromuconazole, each at 0.05, 0.1, 0.5 and 1 µg/ml. Plates were incubated at 20°C in the dark for 10 days, and colony growth assessed. Linear growth of each isolate was determined by calculating the mean of the two perpendicular diameters for each of the three colonies at each concentration. The fungicide concentration which inhibited the colony growth of the isolates by 50%

(EC_{50} value), compared to the control, was determined by regression analysis of the log inhibition.

RAPD analysis

DNA extraction. Mycelium was scraped from actively growing colonies on PDA (approximately 4 cm diam.), placed in Eppendorf tubes containing 1000 µl SDS extraction buffer, boiled for 3 min, cooled on ice for 10 min and incubated at 65°C for 1.5 h. Phenol (600 µl) and chloroform (400 µl) were subsequently added, and mixed thoroughly. The mixture was spun at 13000 rpm for 15 min at room temperature, the upper aqueous phase containing the DNA removed, and the DNA precipitated by adding 20 µl of 3 *M* NaOAc and 600 µl isopropanol. After 2 min of centrifugation at 13000 rpm, the supernatant was poured off and the DNA pellet resuspended in 1.4 ml of 1x TNE buffer (50 mM Tris, pH 7.5, 5 mM EDTA, 100 Mm NaCl). To remove RNA, 20 µl of RNase (20 mg/ml stock solution) was added and incubated at 37°C for 2 h. The RNase digestion was terminated by adding 10 µl of 20% SDS. Proteinase K (10 µl of 20 mg/ml stock solution) was added and incubated at 37°C for 3 h. The sample was extracted with an equal volume of phenol:chloroform. The DNA was precipitated by adding 0.1 volume of 3 *M* NaOAc and 0.6 volume of isopropanol. After centrifugation the DNA was washed with 70% ethanol, dried in an oven at 65°C for an hour, resuspended in sterile distilled water, and stored at -20°C.

Amplification conditions. Amplification reactions were run in a final volume of 25 µl of reaction mixture. The mixture consisted of 2 µl of each dNTP, 2 µl of $MgCl_2$, 2 µl of the primer, 2.5 µl of buffer, 0.1 µl Taq polymerase, 11.4 µl ddH_2O and 5 µl genomic DNA. The primer (Operon Technologies Inc., Almeda, U.S.A.) that segregates mating type was: OPE 02: 5' GGTGCGGGAA3'. *T. yallundae* is a heterothallic fungus with a 1:1 ratio of MAT1-1 to MAT1-2 ascospores in an ascus (Moreau and Maraite, 1995; Dyer *et al.*, 1993), which this primer could successfully distinguish.

RESULTS

Bromuconazole

The EC_{50} values of the first parental generation (P1) mating strains were relatively similar (0.7633 µg/ml and 0.6815 µg/ml), whereas their progeny proved to have lower EC_{50} values (0.0403 – 0.9283 µg/ml). In contrast, the EC_{50} values of the second parental generation (P2) mating strains varied greatly (0.6214 µg/ml and 0.9283 µg/ml), whereas their progeny all had EC_{50} values between (0.5077 – 0.7298 µg/ml), thus generally being lower than that of the parental strains. On average, only one (F2) isolate (F2-1) had a comparable EC_{50} value (0.7298 µg/ml) to that of the original P1 parental strains (Fig. 1).

Propiconazole

The parental isolates of both generations (P1 and P2) had comparable EC_{50} values between (0.7377 µg/ml and 0.614 µg/ml; 0.7227 µg/ml and 0.6878 µg/ml, respectively). More than 50% of the F1 progeny, however, exhibited a lower resistance to that of their parental isolates. In contrast, the F2 progeny either had comparable or higher EC_{50} values than their parental isolates (Fig. 2).

Flutriafol

As found with propiconazole, parental isolates of the two generations exhibited comparable EC_{50} values (0.9581 µg/ml and 0.8714 µg/ml; 0.9243 µg/ml and 0.8713 µg/ml, respectively). Furthermore, the same trend was also observed in the progeny, where the F1 isolates generally had the same or lower EC_{50} values. In contrast, most F2 isolates were comparable with the parental isolates in having EC_{50} values between (0.539 µg/ml – 1.3296 µg/ml). However, a few isolates were either below or above this range (Fig. 3).

Tebuconazole

As found with propiconazole and flutriafol, parental isolates of the two generations were once again similar regarding their EC_{50} values (0.912 µg/ml and 0.5937 µg/ml; 0.6393 µg/ml and 0.6964 µg/ml, respectively). In this case, however, F1 and F2 progeny had either similar or lower EC_{50} values than the parents. The exception was

isolate F2-14, which had an EC_{50} value of 1.65 µg/ml, and proved to be highly resistant to tebuconazole (Fig. 4).

Flusilazole

Parental isolates of both generations had comparable EC_{50} values between 0.6499 µg/ml and 0.5794 µg/ml; 0.5991 µg/ml and 0.6087 µg/ml), once again exhibiting the trend observed for propiconazole, flutriafol and tebuconazole. Progeny of both the F1 and F2 generations exhibited more or less the same or a much lower EC_{50} value (Fig. 5).

RAPD analysis

From the initial screening, the RAPD primer OPE 02 clearly differentiated progeny as either MAT1-1 or MAT1-2, and furthermore showed mating type of *T. yallundae* to segregate in a Mendelian fashion as reported earlier (Moreau and Mariate, 1995) (Fig. 6). Of the 17 isolates screened, six belonged to one mating type (MAT1-1) (P1-1, P2-1, F1-1, F1-3, F1-4 and F1-6), and eleven to the other (MAT1-2) (P1-2, P2-2, F1-2, F1-5, F1-7 — F1-13).

DISCUSSION

The importance of sexual reproduction to the dynamics of resistance depends as much on the basic resistance as on the frequency of sexual recombination (Milgroom *et al.*, 1989). The occurrence of progeny with EC_{50} values different from that of the parental isolates towards specific fungicides is indicative of sexual recombination. Resistance may be controlled by a single locus, or may be polygenic (Hollomon, 1981; Hollomon *et al.*, 1984; Stanis and Jones, 1984; Crute and Harrison, 1988). In a study by Gafur *et al.* (1998), four strains of *Cochliobolus heterostrophus* resistant to polyoxin were crossed with a sensitive wild-type strain. Progeny was observed to segregate in a 1:1 ratio, indicating that a single Mendelian gene was in control of resistance to polyoxin in *C. heterostrophus*. Furthermore, progeny of allelism tests among resistant mutants segregated in a 3:1 ratio of resistant to sensitive phenotypes, implying that polyoxin resistance in these four strains was controlled by a single gene at different, unlinked loci. Dekker (1993) stated that there is a quantitative build-up of resistance to triazoles, which indicated polygenic control. In terms of resistance dynamics, the

simplest case of sexual reproduction to consider is when resistance is controlled by a single locus in a haploid fungus. If it is assumed that an allele for resistance operates equally in all genetic backgrounds (i.e. equal levels of resistance and fitness costs), then sexual reproduction in a randomly mating population will not have any effect on the genotypic or phenotypic frequencies for resistance. The frequencies will be the same in the progenies as they were in the parents (assuming no selection). On the other hand, if an allele has a different expression depending on the genetic background, then recombination may affect the fitness of resistant individuals and subsequently alter phenotypic frequencies (Milgroom *et al.*, 1989).

If the build-up of resistance is polygenically controlled, the various combinations will result in a continuous distribution of sensitivities, so that distinct subpopulations cannot be recognized. In the present study, where parental strains and the F1 and F2 populations were compared to different triazoles for fungicide resistance, a continuous range of sensitivities was observed (Figs 1-5), indicating polygenic control. The manner in which quantitative resistance is inherited can be correlated with how it develops. Single gene resistance is distributed into a limited number of discrete phenotypes, whereas quantitatively inherited resistance is distributed continuously (Georpoulos, 1985). Moreau and Maraite (1996) observed frequent recombinations between benomyl and triademinol sensitivities in ascospore progeny of *Tapesia yallundae*, which demonstrated that clonal isolation was broken by the sexual cycle, resulting in a strain with multiple fungicide resistance. Quantitative resistance towards the various triazoles screened in the present study was clearly observed in both generations of *T. yallundae* studied.

The purpose of employing RAPD's in the present study was to derive a quick technique to distinguish mating type in field populations, and to determine if there was any correlation between mating type and fungicide resistance. *T. yallundae* is a biallelic hetherothallic ascomycete (Moreau and Maraite, 1995; Dyer *et al*, 1993). In heterothallic ascomycetes mating type is determined by one gene at one locus, and therefore no intermediates are expected when mating type is segregated. In the present study, mating type (single locus) was contrasted to triazole fungicide resistance, which is a polygenic trait. No correlation was found between mating type and fungicide sensitivity, which suggests that different sets of genes control the two phenomena, namely mating type determination and triazole fungicide sensitivity or

fungicide sensitivity in general. The low numbers of progeny investigated in the present study, preclude any further genetic or mating type interpretations of the data obtained. However, MAT1-1 and MAT1-2 isolates were observed to be equally resistant or susceptible to the various triazoles studied.

REFERENCES

Brasier, C.M. (1987). Dynamics of fungal speciation. In *Evolutionary biology of the fungi*, (Eds. A.D.M. Rayner., C.M. Brasier. and D.Moore), pp. 231-260. Cambridge University Press: Cambridge.

Burdon, J.J. and Roelfs, A.P. (1985). Isozyme and virulence variation in asexually reproducing populations of *Puccinia graminis* and *P. recondita* on wheat. *Phytopathology* 75, 907-913.

Burnett, J.H. (1983). Speciation in fungi. *Transactions of the British Mycological Society* **81**, 1-14.

Crute, I.R. and Harrison, J.M. (1988). Studies on the inheritance of resistance to metalaxyl in *Bremia lactucae* and on the stability and fitness of field isolates. *Plant Pathology* **37**, 231-250.

Dekker, J. (1993). The fungicide resistance problem: current status and the role of systemics. In *Pesticide interactions in crop production*. (Ed. J. Altman), pp. 163-180. CRC Press, Inc.: Florida.

Dyer, P.S., Nicholson, P., Rezanoor, H.N., Lucas, J.A. and Peberdy, J.F. (1993). Two-allelle heterothallism in *Tapesia yallundae*, the teleomorph of the cereal eyespot pathogen *Pseudocercosporella herpotrichoides*. *Physiological and Molecular Plant Pathology* **43,** 403-414.

Dyer, P.S., Bateman, G.L., Lucas, J.A. and Peberdy, J.F. (1994). Seasonal development of apothecia of cereal eyespot pathogen *Tapesia yallundae* on straw stubble in the UK. *Annals of Applied Biology* **125,** 489-500.

Dyer, P.S. and Lucas, J.A. (1995). Incidence of apothecia of *Tapesia yallundae* at set-aside sites in England and sensitivity of the ascospore offspring to the fungicides benomyl and prochloraz. *Plant Pathology* **44,** 796-804.

Ennos, R.A. and Swales, K.W. (1988). Genetic variation in tolerance of host monoterpenes in a population of the ascomycete canker pathogen *Crumenulopsis sororia*. *Plant Pathology* **37**, 407-416.

Ennos, R.A. and Swales, K.W. (1991). Genetic variation in fungal pathogen: Response to host defensive chemicals. *Evolution* **45**, 190-234.

Faretra, F., Antonacci., E. and Pallastro, S. (1988). Sexual behaviour and mating system of *Botryotinia fuckeliana*, teleomorph of *Botrytis cineria*. *Journal of General Microbiology* **134**, 2543-2550.

Fitt, B.D.L., Goulds, A. and Polley, R.W. (1988). Eyespot (*Pseudocercosporella herpotrichoides*) epidemiology in relation to prediction of disease severity and yield loss in winter wheat - a review. *Plant Pathology* **37**, 311-328.

Gafur, A., Tanaka, C., Shimizu, K., Ouchi, S. and Tsuda, M. (1998). Genetic analysis of *Cochliobolus heterostrophus* polyoxin resistant mutants. *Mycoscience* **39**, 155-159.

Georpoulos, S.G. (1985). The genetic basis of classification of fungicides according to resistance risk. *EPPO Bulletin* **15**, 513-517.

Goodwin, S.B., Spielman, L.J., Matuszak, J.M., Bergeron, S.N. and Fry, W.E. (1992). Clonal diversity and genetic differentiation of *Phytophthora infestans* populations in Northern and Central Mexico. *Phytopathology* **82**, 955-961.

Grindle, M. (1987). Genetic basis of fungicide resistance. In *Combating Resistance to Xenobiotics-Biological and Chemical Approaches*, (Eds. M.G. Ford, D.W. Hollomon, B.P.S. Kambay and R.M. Sawicki), pp. 75-93. Ellis Horwood: Chichester.

Hollomon, D.W. (1981). Genetic control of ethirimol resistance in a natural population of *Erysiphe graminis* f.sp. *hordei*. *Phytopathology* **71**, 536-540.

Hollomon, D.W., Butters, J. and Clark, J. (1984). Genetic control of triadimenol resistance in barley powdery mildew. In *Procedures of British Crop Protection Conference* pp. 477-482.

King, A. C. (1991). Observations of apothecia of *Tapesia yallundae* and the cultural phenotypes of their progeny. *Plant Pathology* **40**, 367-373.

Leroux, P., Gredt, M. and Boeda, P. (1988). Resistance to inhibitors of sterol biosynthesis in field isolates or laboratory strains of the eyespot pathogen *Pseudocercosporella herpotrichoides. Pesticide Science* **23**, 119-129.

Leroux, P. and Gredt, M. (1997). Evolution of fungicide resistance in the cereal eyespot fungi *Tapesia yallundae* and *Tapesia acuformis* in France. *Pesticide Science* **51**, 321-327.

Lochner, H. (1989). Omlê word onderskat. *Landbouweekblad* **591**, 36-37.

Milgroom, M.G., Levin, S.A. and Fry, W.E. (1989). Population genetic theory and fungicide resistance. In *Plant Disease Epidemiology* (Eds. K.J. Leonard and W.E. Fry) pp. 340-347. McGraw-Hill: New York.

Moreau, J.M. and Maraite, H. (1995). Bipolar heterothallism in *Tapesia yallundae* for the two varieties of the anamorph *Pseudocercosporella herpotrichoides. Mycological Research* **99**, 76-80.

Moreau, J.M. and Maraite, H. (1996). Evidence for heterothallic mating system in *Tapesia acuformis* using benomyl sensitivity and esterase isoenzymes profiles. *Mycological Research* **100**, 1227-1236.

Peever, T.L. and Milgroom, M.G. (1993). Genetic structure of *Pyrenophora teres* populations determined with random amplified polymorphic DNA markers. *Canadian Journal of Botany* **72**, 915-923.

Robbertse, B., Crous, P.W. and Holz, G. (1994). *Tapesia yallundae* collected from wheat stubble in South Africa. *Mycopathologia* **125**, 23-28.

Saunderson, F.R. and King, A.C. (1988). Field occurrence of *Tapesia yallundae*, the teleomorph of *Pseudocercosporella herpotrichoides. Australasian Plant Pathology* **17**, 20-21.

Scott, D.B. (1990). Wheat diseases in South Africa. Republic of South Africa, Department of Agricultural Research, Technical Communication **220**, 17-19.

Sindberg, S., Munk, L. and Schulz, H. (1994). *Tapesia yallundae*, the teleomorph of *Pseudocercosporella herpotrichoides* observed for the first time in Denmark. *Journal of Plant Disease and Protection* **101**, 316-320.

Skylakakis, G. (1985). Two different processes for the selection of fungicide resistant populations. *EPPO Bulletin* **15**, 519-525.

Skylakakis, G. and Hollomon, D.W. (1987). Epidemiology of fungicide resistance. In *Combating Resistance to Xenobiotics*: *Biological and Chemical Approaches* pp. 94-102. Ellis-Horwood: Chichester.

Smith, F.D., Parker, D.M. and Koller, W. (1991). Sensitivity distribution of *Venturia inaequalis* to the sterol demethylation inhibitor flusilazole: Baseline sensitivity and implications for resistance monitoring. *Phytopathology* **81**, 392-396.

Stanis, V.F. and Jones, A.L. (1984). Genetics of benomyl resistance in *Venturia inequalis* from North and South America, Europe and New Zealand. *Canadian Journal of Plant Pathology* **6**, 283-290.

Stanis, V.F. and Jones, A.L. (1985). Reduced sensitivity to sterol biosynthesis-inhibiting fungicides in field isolates of *Venturia inequalis*. *Phytopathology* **75**, 1098-1101.

Van Tuyl, J.M. (1977). Genetics of fungal resistance to systemic fungicides. *Mededelingen Landbouwhogeschool* 77, 2.

Via, S. (1986). Quantitative genetic models and the evolution of pesticide resistance. In *Pesticide Resistance; Strategies and Tactics for Management* pp. 222-235. National Academy Press: Washington D.C.

Welgemoed, Z. (1987). Keer omvalsiekte by koring betyds. *Landbouweekblad* **463**, 16-19.

Wilson, A.D. and Kaiser, J. (1995). Cytology and genetics of sexual compatibility in *Didymella rabiei*. *Mycologia* **87**, 795-804.

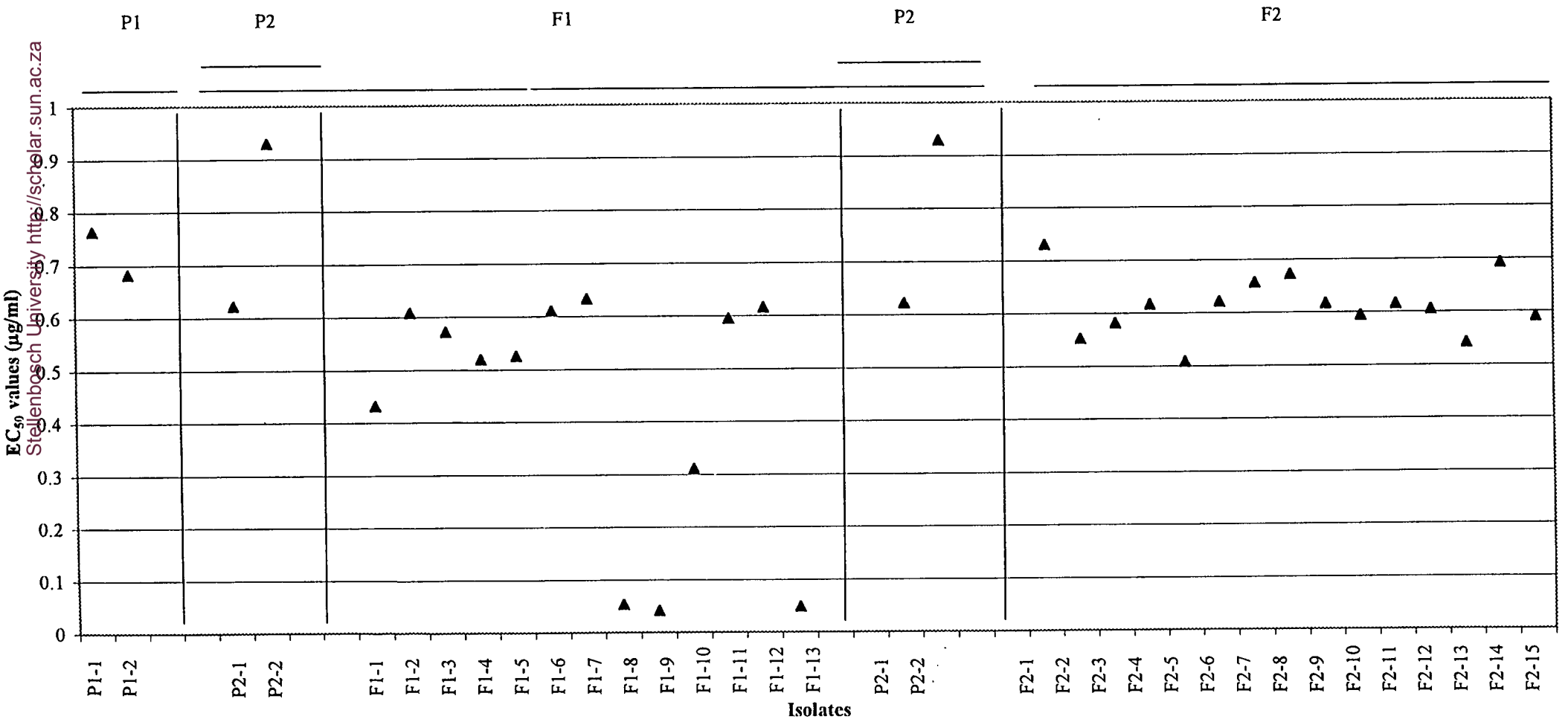


Fig. 1. Fungicide sensitivity of parental isolates and progenies of *Tapesia yallundae* towards bromuconazole. P1 and P2 respectively indicate the first and second parental generations. F1 and F2 respectively indicate the first and second filial generations.

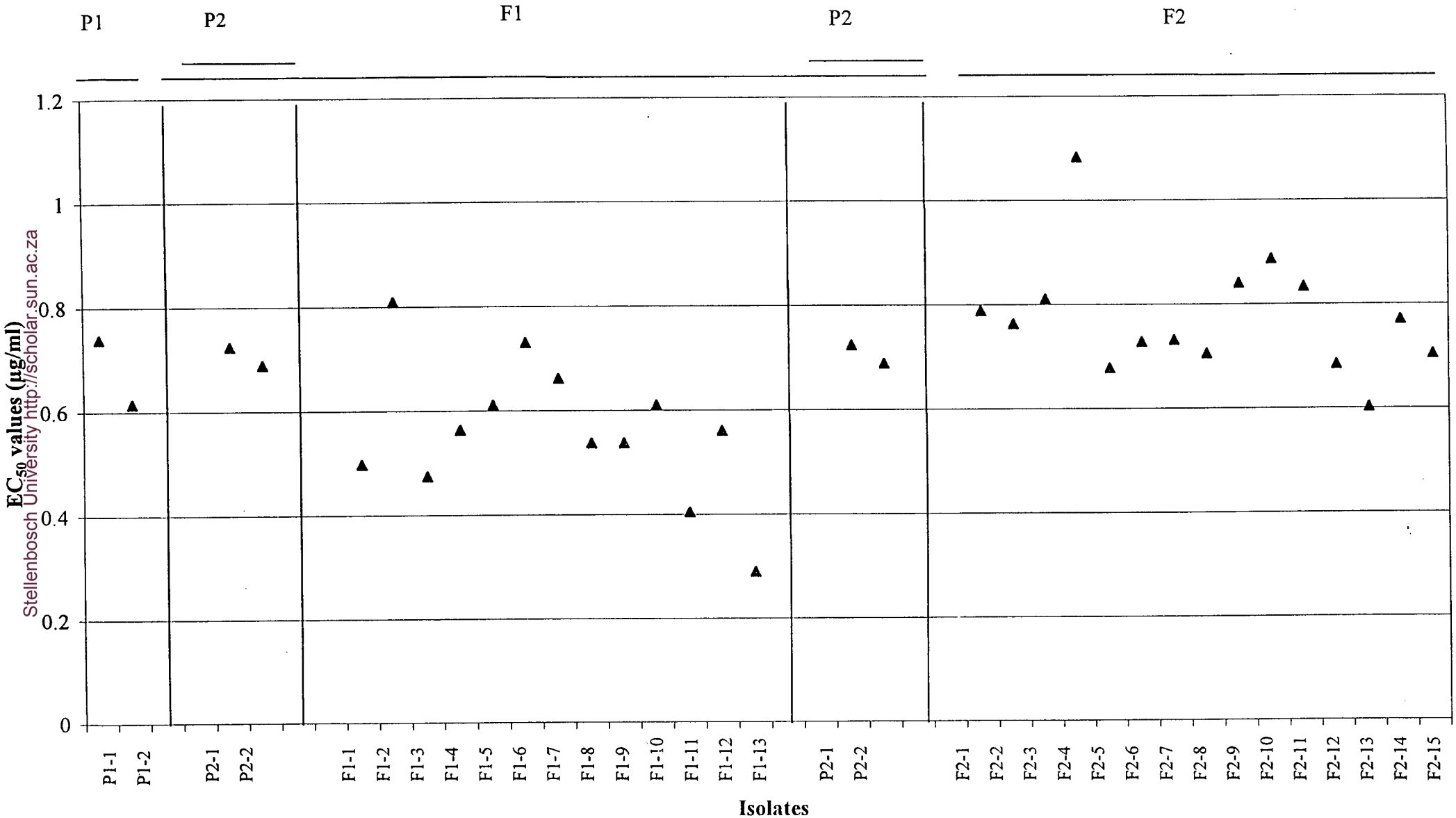


Fig. 2. Fungicide sensitivity of parental isolates and progenies of *Tapesia yallundae* towards propiconazole. P1 and P2 respectively indicate the first and second parental generations. F1 and F2 respectively indicate the first and second filial generations.

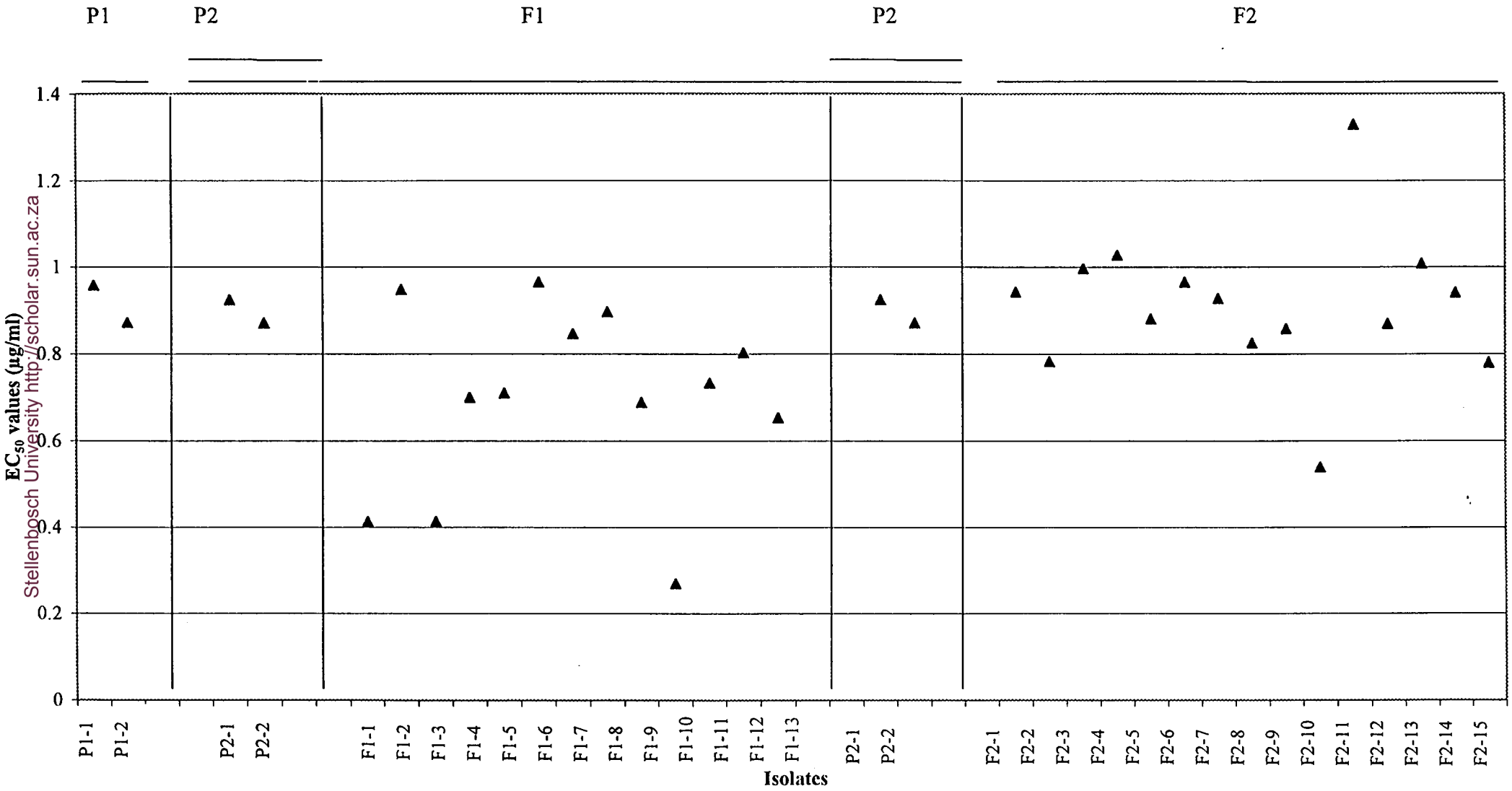


Fig. 3. Fungicide sensitivity of parental isolates and progenies of *Tapesia yallundae* towards flutriafol. P1 and P2 respectively indicate the first and second parental generations. F1 and F2 respectively indicate the first and second filial generations.

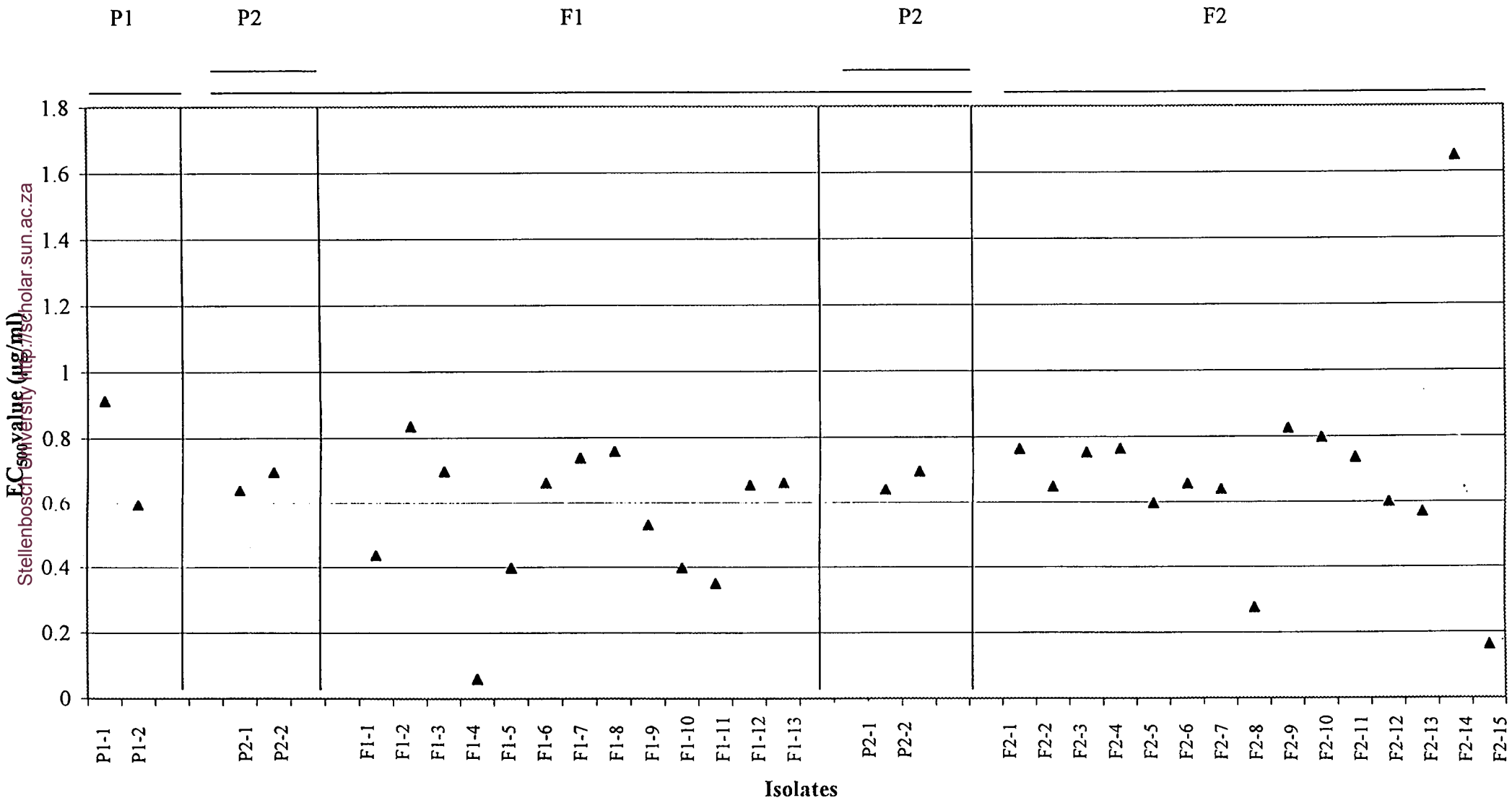


Fig. 4. Fungicide sensitivity of parental isolates and progenies of *Tapesia yallundae* towards tebuconazole. P1 and P2 respectively indicate the first and second parental generations. F1 and F2 respectively indicate the first and second filial generations.

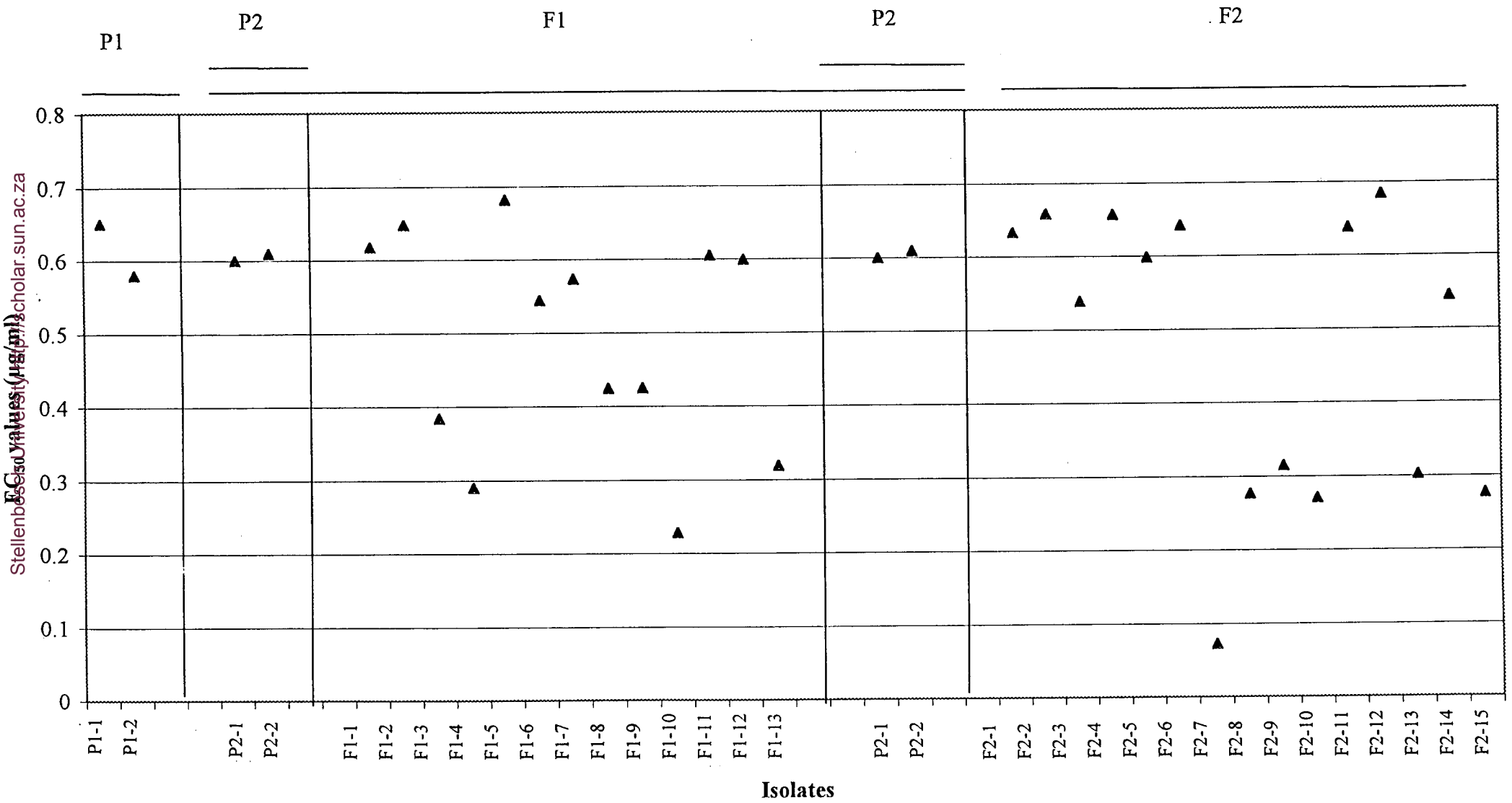


Fig. 5. Fungicide sensitivity of parental isolates and progenies of *Tapesia yallundae* towards flusilazole. P1 and P2 respectively indicate the first and second parental generations. F1 and F2 respectively indicate the first and second filial generations.

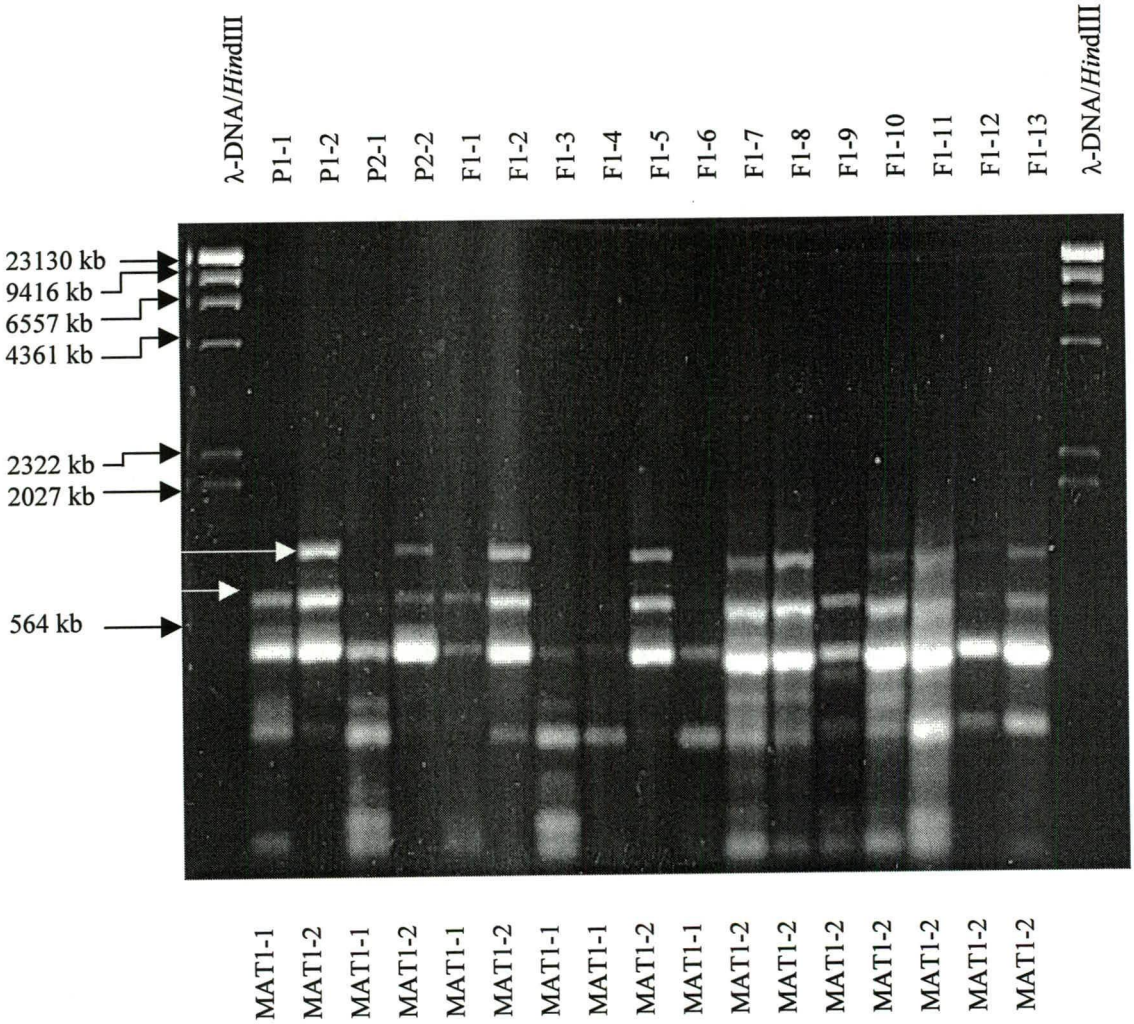


Fig 6. RAPD banding patterns generated on the F1 progeny of *Tapesia yallundae* using the primer OPE 02. Markers are shown on either side of the isolates. White arrows indicate bands distinguishing mating type.

4. DETERMINING THE EFFECTIVE POPULATION NUMBER IN POPULATIONS OF *TAPESIA YALLUNDAE* FROM SOUTH AFRICA AND THE UNITED KINGDOM

ABSTRACT

Tapesia yallundae causes eyespot disease of cereals in cool, maritime regions. In England and other countries in Europe, the sexual phase of this fungus has been commonly collected from the field, but in South Africa, the sexual phase has thus far only been induced in the laboratory. Sexual compatibility studies were conducted using 20 and 45 isolates from England and South Africa, respectively. All isolates were mated with two South African tester strains (MAT1-1 and MAT1-2). The isolates and the tester strains were used both as females (sperm recipients) and as males (sperm donors). Only two of the isolates were found to be hermaphrodites in the U.K. population, while three of the South African isolates tested proved to be hermaphrodites. The effective population number was 7.2 in the U.K. population and 11.2 in the South African population. The inbreeding effective population number was 14.5 for the U.K. population, and 22.5 for the South African population. The variance effective number was 3.6 in the U.K. population and 3.75 in the South African population. These values indicate a lack of female fertility in both populations. The data reflects no correlation between the existence of the sexual stage and the proportion of hermaphrodites. Furthermore, it also does not support the hypothesis that the South African population is any less sexual than the one found in England. These data suggest, therefore, that although sex may be a more common phenomenon in eyespot populations occurring in England, the South African population appears genetically similar to that of the U.K.

INTRODUCTION

The concept of effective population number was introduced by Wright (1931, 1938) and is useful in predicting inbreeding or random genetic drift (Leslie, 1995), and to compare field populations to an idealised population. An idealised member of a heterothallic population of filamentous ascomycetes is a self-sterile hermaphrodite that is capable of producing male gametes and elaborating the female reproductive

structure. The effective population number may be based on the number of strains of different mating types or the relative frequency of hermaphrodites. The female-sterile mutants are at a selective disadvantage every time sexual reproduction occurs, and must be at an advantage during vegetative propagation to persist at significant frequency. When a high frequency of female-sterile strains is observed in field populations, it indicates that vegetative propagation is a significant component of the fungus's natural history (Leslie and Klein, 1996).

A female structure that is fertilized by a male gamete matures to produce ascospores that are derived from the meiotic products of a diploid, biparental nucleus. The parent from whom the male gamete originated is termed the male parent while the parental mycelium upon which the female reproductive structure develops is termed the female parent. The male gamete might be an asexually produced spore, a sexually produced spore, or a mycelial fragment. The female structure is usually more elaborate, requiring a high degree of cellular specialization and the development of a highly organised structure. When sexual reproduction does occur, it is possible for a single self-sterile individual to act as a male, female or both (Leslie and Klein, 1996). Strains that are fertile in both crosses are described as either female fertile or as hermaphrodites (Leslie, 1995). To determine which parent was the male and which was the female in *Gibberella fujikuroi*, strains carrying the pall mutation can be used to distinguish perithicia formed by the different parental strains (Leslie, 1995). A similar mechanism needs to be established in *Tapesia yallundae* Wallwork & Spooner. In real populations, however, parents may have different probabilities of contributing offspring because of differences in their fertility or in the viability of their offspring or, peharps, because of impositions by the breeder. Thus parents will vary in their contributions just by chance (Caballero, 1994).

In the production of sexual progeny, hermaphrodites always contribute more to the next generation than the female sterile strains. Differences among parents in their contributions other than by sampling might be due to non-inherited causes, and the impact of the two situations on the effective size is different. Non-inherited differences of contributions of parents can arise, for example, from environmental causes, randomly allocated parents to each generation, or due to differences in parental fertility (Caballero, 1994). The effective number of a natural population is almost less than the number of adults of reproductive age and may be considerable

less, usually for one or more of three reasons: 1) unequal numbers of males and females, 2) temporal variation in population number, and 3) greater than binomial or Poisson variability in the number of progeny per parent (Crow and Denniston, 1988). Female sterility limits genetic exchange in field populations and reduces the inbreeding effective population size. Loss of the male function on the other hand might require the ability to form any asexual spores, a loss that is likely to be heavily selected against in field conditions. The relative levels of strains that are male only and those that are hermaphrodites may provide a good indication of how often sexual reproduction is occurring under field conditions (Leslie, 1995).

The genetic complexity associated with the elaboration of female reproductive structures provides a large number of loci together with a relatively long time in which the mutations can accumulate. This permits drift and mutation to play a much larger role in determining the frequency of female sterility than is found in single locus processes. The origin of the female sterile strains may not be known, and their high frequency may be due to selection, mutation, drift, or a combination of effects with the same end results (Leslie and Klein, 1996).

Although previous studies have shown that both mating types are represented within South African field populations of *T. yallundae* with 26 out 300 matings succeeding in a study by Robbetse *et al.* (1994), the reproductive status of the South African population is not yet known. It is therefore crucial to understand the sexual reproductive potential in the South African *T. yallundae* population. The purpose of this study is to determine the effective population number in the South African population in which sex has not been observed in the field, and to contrast this with a British field population, in which sex is commonly observed.

MATERIALS AND METHODS

Sampling

Three fields in close proximity to each other in the Swartland were selected, and 15 plants collected from each field (one isolate per symptomatic plant), resulting in 45 isolates representing the South African population. Similarly, 20 isolates were collected from a field at Rothamsted, and accepted as representing the U.K. population.

Isolation and identification

Symptomatic stubble were dipped in 70% ethanol for 30 sec to dissolve the waxy layer, and surface sterilised in 1% NaOCl for 1 min. The NaOCl was removed by dipping the stubble into 70% ethanol for a further 30 sec, and the stubble rinsed in sterile distilled water. Stubble were placed in Petri dishes on moistened filter paper and incubated for 2 weeks at 10°C under near-ultraviolet light to enhance sporulation. After sporulation conidia were suspended in water on water agar (12 g Biolab agar), and left under a laminar flow hood to germinate. Conidia were located using a stereo microscope and single conidial colonies were established. Isolates were grown on potato dextrose agar (PDA) (Biolab) at 25°C in the dark.

Mating system

Isolates of *T. yallundae* from South Africa and from England were mated to each of two South African tester strains, BR14A and BR3 (Robbertse *et al.*, 1994). The 45 isolates from South Africa and the 20 from the U.K. were mated against the two tester strains in two sets of matings. In one series of matings the one tester strain was used as a female (sperm recipient), and in another mating experiment the other tester strain was used as a male (sperm donor). Isolates used as female strains colonize the stubble to which sperm is added (crushed mycelial and conidium suspension). The sperm is dispensed over colonized stubble with a pipette. Matings were done with sterile, autoclaved stubble pieces (5 cm long) on water agar plates. Colonized stubble (25°C for 2 weeks) that were spermatised, were further incubated at 10°C under near-ultraviolet light for 3-8 months.

Successful matings were indentified by the presence of apothecia on stubble. The effective population number (*Ne*) reflects the genetic drift and inbreeding present in the population. The effective population number was calculated based on the mating data using the equation derived from Leslie and Klein (1996).

$$Ne = (4NmNf) / (Nm+Nf)$$

Nm represents the number of isolates with the one mating type allele, and *Nf* represents the number of isolates with the other mating type allele.

The number of hermaphrodites in a population influences the succeeding generation than female sterile strains, and in that function hermaphrodites always

have a greater contribution (Leslie and Klein, 1996). The inbreeding effective number (*Ne(f)*) is based on the numbers of males and hermaphrodites, and can be calculated by the following equation derived from Leslie and Klein (1996).

$$(Ne(f))\ 4N2Nh / (N + Nh)$$

The total number of individuals in a population is represented by *N*, and the number of hermaphrodites is represented by (*Nh*).

The variance effective number (*Ne(v)*) is associated with the number of individuals in the population as well as the fertility of the progeny resulting from sexual reproduction and recombination (Crow and Deniston, 1988; Leslie and Klein, 1996). The variance effective number provides an assessment of the amount of allele-frequency drift currently occurring in the population (Crow and Deniston, 1996). The size of an ideal population should thus have the same amount of random gene-frequency drift as that in the observed population. The variance effective number is based on numbers of males and hermaphrodites, and is calculated by the following equation derived from Leslie and Klein (1996):

$$Ne(v)=2N2 / (N + Nh)$$

RESULTS AND DISCUSSION

Of the 45 South African isolates involved 31 mated successfully with both tester strains. Thirteen out of 20 UK isolates mated successfully with the tester strains. Of the 260 crosses performed only 52 successful crosses were recorded. Three and two hermaphrodites were recorded for the South African and U.K. populations, respectively. The percentage of hermaphrodites was lower in the South African population than in the U.K. population, but the small difference in the percentages, and the relatively small sample sizes made it difficult to draw conclusions. Furthermore, a 20% mating success is also not a convincing result for mating studies aimed at analyzing this trait. Alterations caused by mutation, less suitable substrate, temperature, light, or unfavourable tester strains might be the cause of the low success rate. Usually, compatibility is largely determined by mating type, with similarity in mating type of different strains meaning incompatibility. Genetic factors other than those at the mating type locus may thus determine the productivity of sexual crosses, although this fact is rarely considered (Burnett, 1975).

Several factors could play a role in the polygenic control of compatibility and fertility. Genes encoding for functions necessary for successful sexual reproduction, such as the development of ascocarp initials might be expressed at different levels in different isolates or have multiallelic forms. Certain isolates may also have reduced ability to synthesise the metabolites required for mating (Nelson, 1970; Dyer, 1991; Dyer *et al.*, 1993).

Furthermore, true secondary sexual incompatibility systems may operate. In contrast to the above factor a system of "heterogenic incompatibility" may be present in some fungi. To achieve full fertility, it is necessary that mating partners carry similar alleles at all relevant incompatibility loci. Such an incompatibility mating system might be important in mating isolation between separate races of an otherwise compatible species. Evidence for heterogenic incompatibility has come from an analysis of crossing relationships in *Coprinus bisporus* (Kemp, 1989) and *P. anserina,* in which at least nine loci were identified (Esser and Kuenen, 1967; Fincham, *et al.*, 1979). Evolutionary changes may lead to subgroup fertility barriers, as observed in *C. ulmi,* with incompatibility arising from slight differences in pheromone structure or differences in cell-surface recognition molecules (Brasier, 1984). Cytoplasmic factors may also determine sexual potential. The degree of compatibility between different cell nuclei and mitochondria may be a key factor governing the success of sexual fusions (Rayner and Ross, 1990).

Loss of female fertility limits the genetic exchange in a population and reduces the inbreeding effective number (Leslie and Klein, 1996). The effective population number was found to be 7.2 in the U.K. population, and 11.2 in the South African population. The inbreeding effective number was 14.5 in the U.K. population and 22.5 in the South African population. The variance effective number was 3.6 in the U.K. population and 3.75 in the South African population.

In both the South African and the U.K. populations of *T. yallundae* female fertility seemed to have been lost, as could be seen from the low inbreeding effective population size. The inbreeding effective population size is a function of the fertility of individuals in the population and the number of individuals. No female sterile strains can be crossed successfully. The low mating success rate might be a result of female sterility. Mating between a female-sterile isolate and a hermaphrodite would

have 50% female-sterile offspring and mating of this offspring with a hermaphrodite would have 75% female-sterile offspring. Female-sterile strains, therefore, result in more female-sterile strains than hermaphrodites (Leslie and Klein, 1996). In contrast, compatibility between two hermaphrodites would produce no female-sterile offspring.

Most pathogenic fungi have significant asexual reproduction phases so that a field containing millions of individuals may be comprised of only a limited number of different genotypes. In this case, the population size would be large, but the effective population size (the number of potential breeding individuals) may be small (McDonald and McDermott, 1993). No correlation has been found to exist between the respective presence and absence of the sexual stage in fields in the U.K. and South Africa, and their effective population numbers. In South Africa, wheat is harvested before the onset of a warm, dry summer, which inhibits the fungus of forming apothecia on stubble, for which it needs cold, wet weather conditions for eight weeks (Wallwork, 1987; Robbertse *et al.*, 1994). In the following season, it is possible that temperatures are never low enough for a lengthy period of time to allow the teleomorph to develop. The small differences between the effective population numbers of the South African and UK populations make any correlation analysis between the two populations difficult. However, based on these data, it would seem that the absence of the teleomorph from South African fields is due to environmental conditions and cultural practices, rather than the genetic constitution of the respective populations.

REFERENCES

Brasier, C.M. (1984). Intermycelial recognition systems in *Ceratocytis ulmi*: their physiological and ecological importance. In *Ecology and Physiology of the Fungal Mycelium*, (Eds. A.D.M. Rayner. and D.H. Jennings), pp. 451-497. Cambridge University Press.

Burnett, J.H. (1975). *Mycogenetics. An Introduction to the General Genetics of Fungi*. John Wiley: London.

Caballero, A. (1994). Developments in the prediction of effective population size. *Heredity* **73**, 657-679.

Crow, J.F. and Denniston, C. (1988). Inbreeding and variance effective population numbers. *Evolution* **42**, 482-495.

Dyer, P.S. (1991). Perithecial development in *Nectria haematococca* mating population VI. Ph.D. thesis, University of Cambridge.

Dyer, P.S., Ingram, D.S. and Jonestone, K. (1993). Evidence for the involvement of linoleic acid and other endogenous lipid factors in perithecial development of *Nectria haematococca* mating population VI. *Mycological Research* **97**, 485-496.

Esser, K and Kuenen, R. (1967). *Genetics of Fungi*. Springer-Verlag: Berlin.

Fincham, J.R.S., Day, P.R. and Radford, A. (1979). *Fungal genetics*. Fourth ed. Blackwell Scientific Publications: Oxford.

Kemp, R.F.O. (1989). Incompatibility in the Basidiomycetes. The heterogenic pentax. *Fork Publications* **1**,1-19.

Leslie, J.F. (1995). *Gibberella fujikuroi*: available populations and variable traits. *Canadian Journal of Botany* **73**, S282-S291.

Leslie, J.F. and Klein, K.K. (1996). Female fertility and mating type effects on effective population size and evolution in filamentous fungi. *Genetics* **144**, 557-567.

McDonald, B.A. and McDermott, J.M. (1993). Population genetics of plant pathogenic fungi. *BioScience* **43**, 311-319.

Nelson, R.R. (1970). Variation in mating capacities among isolates of *Cochliobolus carbonum*. *Canadian Journal of Botany* **48**, 261-263.

Rayner, A. and Ross, I. (1990). Sexual politics in the cell. *New Scientist* **129**, 30-33.

Robbertse, B., Crous, P.W. and Holz, G. (1994). *Tapesia yallundae* collected from wheat stubble in South Africa. *Mycopathologia* **125**, 23-28.

Wallwork, H. (1987). A *Tapesia* teleomorph for *Pseudocercosporella herpotrichoides*, the cause of eyespot of wheat. *Australasian Plant Pathology* **16**, 92-93.

Wright, S. (1931). Evolution in Mendelian populations. *Genetics* **16**, 97-159.

Wright, S. (1938). Size of population and inbreeding structure in relation to evolution. *Science* **87**, 430-430.